

Studies on Polyvinyl Alcohol-Starch Composite Films Targeting Wound Dressing Applications

*Thesis submitted in partial fulfillment of the
requirements for the degree of*

DOCTOR OF PHILOSOPHY

by

Aritra Das



**Department of Chemical Engineering
Indian Institute of Technology Guwahati
Guwahati–781039, India**

October 2021



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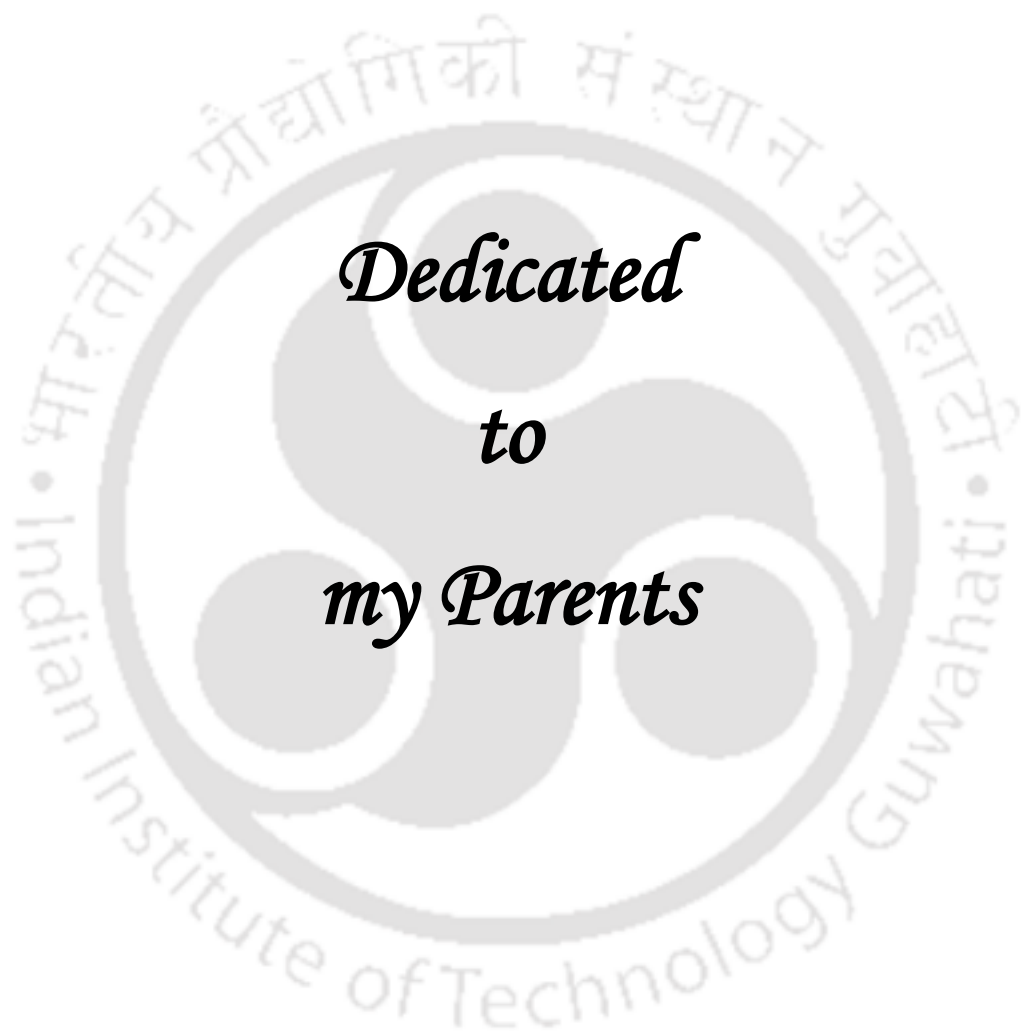
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**Department of Chemical Engineering
Indian Institute of Technology Guwahati
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October 2021





*Dedicated
to
my Parents*





Department of Chemical Engineering
Indian Institute of Technology Guwahati
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CERTIFICATE

It is certified that the work contained in this thesis entitled **“Studies on Polyvinyl Alcohol-Starch Composite Films Targeting Wound Dressing Applications”** submitted by **Ms. Aritra Das** for the award of the degree of Doctor of Philosophy has been carried out in the Department of Chemical Engineering, Indian Institute of Technology Guwahati under our supervision. To the best of our knowledge, this work is not submitted elsewhere for the award of any other degree or diploma.

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Abstract

Certain limitations of biodegradable natural polymers such as affordability, availability, shelf life and mechanical stability potentially restrict their role to replace synthetic polymers that gained wider acceptance in the global economy. On the other hand, the synthetic polymers do suffer with a basic limitation in terms of their disposal and inevitable recycling needs. To circumvent the issue, synthetic polymers need to be supplemented with natural polymers to enhance the biodegradability characteristics of the fabricated composites. Among synthetic and natural polymers, polyvinyl alcohol and starch can serve as potential composite film materials due to their promising features such as film forming capability, non-toxicity and transparency. However, the PVA-St composites need to be further engineered to supplement their higher water solubility and poor mobility characteristics with other additives that further enhance their combinatorial characteristics. Thereby, alternate wound dressing materials can be envisaged through such engineering research and development studies.

Compared to other alternate commercial wound dressing products, the polymer hydrogel films have the edge in terms of their ability to imbibe wound exudates and lower costs of materials and fabrication. Thus, functional PVA-St based composite hydrogel films need to be engineered with suitable low cost non-toxic crosslinker additives such as glycerol and organic acids constituting carboxylic and hydroxyl groups. Such additives contribute towards the crosslinking, plasticizing and antibacterial efficacy characteristics of the composite hydrogel films.

This Ph.D. thesis devotes towards the experimental and numerical optimization of PVA-St blended composite films targeting wound dressing applications. The appropriate constitution of Gl, alternate organic acid (Citric/Malic/Tartaric acid) in conjunction with PVA and St has been targeted for solution casting based polymer hydrogel films. Addressed characterizations include physical characteristics such as appearance and rheological characteristics, film thickness, ATR-FT-IR spectral analysis, swelling index, solubility, in-vitro degradation tests, gel fraction, mechanical strength (tensile strength and percentage elongation) and water vapour transmission rate and qualitative characteristics in terms of antibacterial effectiveness and relative growth rate of HEK cells.

The Ph.D. thesis targeted the accomplishment of five major objectives namely (a) Optimization of drying time, crosslinking temperature and CA concentration for fixed choice of PVA, St and Gl concentrations to achieve maximal combinations of SI, solubility, in vitro degradation or weight loss, GF, mechanical strength, antibacterial effectiveness and WVTR (b) Application of response surface methodology towards the experimental design based optimality of all film constituent concentrations and responses such as SI, in vitro degradation and TS characteristics (c) Extending RSM based experimental design towards the consideration of %E as an additional response variable (d) Comparative efficacy of alternate organic acid based PVA-St composite films being fabricated with the experimental and numerical optimization based film constitutions and (e) RSM design based optimality of PVA, St and MA constitutional variation based PVA-St composite films. Design expert software was deployed to achieve relevant experimental designs in the thesis. Thereby, optimal film constitutions and responses have been compared and contrasted with the most relevant optimal prior art data. The critical findings of the thesis have been summarized as follows:

The first objective related experimental investigations affirmed that the optimal solution casting process parameters refer to 35.58 wt% CA concentration, 50 °C crosslinking temperature and 12 h drying time to achieve PVA composite films with significantly higher SI (260.5 %), solubility (67.26 %), in-vitro degradation (59.77 %), good antibacterial activity against both *Escherichia coli* (Gram-negative bacteria) and *Listeria monocytogenes* (Gram-positive bacteria) but lower TS (2.54 MPa). Compared to the most relevant prior art, these findings translate into a 35 % reduction in energy costs through the deployment of 10 % expensive constituents.

The targeted second objective based experimental affirmed that while SI and WL followed quadratic expressions with respect to variations in the quaternary compositions, the TS was relatively less complex with bilinear and linear terms of the independent variables. Thereby, the optimal film constitution based on the adopted methodology refers to 5 w/w% PVA, 10 w/w% St, 15 wt% CA and 15 wt% Gl to achieve composite hydrogel films with excellent SI (338.37 %), acceptable in-vitro degradation (53.27 %) and higher TS (7.65 MPa).

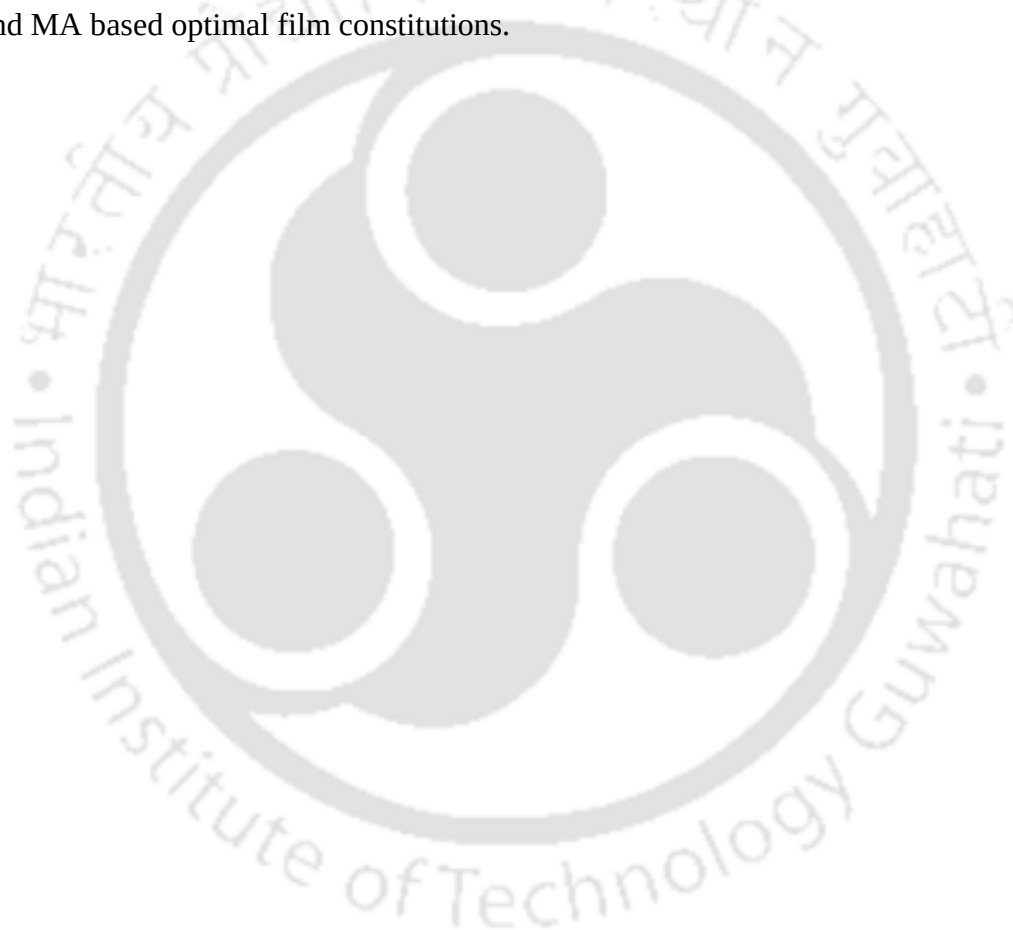
The third objective of the Ph.D. thesis affirmed that the consideration of other characterizations (ATR-FT-IR analysis, solubility and GF) corroborated with the second objective based trends

associated to solubility, WL, GF and TS but not %E. Thereby, the best experimental data set referred to 7.5 w/w% PVA, 7.5 w/w% St, 27.5 wt% CA and 27.5 wt% Gl (EXP+%E case) for which optimal responses were achieved as 297.88 % SI, 53.14 % WL, 5.31 MPa TS and 82.13 % elongation. Further, numerical optimization of the data set measured without considering %E as an additional response variable affirmed alternate film constitution of 5.46 w/w% PVA, 8.09 w/w% St, 22.77 wt% CA and 22.69 wt% Gl (RSM-%E case). Corresponding optimal responses were 305.73 % SI, 56.41 % WL and 5.39 MPa TS. Comparatively, for the RSM+%E case, alternate film constitution of 7.45 w/w% PVA, 6.96 w/w% St, 28.57 wt% CA and 18.38 wt% Gl was obtained along with significantly variant optimal response data of 300.48 % SI, 51.4 % WL, 4.98 MPa TS and 87.54 % elongation. Thus, RSM+%E and EXP+%E cases affirmed similarities in the film constitution and response optimality, which was not the case for the comparative trends of data associated to RSM-%E and EXP-%E cases. Among all optimized formulations, RSM+%E constitution indicated promising results in terms of both antibacterial activity and RGR (145.48 % with HEK cells for 48 h extracted sample).

The fourth objective of the Ph.D. thesis indicated that among alternate organic acids such as CA, MA and TA, the PVA-St-organic acid-Gl composite films with MA performed the best with respect to the relevant data being obtained for all other optimal film constitution cases (EXP-%E, EXP+%E, RSM-%E). For the RSM+%E case, the optimal responses referred to SI 739.29 %, WL 66.56 %, TS 4.88 MPa and %E 103.68 % and 104.78 % RGR with HEK cells for the 48 h extracted sample.

The final objective of the Ph.D. thesis targeted the compositional optimality of CCD-RSM based MA, St and PVA varied film constitutions and for fixed choice of Gl in the hydrogel films. Additional constraints have been imposed for the responses to achieve compositions with improvised characteristics during numerical optimization. The optimal film constitution referred to 6.92 w/w% PVA, 6.67 w/w% St, 25.96 wt% MA and 18.38 wt% Gl for which the best responses were being obtained as 583.83 % SI, 65.47 % WL, 5.57 MPa TS and 99.53 % elongation, 181.95 % RGR with the HEK cells after 48 h of extraction with the optimal film constitution. The optimal MA based film constitution has been 4.56 % inexpensive with respect to the optimal CA based film constitution.

In summary, the Ph.D. thesis affirmed that the comparatively expensive MA additive did indicate optimal film constitution with lower retail cost of the composite hydrogel film and enhanced properties. Thereby, compared to best available prior art data and those being inferred in the earlier objectives of the thesis, the MA based optimal film constitution can be inferred to be the most inexpensive polymer hydrogel film for targeted wound dressing application. In summary, the Ph.D. thesis demonstrated that the RSM has been a potential tool to assist physical characterizations and desired responses for the fabrication of low cost PVA composite films. Thereafter, further research towards process scale up and product development can be targeted for the CA and MA based optimal film constitutions.



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Abbreviation

–OH	Hydroxyl
–COOH	Carboxyl
–NH ₂	Amino
PVP	Polyvinyl pyrrolidone
PEG	Polyethylene glycol
PVAc	Hydroxylated polyvinyl acetal
PP	Polypropylene
PLGA	Poly(lactic-co-glycolic acid)
PCL	Polycaprolactone
PGA	Polyglycolide
PVA	Polyvinyl alcohol
PVA-St composite	SP composite
St	Starch
CMC	Carboxymethyl cellulose
Cs	Chitosan
Gel	Gelatine
CC	Collagen
Ag	Silver
AgNps	Silver nanoparticle
AgSD	Silver sulfadiazine
ZnO	Zinc oxide
nZnO	Zinc oxide nanoparticle
TiO ₂	Titanium dioxide nanoparticle
AV	Aloe vera
Gl	Glycerol
SO	Sorbitol
CA	Citric acid
MA	Malic acid

TA	Tartaric acid
SA	Succinic acid
HA	Hyaluronic acid
BA	Boron
GA	Glutaraldehyde
HP β CD	Hydroxypropyl- β -cyclodextrin
TH	Tetracycline hydrochloride
DI water	Deionized water
DMEM	Dulbecco's modified eagle's medium
FBS	Fetal bovine serum
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide
PBS	Phosphate buffer saline
F-T	Freeze-thaw
FTIR	Fourier transform infrared spectroscopy
BCI	Blood clotting index
HDF	Human dermal fibroblasts
S-MSCs	Skin mesenchymal stem cells
HEK	Human embryonic kidney
<i>E. coli</i>	<i>Escherichia coli</i>
<i>S. aureus</i>	<i>Staphylococcus aureus</i>
<i>P. aeruginosa</i>	<i>Pseudomonas aeruginosa</i>
<i>L. monocytogenes</i>	<i>Listeria monocytogenes</i>
<i>C. albicans</i>	<i>Candida albicans</i>
<i>B. subtilis</i>	<i>Bacillus subtilis</i>
USA	United states of america
NCCS	National centre for cell science
FDA	Food and drug administration
ISO	International organization for standardization
ANOVA	Analysis of variance
RSM	Response surface methodology
CCD	Central composite design
3D	Three dimensional

SD Standard deviation

Nomenclature

<i>A</i>	PVA concentration as an independent variable
<i>B</i>	St concentration as an independent variable
<i>C</i>	CA/MA concentration as an independent variable
<i>D</i>	Gl concentration as an independent variable
EXP-%E	Experimental investigation based optimal formulation that did not consider %E as an additional response variable
EXP+%E	Experimental investigation based optimal formulation that considers %E as an additional response variable
RSM-%E	Design expert software based optimal formulation that did not consider %E as an additional response variable
RSM+%E	Design expert software based optimal formulation that considers %E as an additional response variable
Opt 1	Design expert software based optimal formulation that did not consider additional compositional constraints (general approach)
Opt 2	Design expert software based optimal formulation that considers additional compositional constraints

Notations

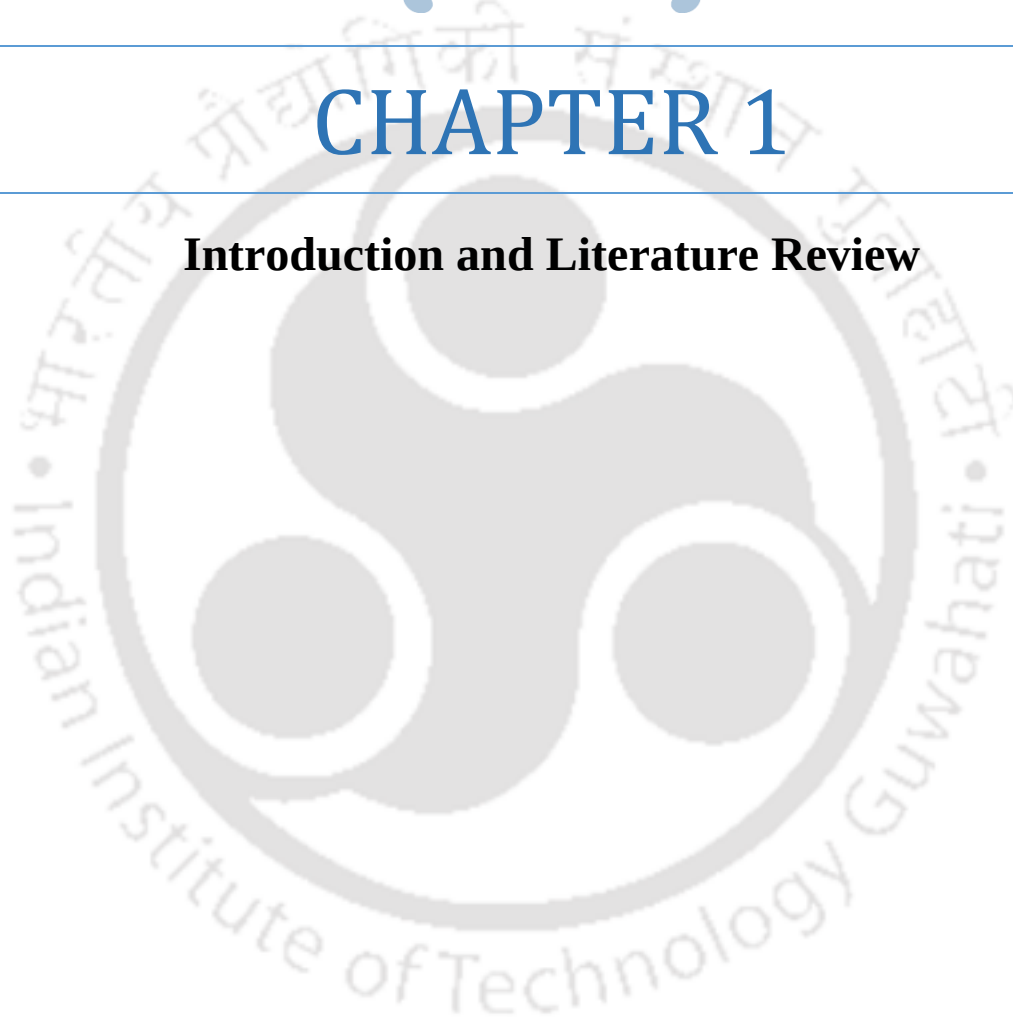
T_g	Glass transition temperature, °C
pK_a	Acid dissociation constant
WVTR	Water vapor transmission rate, g/m ² .day
WVP	Water vapor permeability, g.m/m ² .Pa.s
OTR	Oxygen transmission rate, g/m ² .day
TS	Tensile strength, MPa
%E	Percentage elongation or elongation at break, %
WS	Water solubility, %

WL	Weight loss, %
S	Solubility, %
DS	Degree of swelling, %
SI	Swelling index, %
GF	Gel fraction, %
RGR	Relative growth rate, %
G'	Storage modulus, Pa
G''	Loss modulus, Pa
W_0	Initial dry weight of the samples, g
W_s	Weight of the sample soaked in PBS for 24 h, g
W_d	Weight of the water-soaked sample measured after drying at 37 °C and 6 h, g
W_f	Weight of the PBS-soaked sample measured after drying at 37 °C and 6 h, g



CHAPTER 1

Introduction and Literature Review





Introduction and Literature Review

Section 1.1 of the chapter presents a brief summary with respect to the need for bio-degradable polymer films and their application towards wound dressing applications. The section also enlightens upon the conventionally used traditional material, wound healing process, mechanism and the desirable attributes of wound healing. Further, section 1.2 presents an overview of various alternate wound dressing materials. Section 1.3 devotes towards crosslinking based alternate fabrication methods. Section 1.4 details upon the efficacy of alternate low cost polymers, additives and the challenges associated with wound dressing film fabrication. Section 1.5 addresses the available prior art in the specific field of the effect of alternate base polymer, additives and variations in crosslinking time and temperature on the characteristics of bio-composite wound dressing films. Thereafter, section 1.6 devotes towards the scope for further research in the primary area of the synergy between various polymers and additives. Finally, section 1.7 presents objectives of the Ph.D. thesis followed with thesis organization related details in section 1.8.

1.1 Overview of wound dressing compatible polymer films

Polymeric films have numerous applications in the diverse fields of wastewater treatment, process industries, biomedical industries and packaging industries. The lower cost of polymeric films fosters and catalyzes upon their abundant usage. However, this same risks upon the well known serious ecological issues. To circumvent this issue, a transitional phase is apparent that attempts to enhance utilization of polymers as composites prepared with natural and bio-

degradable synthetic polymers (Torres et al., 2013). Apart from bio-degradability, such bio-composite polymer films have better combinations of properties that are contributed by each precursor in the polymer matrix.

The real world applications of bio-composite films necessitates towards a careful manipulation of polymeric materials to enhance their desired set of characteristics such as reduced water solubility, adaptability to simpler and effective processing techniques, shape stability and acceptable range of mechanical properties (Kulkarni et al., 2012). For example, composite films prepared with natural and synthetic polymers do not possess stability in aqueous media due to the inability of the natural polymers to possess an intriguing structure despite being crosslinked with the synthetic polymers. Therefore such bio-composites with poor aqueous stability cannot be deployed in wastewater treatment applications. However, such bio-composites possess excellent bio-compatibility and toxicologically safe properties. Hence, they can be effectively targeted in the field of packaging, pharmaceutical and bio-medical industries (Kaushik et al., 2016, Vroman and Tighzert, 2009).

Fine-tuned research in the field of natural and synthetic polymer based bio-composites indicate towards the relevance of polymer based composite hydrogel films. These hydrogel films have been characterized to possess promising features of transparency, near impermeability against harmful bacteria, ability to facilitate a moist environment for healing and ability to promote autolytic debridement for the effective imbibing of wound exudates (Kamoun et al., 2017).

To further augment the application of natural polymer-synthetic polymer based bio-composites as wound dressing materials, additional additives are often required to achieve other desirable attributes such as mechanical stability, protection against microbial contamination, enhanced bio-degradability and enhanced barrier properties. Several additives or crosslinkers have been

investigated till date to indicate upon pertinent molecular interactions and their synergistic compatibility. Thereby, the mentioned bio-composites with such additives have even better combinations of desired functionalities such as water resistance, flexibility, plasticity, physicochemical and mechanical properties. Despite indicating an improvement in many desired attributes, such polymeric bio-composites are not toxicologically safe. Therefore, contemporary research in the field of wound dressing applicable polymer bio-composite film fabrication needs to address upon the optimality of the additive constitution so as to simultaneously enhance antibacterial characteristics and facilitate the growth of healthy cells and new tissues in due course of the healing process.

In summary, in the vast field of natural polymer and/or synthetic polymer based bio-composites for wound dressing film fabrication and characterization, contemporary research needs to address the development of newer and smarter inexpensive materials with desired and improved combinations of wound exudates uptake capacity, biodegradability, mechanical and pH stability, flexibility, transparency, non-cytotoxicity, antimicrobial activity and semi-permeability that enables transport of oxygen and nutrients for the critical survival of biological cells.

1.1.1 Conventional materials

With good absorbency characteristics and ability to manage wounds from contaminations, natural and synthetic resources such as cotton wool, lint and gauzes are adopted as adequate primary and secondary dressing materials (Xu et al., 2017). Among these, gauze dressings are relatively expensive and are usually made as woven and non-woven fibers of cotton, rayon and polyesters to offer inadequate protection against bacterial infection. To further enhance their ability to protect wounds from contamination, sterile gauze pads are used to absorb exudates and fluids of open wounds in the fiber matrix. However, the frequent replacement of these materials

is inevitable to prevent maceration of healthy tissues. Also, non-occlusive and exogenous bacteria are well known to penetrate up to 64 layers of gauze. Hence, wound dressing gauzes will not be able to offer a highly protective environment to safeguard the wound from further infection. Moreover, due to excessive wound drainage, gauze dressings do get significantly moistened and adhere with the wound to thereby make their removal as a painful exercise (Sood et al., 2014). To overcome these issues, synthetic bandages are often used.

The primary functionality of natural and synthetic bandages are diverse and distinct. Natural bandages made of cotton wool and cellulose are deployed for retention of light dressings, high compression bandages and short stretch compression bandages that involve sustained compressions associated to venous ulcers. Synthetic bandages prepared with polymeric materials such as polyamide serve towards sensitive applications. With 3 % bismuth tribromophenate, Xeroform™ is a non-occlusive dressing being suggested for non-exuding and moderately exuding wounds. Similarly, Bactrigras, Jelonet, Paratulle are few tulle dressings that are available commercially. These materials are impregnated with paraffins to serve superficially clean wound dressing systems. In other words, traditional dressings are often utilized for clean and dry wounds with mild exudate levels. Therefore, they can be classified as primary and secondary dressings. However, with the advancements in science and technology, greater knowledge base has been developed with respect to the functional healing methods. Thereby, traditional dressings have been regarded to be ineffective to custom serve the generic needs of wound healing (Dhivya et al., 2015). In summary, a typical synthetic wound dressing material shall have desired attributes to quickly heal the wounds and mitigate infections and other similar issues.

1.1.2 Desired attributes of functional wound dressing materials

A functionally efficient wound dressing material actively controls the biochemical state of the wound to effectively aid and accelerate healing process. Wound dressing material is often anticipated to facilitate rapid healing, be available at affordable cost to the patient, shall have aesthetic appearance and must prevent infection. The injured skin involves breaching of the skin barrier from foreign invasion. For this reason, without sequestered just at the skin surface, microorganisms such as *Staphylococcus aureus* or *Escherichia coli* enter the wound, colonize and contaminate it. Thereby, blood flow gets reduced in the area of the wound due to invasion of microbes. These microbes reduce healthy cellular oxygen and nutrient content and eventually lead to tissue necrosis (Torres et al., 2013). Thus, improper microbial removal from wounds translates into enhanced inflammatory cytokines and matrix metalloproteinases and thereby degrade the growth factors associated to healthy human cells. Thus, an efficient dressing material stimulates cell proliferation and histoarchitectural tissue organization and simultaneously prevents bacterial infection (Mir et al., 2018).

Rapid advances in bio-sciences enabled the development of non-toxic biocompatible and biodegradable material based scaffolds that improve tissue reconstruction and transplantation in conjunction with living cells and controlled drug delivery system facilitated with enhanced biodegradability (Sezer and Cevher, 2011). With the establishment of the clinical procedures, tissue engineering enabled bettering wound healing and skin regeneration. Also, the prevention of maceration of the skin surrounding a wound is another important issue that needs to be cautiously addressed by the wound dressing materials. The skin maceration is very likely to occur for a dressing with a lower absorptive capacity (Jones et al., 2006). In addition, it is well known that a wound upon direct exposure to air would dehydrate and form a scab that protects

the wound from bacterial infection. However, such dry or low moisture microenvironment would lose its vitality and may even be lost. This affirms that healing under wet conditions is faster than in dry scab conditions (Xu et al., 2017). Thus, a suitable wound dressing material shall optimize absorption of the excess wound exudates and control water evaporation effectively to enable the wound to remain reasonably wet and undergo quick healing.

In summary, an ideal wound dressing materials should (i) provide a local moist environment to avoid the wound dryness by allowing gaseous exchange between wounded tissue and environment, (ii) protect the wound from any kind of contamination, (iii) absorb excess wound exudates without leakage, (iv) stimulate epidermal migration, (v) possess adequate mechanical strength and flexibility, (vi) be non-toxic and non-allergic, (vii) be bio-compatible, bio-degradable, sterile and (ix) be commercially affordable (Sezer and Cevher, 2011, Kamoun et al., 2017).

1.1.3 Illustrative summary of wound healing process

Scientific analysis of wound healing mechanism involves a dynamic process that comprises of six continuous but overlapping phases occurring in a regulated time bound framework. In this regard, it shall be noted that medical research indicate upon four continuous but overlapping phases and do not elaborate certain stages that are intermediate and highly contributory. The onset of unwanted interruptions, aberrancies or prolongations can seriously jeopardize the time frame of these continuous and overlapping phases. Thereby, such unwanted scenarios profusely contribute to either delayed healing or a non-healing chronic wound. The six continuous and overlapping phases associated towards the gradual recovery of a wound into a healed wound have been indicated to be as follows: (Guo and DiPietro, 2010)

- a) **Rapid hemostasis:** This stage involves coagulation of blood to transform into a gel from the liquid phase.
- b) **Appropriate inflammation:** During this phase, the human immune system triggers a protective response of immune cells, blood vessels and molecular mediators to fight against the harmful stimuli such as pathogens, irritants and damaged cells. Consequently, the response translates into many side effects such as pain, redness, swelling and heat.
- c) **Mesenchymal cell growth:** The phase involves differentiation, proliferation (rapid reproduction of a cell), and migration of the cells to the wound site.
- d) **Angiogenesis:** This step involves the formation of new blood vessels.
- e) **Re-epithelialization:** This phase facilitates re-growth of epithelial tissue over the surface of the wound.
- f) **Strengthening of the healing tissue:** This phase involves appropriate synthesis, crosslinking and alignment of the collagen to enhance strength characteristics of the healing tissue.

Enabling the facilitation and improvisation of a relevant environment that necessitates upon the onset of the above mentioned six overlapping phases, functional wound dressing film would significantly accelerate wound healing process. In this regard, a functional wound dressing films can be analyzed to affirm upon the following characteristics:

- a) An environment that improvises blood clotting index in the wound area and thereby fasten hemostasis.
- b) A safe environment for the wound to restrict contamination and toxicity. Thereby, the material hinders cell proliferation and bacterial contamination that eventually enhance the infection of the wound.

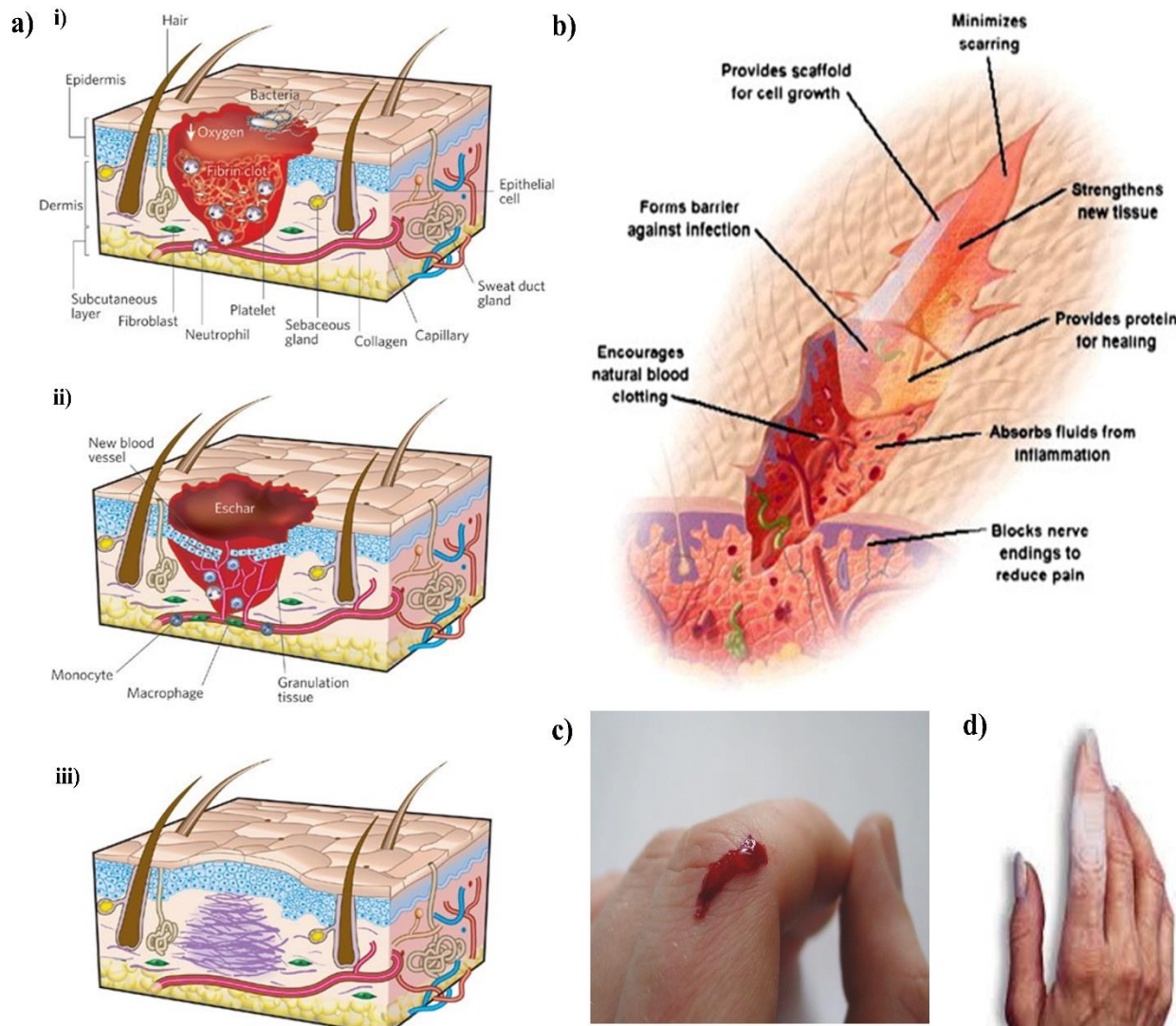


Figure 1.1: (a) Schematic of stage wise wound healing involving the sequence of (i) inflammation, (ii) repair and (iii) remodeling (Reproduced with permission from (Gurtner et al., 2008); Copyright by Springer Nature 2008) of the wound; (b) Schematic representation of functional hydrogel wound dressing material (Reproduced with permission from (Jayakumar et al., 2011); Copyright by Elsevier 2011); (c) Photograph of exudates oozing out from an open wound (Image Courtesy: Wiki Commons (IR1)); (d) Photograph illustrating tissue maceration on human hand (Image Courtesy: Wiki Commons (IR2)).

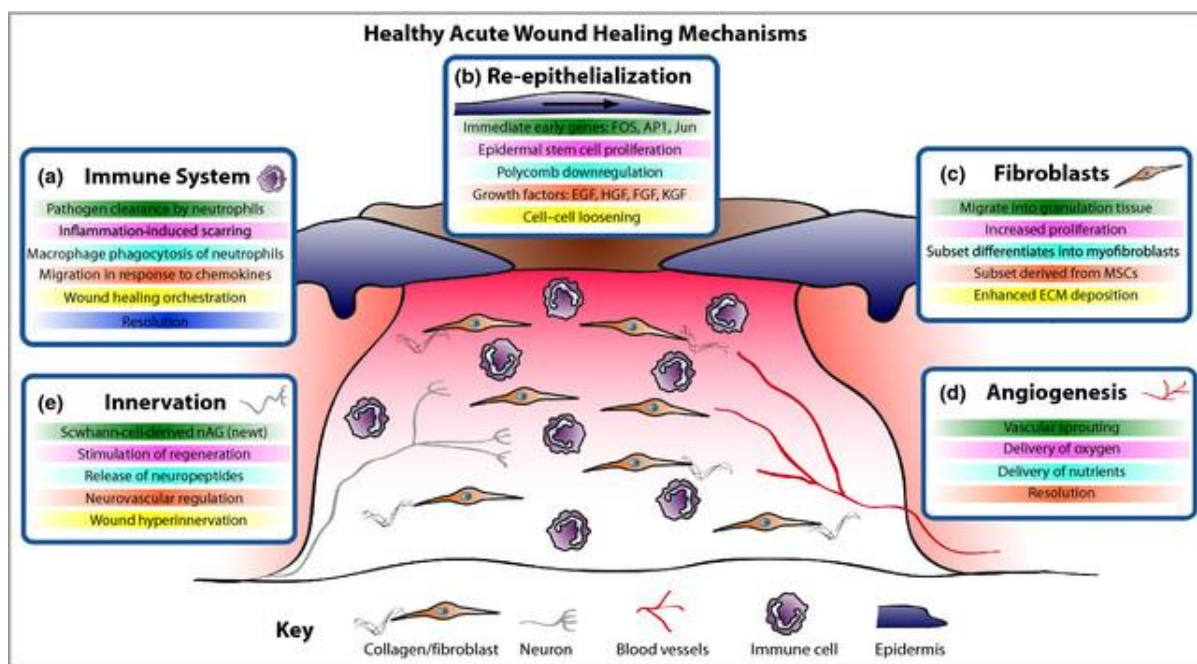


Figure 1.2: Schematic of wound healing mechanism (Reproduced with permission from (Martin and Nunan, 2015); Copyright by John Wiley & Sons Ltd 2015 on behalf of British Association of Dermatologists).

- c) Prevention of the accumulation of the wound fluids in the wound-dressing material interface. This is due to the absorption or swelling characteristics of wound dressing materials. Thereby, the films foster greater absorption and soaking of exudates in the dressing material. Otherwise, newly formed tissue in the wound area will get soaked with the accumulated exudates and fosters maceration. The wound fluid accumulation can be further regulated through appropriate variations in the water vapor transmission rate of the dressing material. High WVTR of functional wound dressing materials enable quicker transfer of wound exudates from the wound and tend to dry the wound. Thereby, gel dehydration (scub) and shrinkage of the dressing material occurs to eventually reduce bacterial contamination of the wounds. However, tissue reorganization will not be effectively facilitated in such dry environment for quicker healing. Contrary to this effect,

a low WVTR verily enhances exudates residence in the wound and thereby detriments healing due to maceration. In summary, a moderate WVTR appears to be promising to foster wound healing process to mitigate maceration and allow retention of relevant moisture content for tissue reorganization.

1.2 Salient features of polymeric wound dressings

Till date, wide varieties of wound dressing material products have been prepared through adequate research in synthetic polymer chemistry and fabrication, functional bio-chemistry associated to wound dressing, bioscience research and pharmacology. These products have diverse features and characteristics. The following sub-sections highlight few salient features of such functional products.

1.2.1 Hydrogel dressings

Being prepared using synthetic polymers, hydrogels are insoluble hydrophilic materials with high water content (70 – 90 %). Thereby, hydrogels foster tissue granulation and epithelium in moist environment. Due to their soft elastic property, they can be easily applied and removed from wounds. Hence, hydrogels facilitate healing without causing further damage to the wound and inflict minimal pain during removal. Being non-irritant, non-reactive to biological tissue and permeable to metabolites, hydrogels provide soothing and cooling effect and reduce temperature of dry chronic, necrotic wounds, pressure ulcers and burn wounds. Few issues with hydrogels dressings refer to exudate accumulation (leading to maceration) and bacterial proliferation (enables foul smell emanating in wounds). Further, with poor mechanical strength, it is difficult to handle such materials. Therefore, they are not deployed for wounds characterized with heavy infection and drainage symptoms. Thereby, they are deployed for all other four stages of healing.

Intrasite™, Nu-gel™, Aquaform™ polymers, sheet dressings, impregnated gauze and water based gels are few examples of hydrogel wound dressing materials (Dhivya et al., 2015).

1.2.2 Hydrocolloid dressings

Constituting an inner colloidal layer and an outer water impermeable layer, hydrocolloid dressing materials can be inferred to be widely deployed interactive dressings. These are fabricated as a composite material using gel forming agents (carboxymethylcellulose, gelatin and pectin), elastomers and adhesives. Hydrocolloids enable water-vapor but not bacteria permeation. With these functional characteristics, they have good debridement property to absorb wound exudates. Therefore, hydrocolloids can be used for light to moderate exudate wounds and do not cause pain upon removal. Upon being in contact with the exudate, they form gels and provide moist environment to foster selective absorption and retention of exudates and hence effective tissue granulation. Commercial materials such as, Granuflex™, Comfeel™ and Tegaserb™ are well known hydrocolloid dressing. They are adopted as sheets or thin films for application as secondary dressing but not for highly exuding wounds (Dhivya et al., 2015).

1.2.3 Alginate dressings

Alginate dressings are fabricated using sodium and calcium salts of mannuronic and guluronic acids and seaweed. With good combinations of absorption and biodegradability, the strong hydrophilic gel formation ability of the dressing fosters higher absorption capacity, wound exudate restriction and minimization of bacterial contamination. Alginates are well known for active macrophages that produce TNF- α . Thereby, the TNF- α initiates inflammatory signals and promotes faster healing. Also, certain ions of the alginate get exchanged with blood constituents to enable the formation of a protective film. These dressings can dehydrate a wound and hence delay healing process by drying the wound. Therefore, secondary dressing are required to

support these materials. Sorbsan™, Kaltostat™, Algisite™ are well known commercially available alginate dressing materials (Dhivya et al., 2015).

1.2.4 Semi-permeable foam dressings

Foam dressing constitutes a hydrophobic outer layer (to allow gaseous and water vapor exchange) residing upon a hydrophilic bottom layer (to absorb wound exudates). At times, adhesive borders are being used to sandwich upon two layers. It is well known that silicone based rubber foam enables better moulding, contouring and alignment with the wound shape. In general, foam dressings are deployed for granulating wounds and as primary dressings. Foam dressings require frequent dressing and are not suitable for low exuding or dry wounds/scars that heal faster due to the presence of exudates. Lyofoam™, Allevyn™ and Tielle™ are well known commercial semi-permeable foam dressings (Dhivya et al., 2015).

1.2.5 Semi-permeable film dressings

Prepared from transparent and adherent polyurethane that enables efficient removal of water vapor and gases from the wound, the semi permeable film dressings are impermeable to bacteria and provide autolytic debridement of eschar. Due to being transparent, a physician or a nurse can inspect the wound healing process without removing the film dressing. Nylon based films are not deployed for highly exuding wounds. This is to their limited absorption capacity. However, such dressing are highly elastic and are flexible to conform towards any desired shape application. Hence much effort is not required to apply them. Opsite™, Tegaderm™, Biooclusive™ are few commercial semi-permeable film dressings. However, they differ widely in terms of their vapor permeability, adhesive characteristics, conformability and extensibility (Dhivya et al., 2015).

1.3 Alternate crosslinking methods for bio-composite film fabrication

Crosslinking enables hydrogel films to achieve hydrophilicity and insolubility in aqueous media. This is due to the chemical or physical crosslinking (tie points) of the polymer chains that fosters a flexible tri-dimensional structure. Thereby, solvent diffusion gets altered in the material and facilitates variant degree of swelling in aqueous media (Siqueira et al., 2018).

A brief account of the alternate crosslinking methods in due course of fabrication of bio-composite films with superior characteristics has been presented in the following sub-sections.

1.3.1 Crosslinking by Radiation

Radiation crosslinking is a widely deployed technique to achieve crosslinked polymer films. The method eliminates deployment of chemical additives. Hence, bio-compatibility characteristic of the bio-polymer are well retained. Further, the method fosters modification and sterilization to occur in a single step process (Gulrez et al., 2011). Relying verily on the external high energy radiation source, the process enables to generate intermediate excited transition state species. These species eventually decompose to generate hydrogen atoms and organic free radical species. To do so, usually, either electromagnetic radiation (γ radiation) from a radioactive isotope or high energy electrons (β radiation) from an electron accelerator is deployed to transfer energy into the polymer molecular chain. Due to the utilization of diverse sources of radiation, these processes have been named as 'nuclear' and 'electron beam' crosslinking processes respectively. Further, room temperature processing allows concentration in the amorphous regions of the material. Such mechanisms promote higher degree of crystallinity. Hence, the process fosters the formation of a chemically crosslinked molecular chain, which has the attribute of either physically or mechanically tangled (intertwined) structure. Radiation assisted

crosslinked polymer products have improved combinations of elevated temperature performance and stress cracking resistance, improved impact properties, enhanced abrasion resistance and reduced deformation under load (creep) (Walsh, 2011).

1.3.2 Physical crosslinking by crystallization

Natural hydrophilic polymer aqueous solutions enable the formation of a gel. Under room temperature based storage conditions, the gel possesses lower mechanical strength. To enhance its mechanical strength, a freeze-thaw process can be adopted. The process transforms the gel into a tougher gel with greater elasticity (Akhtar et al., 2016). A typical F-T process involves following a repeated sequence of quicker freezing of a solution (at about -80 to -85 °C) and subsequent thawing in a normal water bath to reach 4 °C (Ahmed et al., 2017). Due to this effect, the F-T cycle favors a mild homogenization and enables microcrystals to form in the polymer structure (Gulrez et al., 2011). The characteristics of freeze-thawed polymer films are highly sensitize towards several important process parameters such as freezing temperature, freezing time, thawing time and number of cycles. Thereby, these process parameters verily influence the characteristics of fabricated bio-composite polymer films.

1.3.3 Thermal crosslinking process

During thermal treatment, polymer chains possessing unsaturated bonds in the main or side chains undergo polymerization. Thereby, the thermal and chemical stabilities of the polymer film get enhanced. During thermal crosslinking, thermal rearrangement and crosslinking the polymer chain segments foster towards the formation of a three dimensional network structure (Jin et al., 2018). Several variants of thermal crosslinking exist. Among these, oxidation crosslinking process, is famous. In this process, crosslinking is being targeted in an oxidative atmosphere of

about 200 °C. However, due to oxidation of polymer chains and subsequent thermolytic degradation, the crosslinking process is not effective to enhance polymer properties. Thus, oxidative thermal crosslinking detrimentally influences polymer characteristics through undesired heat treatment characteristics.

1.3.4 Group inducing chemical reaction based crosslinking process

Chemical crosslinking is the most common form of crosslinking. The process either grafts monomers onto polymers or crosslinks two different polymer chains using a suitable chemical agent. Functional groups such as hydroxyl, carboxyl and amino can be suitably crosslinked to natural and synthetic polymers by deploying crosslinking agents such as aldehydes and –COOH compounds (Gulrez et al., 2011).

Process conditions have a greater role to foster effective crosslinking. Intense process conditions such as low pH, methanol based quenching and higher temperature are often required to crosslink aldehydes with hydrophilic polymers possessing –OH groups (e.g. polyvinyl alcohol). However, mild process conditions involving Schiff bases are sufficient to enable crosslinking of aldehyde groups with polymers possessing amine groups. Further, higher or Bis functional group possessing crosslinkers are adopted to functionalize hydrophilic polymers such as polysaccharides through addition reactions. On the other hand, condensation reactions are meticulously followed to achieve functionalization of polyesters and polyamides with –OH or –NH₂ or –COOH or their derivatives (Akhtar et al., 2016).

1.3.5 Summary

To serve as adequate wound dressing materials, bio-composite polymer films must possess very good characteristics such as bio-compatibility, non-toxicity, sterility and microbe

impermeability. During hydrogel synthesis, compared to chemical crosslinking methods, physical crosslinking methods are often preferred. This is due to comparatively poor biocompatibility and cytotoxicity of the films fabricated with chemical crosslinking process. It is well known that chemical crosslinkers such as glutaraldehyde (and other chemicals that enable fictionalization with aldehyde groups), boric acid and epichlorohydrin are highly toxic and thereby enhance cytotoxicity characteristics. On the other hand, among physical crosslinking processes, F-T (Ahmed et al., 2017, Fahmy et al., 2015) and γ -radiation (Fan et al., 2016) based crosslinking processes are highly expensive and complex processes that require sophisticated equipment and/or safety procedures. Thus, among physical crosslinking techniques, thermal crosslinking is highly promising from the perspective of processing cost efficacy and simpler equipment. However, it is well known that the thermal crosslinking process suffers due to the basic limitation of thermolytic degradation. Hence, a combination of physical and non-toxic crosslinking agent induced chemical crosslinking would be beneficial to offer advantages of lower processing cost and improvisation of desired characteristics such as mechanical strength. The available prior art is encouraging towards this preposition. Few literature affirm upon the efficacy of heating and non-toxic chemical coupled crosslinking towards fabrication of hydrogen wound dressing films with better structural properties and desired characteristics (Birck et al., 2014, Shi et al., 2008). Moreover, the availability of wider choice of organic acids as crosslinking agents foster towards effective functionalization with -COOH groups. Thereby, the approach enables desired biological integrity of the hydrogel films, despite being subjected to processing at a higher temperature.

1.4 Competent low cost materials for wound dressing applications

1.4.1 Alternate polymers

Wound dressing materials can be broadly classified into hydrogel, hydrocolloid, alginate, foam, collagen and film type materials. Based on extensive literature survey, Table 1.1 summarizes various types of alternate polymers that can be deployed as major constituents in due course of the fabrication of wound dressing materials. The table also details upon the relevance of various dressing materials to serve as excellent materials for specific types of wounds. Also, other promising features of the materials and their detriments and cost efficacy has also been summarized for each material in the table. A detailed account of the content presented in the table along with precise prior art has been elaborated in Tables A1 – A6 for hydrogel, hydrocolloid, alginate, foam, collagen and film type materials respectively.

Table 1.1: A critical summary of alternate wound dressing materials.

S. No.	Dressing type	Polymer constituents	Applicability and promising features	Detriments	Cost
1.	Hydrogel ¹	Highly absorbent rayon/cellulose, carboxymethyl cellulose, high density polyethylene, polyacrylamide, polyvinyl pyrrolidone, sodium carboxymethylcellulose, acemannan, agar, polyethylene oxide, polyethylene glycol, propylene glycol,	Suitable for: most wounds (dry to moderately exuding wounds) and burns types Act as soothing and cooling agent, non-adherent, easily removed from wound, accelerate the healing, pain and inflammatory reduction, easily developed and handled	Semi-transparent, semi-permeable to gases and water vapor, poor bacterial barrier, can lead to maceration and sometimes poor mechanical stability thus difficult to keep in place	Low

¹ Modification of Hydrogel dressing: To overcome the detrimental issues like, bacterial contamination and poor mechanical stability; some commercial hydrogel dressings have been improvised with different additives and protective agents; like: methylparaben (food grade), Imidazolidinyl urea, glycerine, sorbitol, sodium dehydroacetate, propylparaben, sodium hydroxide

S. No.	Dressing type	Polymer constituents	Applicability and promising features	Detriments	Cost
		starch, sodium alginate, calcium alginate, sodium hyaluronate, carbomer 980, pectin, hemicellulose			
2.	Hydrocolloid	Cadexomer iodine, iodine, PEG 300, PEG 1500, cellulose, sodium carboxymethylcellulose, gelatin, pectin, polyurethane, calcium alginate, dextran, polyester, polyisobutylene, CMC	Suitable for: chronic ulcers and burns High absorbent materials, easily removed by saline or sterilized water, non-adherent, high density, waterproof, no pain dressings and changed infrequently Suitable for: surgical wounds and full burns	Little cytotoxicity, varied antimicrobial activity, volumetrically unstable, high leakage exudates, dextran hydrocolloid delays healing, can promote excessive granulation tissue, malodorous and color changes, impermeable to gases	Low to high
3.	Alginate	Sodium and calcium salts of alginic acid, mannuronic and guluronic acid	High absorbent, non-adherent, high mechanical stability, stable for long time, easily removed by saline solution, some has hemostatic properties and good bacterial barrier	Unpleasant odour, can dehydrate dry wound and unavailable due to their scarcity presence	High
4.	Foam	Polyurethane, hydroxylated polyvinyl acetal, chlorhexidine gluconate, polyacrylate	Suitable for: burns, chronic wounds, cavities wound shape and deep ulcers Depending on density high absorbance capacity, keep moist environment, non-leakage against bacterial invasion, very easy to use	Can lead to maceration if too much fluid collects between changes, very adherent, forming opaque layer which complicates wound monitoring, semi-permeable for gases, non-applicable for dried wounds and poor stability	Low

S. No.	Dressing type	Polymer constituents	Applicability and promising features	Detriments	Cost
5.	Collagen	Different type of collagen and β -glucan	Suitable for: necrotic wounds, skin grafts and second-degree burns Biodegradable, good absorptivity, provides moist environment and flexible	Secondary dressing required which can be adherent, limited shelf life	High
6.	Film	Guar gum, polypropylene, polyurethane, polyester, ethyl-methyl acrylate, polyolefin	Suitable for: uncontaminated, laser and superficial wounds Thin, flexible, transparent thus easy wound monitoring, self-adhesive, impermeable to bacteria and fluids, good gases permeability, less maceration and painless	Difficult to handle, adherence to wound bed, non-absorbent allowing wound exudates accumulation, easy bacterial invasion and infection and impermeable for proteins and drugs	Low to high

Based on available information in the relevant prior art, the following can be noted to be the most relevant information to identify competent low cost polymers for wound dressing film fabrication:

- a) Polyurethane has been adopted in various commercial wound dressing materials. However, the polymer is well known to be non-toxic but non-biodegradable polymer (Kamoun et al., 2017). Hence, polyurethane cannot be regarded to be a useful polymer for wound dressing applicable bio-composite film fabrication.
- b) Polyethylene, polyacrylamide, polyisobutylene, polypropylene, poly methyl methacrylate and polyester are commonly deployed synthetic polymers. However, due to being non-biodegradable, they are irrelevant to serve as useful ingredients in due course of bio-composite film fabrication for wound dressing applications.

- c) Compared to other synthetic bio-degradable polymers such as poly(lactic-co-glycolic acid), polycaprolactone, polyglycolide, polyvinyl alcohol is inexpensive, toxicologically safe, biocompatible, affordable, easily processable to achieve transparent film and possesses decent mechanical strength (Wei et al., 2018, Mir et al., 2018).
- d) Given the greater hazard associated to the disposal of synthetic polymers and their expensive recycling costs, synthetic polymers need to be blended along with biodegradable and renewable natural polymers to overcome demand and cost related issues associated to wound dressing films (Mittal et al., 2016). Natural polymers are often derived using complex extraction processes from alternate plant or animal sources (Kulkarni et al., 2012). Verily dependent upon the scarcity of the very source (such as seaweed), natural polymers extracted from animal sources are often expensive in comparison to those that are derived from plant sources (such as polysaccharides derived from vegetables). Thus, bio-degradable natural polymers are as well expensive to accommodate in the functional bio-composite films for wound dressing applications (Kamoun et al., 2015, Kamoun et al., 2017). Therefore, onus shall be upon the identification of low cost bio-degradable natural polymers for effective blending with synthetic polymers.
- e) Table 1.1 also summarizes many other natural polymers such as cellulose, hemicellulose, agar, starch, pectin and guar gum. Being derived from plant sources, these are inexpensive natural polymers that can be effectively blended into polymers such as PVA. Other natural polymers such as chitin, alginate and collagen are frequently used in biomedical applications. However, these are as well expensive materials due to non-abundance of their sources and complex extraction process (Kamoun et al., 2017).

- f) Despite polysaccharides being verily inexpensive natural polymers being extracted from readily available plant sources, they are characterized to possess high solubility in water and poor physico-chemical characteristics. Hence, they cannot be deployed directly for desired applications (Kulkarni et al., 2012). However, these materials can be blended with synthetic materials to achieve useful low cost bio-composite materials. A striking feature of such bio-composites is their enhanced bio-degradability (Torres et al., 2013).

Among alternate polysaccharides such as St, pectin and guar gum, St is the most affordable and plausible due to ease of production from sustainable natural biological resources such as corn, potato, wheat and rice (Torres et al., 2013, Mogoşanu and Grumezescu, 2014). St has promising features such as being non-toxic, biodegradable, easily available and economically feasible.

Numerous investigations conducted till date affirm upon the applicability of St as a key ingredient to fabricate functional materials for bio-medical applications. These include substrates for cell seeding, scaffolds for tissue engineering, drug delivery systems, and bone replacement implants. However, similar to other polysaccharides based materials, the St based composites have detrimental characteristics in terms of relatively poor mechanical properties and higher degree of hydrophilicity. To overcome these issues, modified versions of St have been proposed (Torres et al., 2013). Conversely, PVA exhibits remarkable promising characteristics for applications in bio-medical systems. These are higher water solubility, lower cost, non-carcinogenicity, biodegradability, excellent integrity in acidic and alkaline environments, capability to simulate natural tissues, flexibility for blending with other polymers to form transparent film/fiber and decent mechanical strength (Hajian et al., 2017, Shi et al., 2008).

In summary, among all synthetic polymers, PVA is the most relevant polymer to serve as a key ingredient to achieve bio-degradable wound dressing films. Further, among all natural bio-

degradable polymers, St is the most affordable due to promising characteristics. Thus, compared to all other synthetic polymer-natural polymer based bio-composite films for wound dressing applications, the PVA-St composite film possesses better combinations of cost affordability, biocompatibility, mechanical and pH stability, ease of customization and semi-permeability that enables transport of oxygen and nutrients for critical survival of biological cells (Popescu et al., 2018).

1.4.2 Efficacy of alternate additives

Commonly deployed additives in due course of efficient wound dressing materials include antibiotics, inorganic additives and organic additives. Tables B1, B2 and B3 in the Appendix, respectively summarize the influence of alternate antibiotics, inorganic and organic additives to functionally influence wound dressing film characteristics.

With respect to antibiotics infused wound dressing materials, it is well known that the antibiotics such as erythromycin (Fathollahipour et al., 2013), gentamicin (Hwang et al., 2010), minocycline (Kaur et al., 2019), tetracycline hydrochloride and silver sulfadiazine (Ma et al., 2017) are useful to prevent wound contamination and accelerate wound healing. Antibiotics have a functional challenge due to the genetic mutation of microbes. Thereby, the greater antimicrobial resistance of the microbes is detrimental towards their desired functionability in due course of time. Agreeing upon this hypothesis, the USA Centers for Disease Control envisaged that, by 2050, antimicrobial resistant bacteria will be responsible for all major deaths in comparison to the deaths that are caused due to various types of cancers. Thus, there is a paradigm shift to address improved chronic wound care that does not emphasize upon traditional antibiotics based functional materials. Therefore, newer wound dressing materials do not emphasize upon verily relying upon antibiotics (Kalantari et al., 2020).

Table B2 in the appendix summarizes the influence of various inorganic additives such as nanoparticles and inorganic compounds on the characteristics of wound dressing materials. Nanoparticles with greater surface area promote faster healing due to enhanced antimicrobial characteristics. Silver nanoparticle can be considered as the best among commercially adaptable synthetic additives in the bio-medical materials fabrication (Cencetti et al., 2012). This is due to the unique antibacterial activity of silver (Nguyen et al., 2019). However, AgNPs do not discriminate between various microbial strains and detrimentally destroy microbes that are beneficial to the human body system. Also, similar to borax (Rezvan et al., 2018) and GA (Bursali et al., 2011), silver has been characterized to detrimentally influence the proliferation and expression of cytokines through the peripheral blood mononuclear cells. Thereby, silver is as well considered to be toxic (Kalantari et al., 2020). Further, nanoparticle systems (such as Ag, ZnO, titania nanoparticles (Rinehart et al., 2016)) embedded into a polymeric matrix are well known to get leached in due course of time. Thereby, through the release of hazardous inorganic components, these nanoparticles tend to create a harmful environment to the ecology. Also, nanoparticle fabrication is expensive and so is their storage. Considering all these, the inorganic additives can be regarded to be expensive and ineffective to facilitate the fabrication of low cost wound dressing materials. Similar analogies are applicable for antibiotics induced materials.

Table B3 in the appendix summarizes the utilization of various organic additives such as honey, aloe vera, *Centella asiatica*, hyaluronic acid, curcumin, and various organic acids. These additives are abundantly available and can be extracted on an economic basis. Further, they have many promising features such as anti-inflammatory, immunomodulatory and antimicrobial properties. Functionally, each class of organic additives can promote the desired characteristics of wound dressing materials. These can be classified as follows:

- a) Naturally derived additives such as, glycerol and sorbitol (Kozłowska et al., 2018) profusely contribute towards enhancing the physical properties of the dressing materials. These include swelling, solubility and mechanical stability.
- b) Other organic additives such as AV and organic acids invoke a combinatorial improvement in various desired characteristics of wound dressing films. These include enhanced formation of intrigued networks, improved mechanical stability and elasticity, enhanced anti-inflammatory and anti-microbial properties. Such promising features render them to be highly effective to accelerate wound healing without jeopardizing upon ecological sustainability (Georgescu et al., 2016). Further, being an abundant source of biologically active compounds, these materials provide useful directions for the generation of newer drugs in the pharmaceutical industry. Thus, drug delivery systems using biological macromolecules that are abundantly available in the nature's flora and fauna can be as well targeted to enrich functionality of wound dressing materials.

1.4.3 Challenges associated to PVA based wound dressing film fabrication

Many challenges exist to achieve defect free functional wound dressing bio-composite films using polymers such as PVA and cellulose. Solution casting is a simple process that can be adopted to achieve good quality functional wound dressing films. In due course of solution casting process, the prepared polymer blends possessing bubbles offer a big challenge to achieve defect free wound dressing films with uniform properties and characteristics. The bubble formation in polymer blends is due to the incremental variation of the viscosity of the blend and is indicative of the non-compatibility of the blended ingredients to achieve a uniform solution. Hence, concentration range of the blend needs to be appropriately optimized to avoid bubble formation. Films prepared with bubble containing solutions have poor mechanical strength,

durability and flexibility characteristics. All these are very important for the actual application of the fabricated films.

While PVA-St bio-composite films possess better characteristics such as degradation, strength, flexibility and water resistance in comparison with PVA and St films (Chiellini et al., 2004), the same could not be achieved due to non-compatibility of the polymers (Raj and Somashekar, 2004) in terms of mechanical stability. Hence, plasticizers such as Gl are often adopted to enhance properties of the PVA-St composite films. With three –OH groups that enable hydrogen bond formation with biopolymer molecules and negation towards formation of covalent linkages, Gl serves as an ideal plasticizer to enhance free volume of the system (Wu et al., 2017). Also, both PVA and St offer higher water solubility and hence higher water uptake characteristics. However, with the PVA-St bio-composite possessing superior absorption characteristics of wound exudates, the bio-composite has detrimental characteristics in terms of shape stability and shelf life. Further, the PVA-St bio-composite does not have adequate antibacterial characteristics so as to protect wounds from microbial contamination. Thereby, to enhance the PVA-St bio-composite film functionality in terms of antimicrobial characteristics, organic additives such as AV and organic acids can be adopted using suitable methods such as crosslinking procedures. In summary, all challenges associated to PVA based bio-composite films including cost efficacy can be effectively targeted by considering the inclusion of St, Gl and organic acids such as citric acid, malic acid, tartaric acid, succinic acid and oxalic acid in the wound dressing film fabrication process. Thereby, the specific area of research that would be addressed in the Ph.D. thesis are the following:

- a) Modification of fabrication parameters of the PVA-St composite film

- b) Effect of the various individual constituents and their influence to each other towards modification or disputation of the properties of the PVA-St composite film
- c) Effect of various organic acid as additives to influence upon the properties of the PVA-St composite film

1.5 Prior art

1.5.1 Fabrication and modification of PVA films

1.5.1.1 Effect of physical crosslinking on the properties of PVA composite films

The available state of the art for PVA composite film fabrication target alternate expensive physical crosslinking approaches such as F-T (Ahmed et al., 2017, Fahmy et al., 2015) and γ -radiation (Fan et al., 2016). In addition, conventional inexpensive drying based crosslinking has also been carried out and the relevant literatures can be summarized and tabulated (Table 1.2a) as follows.

Tavakoli et al. (2017) and Hajian et al. (2017) prepared PVA hydrogel with honey and AV by drying the films in an oven at 50 and 40 °C, respectively (Tavakoli and Tang, 2017, Hajian et al., 2017). Birck et al. (2014) investigated crosslinked PVA-CA films at 130 ± 1 °C in the range of 0 to 960 min drying time. The authors concluded that 120 and 40 min of crosslinking time duration were required to bind the entire CA for the chosen concentration range (PVA/CA: 90/10 and 60/40). After 40 min of crosslinking with 40 wt% CA, the film turned yellow and subsequently became brittle after 60 min drying time (Birck et al., 2014).

Shi et al. (2008) used high temperature drying at 140 °C for 1 h during fabrication of the PVA-St-CA-Gl composite films (Shi et al., 2008). Yoon et al. (2006) targeted efficacy of two drying

temperatures for the molding of PVA-St composite films. While the first method followed drying in a ventilated oven at 50 °C for 12 h, the other method involved using a cold lab chamber at 5 °C for 72 h. PVA-St blended film demonstrated high tensile strength and percentage elongation or elongation at break for the films dried at 5 °C. This is due to activation of hydrogen bonding at low temperature (Yoon et al., 2006b).

Wu et al. (2017) fabricated crosslinked PVA-St-CA-Gl composite films by drying the samples at room temperature for 24 h. Later, the authors targeted the influence of baking time (120 – 300 min) on the film properties such as TS, water vapor permeability and water solubility. It was observed that the WVP enhanced ($1.15 - 1.64 \times 10^{-9}$ g.m/m².Pa.s) for the film compositions targeted without CA (7.39 – 9.92 w/w% for PVA, 3.31 – 7.64 w/w% for St and 5.08 – 6.61 w/w% for Gl). For all film compositions, considering fixed CA concentration (2.64 w/w% for CA) and variant PVA, St and Gl blend constitution; the WVP ($0.36 - 1.95 \times 10^{-9}$ g.m/m².Pa.s) and TS (19.58 – 45.54 MPa) enhanced up to a baking time of 270 min. Hence, the said baking time has been inferred by the authors to be optimal to achieve max TS and WS. Any further increment in baking time resulted in cracks on the film surface and hence sharp reduction in TS and WS (Wu et al., 2017).

1.5.1.2 Effect of chemical crosslinking on the properties of PVA composite films

In the broad field of PVA composite films targeted with natural materials and nanoparticles as additives, the available prior art emphasizes towards the specific role of the additives in improvising upon desired characteristics. To further augment the real world applications of PVA composite films for wound dressing application, additives that serve as plasticizers and crosslinkers have been presented in Table 1.2b. Crosslinking with agents such as GA, boric acid, epichlorohydrin is not desired due to their toxicity and synthetic origin.

Table 1.2a: Effect of physical crosslinking on the properties of PVA composite films.

S. No.	Author	Fabrication criteria	Property range	Critical findings
1.	Ahmed et al. (2017)	F-T cycles (each 16 h freezing at -80°C and 8 h thawing at room temperature): 3 to 5	- WVTR: Increases; 735.37 to 2508.88 $\text{g/m}^2\cdot\text{day}$ - TS: Increases; 191.67 to 1000.57 g/cm^2	- Affect the crosslinking ability of the polymeric chains. - Interaction between PVA with F-T cycles and PEG with F-T cycles had no significant effect on GF. - Optimum fabrication process: 5 F-T cycles
2.	Fahmy et al. (2015)	18 h freezing at -20°C and 6 h thawing at 25°C and lastly kept unfrozen for 1 h. - F-T cycles: 3	-	-
3.	Fan et al. (2016)	γ-radiation: absorbed dose of 40 KGy using a ^{60}Co facility, at room temperature, dose rate of 20 Gy/min	-	-
4.	Tavakoli et al. (2017)	Overnight at 50°C	-	-
5.	Hajian et al. (2017)	Oven drying at 40°C	-	-
6.	Birek et al. (2014)	Heat treatment at $130 \pm 1^{\circ}\text{C}$; increasing times: 0 to 960 min.	- With lower and higher concentration of crosslinker: yellowing = from 120 and 40 min; brittleness = never and from 60 min, respectively. 120 and 40 min of crosslinking time were required, respectively.	- Yellowing is due to CA dehydration and formation of a double bond sensitive to oxidation. T_g progressively increases with crosslinking time.

S. No.	Author	Fabrication criteria	Property range	Critical findings
7.	Shi et al. (2008)	Ventilated oven, at 140 °C, for 1 h	-	-
8.	Yoon et al. (2006)	- Oven, at 50 °C, for 12 h - Cold lab chamber, at 5 °C, for 72 h	❖ TS and %E of the film dried at 5 °C were better than those dried at 50 °C.	➤ Hydrogen bonding was activated at low temperature.
9.	Wu et al. (2017)	- Drying at room temperature for 24 h - Baking (120 – 300 min)	❖ WVP: 1.15 – 1.64 ($\times 10^{-9}$) g.m/m².Pa.s for the film without CA. ❖ Film with CA; WVP: 0.36 – 1.95 ($\times 10^{-9}$) g.m/m².Pa.s and TS: 19.58 – 45.54 MPa for baking time 120 – 270 min.	➤ Optimum baking time: 270 min. For further increase in baking time, films got cracked and resulted a sharp drop in TS.

Table 1.2b: Effect of chemical crosslinking on the properties of PVA composite films.

S. No.	Author	Chemical crosslinker	Concentration range	Property range	Critical findings
1.	Tavakoli et al. (2017)	Borax	0.3, 0.6 and 1 w/w%	❖ Swelling ratio: 23, 36 and 32 %, respectively. ❖ Erythromycin release: for low borax density <8 h, for high borax density over 24 h. ❖ For 1 % borax film; TS: 201.4 N/m², %E: 120.41 % and moisture permeability: 1496.8 g/m².h.	➤ For the swelleable system, the electronegative oxygen of borate forms weak hydrogen bonds with some –OH groups of PVA and honey. ➤ After swelling, PVA-borax structure remain intact.
2.	Fahmy et al. (2015)	HA	0, 10, 20, 30, 40 and 50 m/v%	❖ WL: 32 – 75 %, for 2 days	➤ HA reduced the crosslinking reaction, consequently the gelation process.
3.	Hajian et al. (2017)	AV	0, 10, 20, 30, 40 and 50 wt%	❖ WL: 22 – 77 %, during 27 days	➤ As AV gradually released from the matrix, structure become more porous. ➤ Higher porous structure facilitate higher water penetration, so higher rate of hydrolytic degradation.

S. No.	Author	Chemical crosslinker	Concentration range	Property range	Critical findings
4.	Birek et al. (2014)	CA	Weight compositions of PVA/CA: 90/10 and 60/40	❖ 10 wt% CA films, after 40 min, CA not detected. Whereas PVA presents after 960 min. ❖ PVA exhibits a T_g at 57 °C, while it decreased to 42 °C and 28 °C for CA-10 and CA-40, respectively.	➤ For 10 wt% CA films, a weak amount of non-crosslinked PVA chains remains.
5.	Yoon et al. (2006)	CA or Gl	10, 20, 30, 40, and 50 %	❖ For CA: DS = 2.4 – 1.8, S = 0.2 – 0.37, TS = 20 – 13 MPa & %E = 40 – 148 % ❖ For Gl: DS = 2.4 – 0.8, S = 0.2 – 0.54, TS = 20 – 4 MPa & %E = 40 – 116 %	➤ Except for solubility, CA was better than Gl for, TS, %E and DS. ➤ –OH group and –COOH group of CA increased the inter/intra-molecular interaction between St, PVA and additives.
6.	Shi et al. (2008)	GA; along with 40 % CA or Gl	0.045, 0.09, 0.19, 0.375, and 0.75 %	❖ In presence of CA; DS = 2 – 0.65, S = 0.36 – 0.14, TS = 13.5 – 23 MPa & %E = 128 – 56 % ❖ In presence of Gl; DS = 1.05 – 0.8, S = 0.54 – 0.4, TS = 5.5 – 8 MPa & %E = 100 – 66 %	➤ Except for TS, Gl is detrimental for all other properties (DS, S and %E). ➤ Influence of GA on the properties is far more prominent while blending with CA, rather than Gl.
7.	Wu et al. (2017)	CA	5, 10, 20, 30 wt% of the dry weight	❖ DS: 32.6 – 19.6 %, TS: 48 – 42 MPa & %E: 101.8 – 207.8 %	➤ The residual free CA acted as a plasticizer for the St and PVA.
			0 – 2.64 w/w%	❖ S: 7 – 45.6 %, TS: 23.25 – 45.54 MPa, elastic modulus: 31 – 74 % & WVP: 0.42 – 1.95 (x10⁻⁹) g.m/m².Pa.s	➤ The distribution of CA with high elastic modulus generate tremendous interfacial contacts with the polymer matrices, leads to effective stress transfer resulting in an increase in the TS.

Tavakoli et al. (2017) prepared honey-PVA hydrogel, using variant borax crosslinker concentrations (small amount; 0.3, 0.6, 1 %). The optimized film constitution with 1 % borax refers to good swelling behavior, controlled erythromycin release and good moisture permeability (about $1496.8 \pm 180.1 \text{ g/m}^2\cdot\text{h}$). After 24 h, the honey concentration became zero due to swelling. Such an effect reduced TS but enhanced of %E. The composite films were antibacterial in nature and exhibited better resistance against *S. aureus* in comparison with *E. coli*. More than 90 % cell viability was achieved for films with variant borax crosslinker concentrations (Tavakoli and Tang, 2017).

Fahmy et al. (2015) and Hajian et al. (2017) prepared PVA hydrogel membranes with variant concentrations of hyaluronic acid (0 – 50 %, m/v) and AV (10 – 50 wt%), respectively and investigated upon their in-vitro degradation characteristics. For the AV addition case, the weight loss initially involved very rapid loss in one day followed by steady degradation (22 – 77 %, with respect to variant HA concentration) during 27 days of incubation. Similarly, HA addition enabled fast initial degradation followed with a steady increment in WL (32 – 75 %, with respect to variant HA concentration) up to 2 days (Fahmy et al., 2015, Hajian et al., 2017).

Birck et al. (2014) targeted PVA-CA composite film fabrication by varying PVA concentration (1, 5, 17 wt%) and CA concentration based on PVA (PVA/CA: 90/10 and 60/40). The film turned yellow due to CA dehydration and pertinent formation of an oxidation sensitive double bond. Films with 40 and 10 wt% CA exhibited brittle and ductile behavior, respectively. PVA/CA/Hydroxypropyl- β -cyclodextrin films (80/10/10 weight proportion) not at all influenced the physical and mechanical properties of the control film (Birck et al., 2014).

Yoon et al. (2006) prepared PVA-St blended films with variant in CA or Gl (10, 20, 30, 40, and 50 %) or GA (0.045, 0.09, 0.19, 0.375, and 0.75 %) additive concentrations. It was inferred that,

except for solubility, CA was better than Gl for all measured properties such as, TS, %E and degree of swelling. With increasing CA and Gl content, the DS (2.4 – 1.8 for CA and 2.4 – 0.8 for Gl), TS (20 – 13 MPa for CA and 20 – 4 MPa for Gl) reduced and solubility (0.2 – 0.37 for CA and 0.2 – 0.54 for Gl) and %E (40 – 148 for CA and 40 – 116 for Gl) increased. Also, while TS (13.5 – 23 MPa for CA and 5.5 – 8 MPa for Gl) increased with GA enhancement along with CA or Gl, the %E (128 – 56 % for CA and 100 – 66 % for Gl), DS (2 – 0.65 for CA and 1.05 – 0.8 for Gl) and solubility (0.36 – 0.14 for CA and 0.54 – 0.4 for Gl) characteristics of Gl and CA blended films reduced (Yoon et al., 2006b).

Shi et al. (2008) prepared PVA composite films using distinct CA concentrations (5, 10, 20, 30 wt% of the dry weight). The authors concluded that a small quantity of CA has less effect on the DS. However, the DS decreased (32.6 – 19.6 %) with increasing CA concentration. On the other hand, correspondingly %E (101.8 – 207.8 %) increased and TS (48 – 42 MPa) decreased due to the plasticizing effect of the residual free CA. All film samples having distinct CA concentration exceeded a relative growth rate of 80 % after 7 days of incubation (Shi et al., 2008).

Wu et al. (2017) targeted variant constitution of PVA, St and Gl for fixed choice of CA concentration (2.64 w/w%) to evaluate WVP ($0.42 - 1.95 \times 10^{-9}$ g.m/m².Pa.s.), mechanical strength (TS to be 23.25 – 45.54 MPa and elastic modulus to be 31 – 74 %) and antibacterial effectiveness of the composite films (Wu et al., 2017). In summary, for the precisely targeted research in this work with respect to PVA, St, CA and Gl composite films for wound dressing applications, the most relevant literature refer to Shi et al. (2008) and Wu et al. (2017).

1.5.1.3 Summary

With three –COOH groups and one –OH group per monomer, CA can efficiently facilitate esterification reaction with –OH groups of the PVA-St blend and facilitate both crosslinking and

plasticizing effect to improve the properties of the composite film. Therefore, CA could be deployed to fine tune and improve the combination of strength and flexibility characteristics of PVA-St composite (Shi et al., 2008). Also, through the facilitation of the local antiseptic effect, an ideal wound dressing film should mitigate the potential danger of infection. With promising acidity and FDA approval as a toxically safe organic acid, CA in appropriate proportion shall also enhance cell cytotoxicity characteristic of the wound dressing film, which significantly improves the development of new tissues in a healing wound (Khoswanto et al., 2008, Wu et al., 2017). The available prior art indicates that St is a highly inexpensive natural polymer and compatible with PVA to fabricate wound dressing films (Popescu et al., 2018). Due to its ability to improve film flexibility, Gl can also be considered as an additional constituent to improvise upon the film flexibility characteristics (Wu et al., 2017).

1.5.2 Synergy and competitiveness of additives with the base polymer in composite films

In the field of PVA composite films, the available prior art does not elaborate upon the synergy and competitiveness of additives with respect to the base polymer. Very few investigations have been reported (Table 1.3) till date that remotely indicate upon the role of additives to enhance characterization parameters associated to wound dressing compatible PVA composite films.

1.5.2.1 Influence of polymers or additives on the physical properties of PVA composite films

Baghaie et al. (2017) investigated PVA-St-Cs hydrogel membranes by considering variant concentrations of chitosan (4, 7 and 10 w/v% dry weight of PVA-St polymers) with PVA-St blends. Thereafter, the authors embedded commercial zinc oxide nanoparticles into the polymer composite matrix. Three continuous F-T cycles were adopted to fabricate the PVA composite

Table 1.3: Synergy and competitiveness of additives with respect to the base polymer of composite films.

S. No.	Author	Polymer	Additives	Concentration range	Property range	Critical findings
1.	Baghaie et al. (2017)	PVA, St and Cs	nZnO	Cs: 4, 7 and 10 w/v% nZnO: 0.1 w/v%	❖ For only variation of Cs; DS: 1.5 – 2.1, WVTR: 600 – 1350 g/m².day, TS: 5.6 – 3 kgf & strain: 7.6 – 6.2 ❖ In addition of nZnO; DS: 0.9 – 1.5, WVTR: 500 – 850 g/m².day, TS: 6.5 – 3.4 kgf & strain: 6 – 4.9	➤ Filling of pores by nZnO inside the network stop water molecules from entering; hence reduced water absorption, WVTR.
2.	Fan et al. (2016)	PVA, Cs and Gel	-	Cs to Gel 1:3, 1:2, 1:1, 2:1 & 3:1	❖ Gel content: 84 – 57 % & water lost: 77.5 – 88 % ❖ Swelling ratio: 17 – 12.5 at pH 6 ❖ Swelling percentage: 20.5 – 40 % after 50 h ❖ TS: 2.2 – 0.9 MPa & %E: 260 – 160 %	➤ Increasing weight ratio, increases hardness. Hence, becomes brittle and decrease of TS.
3.	Fahmy et al. (2015)	PVA	HA	0, 10, 20, 30, 40 and 50 m/v%	❖ Swelling ratio: 200 – 1700 % ❖ GF: 65 – 30 % ❖ TS: 30 – 25 MPa & %E: 275 – 95 % ❖ Protein adsorption: 0.75 – 2.075 mg/mL	-
4.	Hajian et al. (2017)	PVA	AV	0, 10, 20, 30, 40 and 50 wt%	❖ Swelling ratio: 3.2 – 4.1 up to 30 wt%; then decreased to 2.5 ❖ WVTR: 1200 – 1500 g/mm² h ❖ TS: 22.99 – 40.85 MPa up to 30 wt%; then decreased to 8.84 MPa ❖ %E: 11.59 – 20.33 % up to 30 wt%; then decreased to 9.32 %	➤ Increase in AV content up to 30 % increased the film's water absorption, mechanical properties (TS & %E)
5.	Ahmed et al. (2017)	PVA and PEG 400	-	PVA: 6 – 10 % PEG 400: 1 – 5 %	❖ WVTR: 735.37 – 2508.88 g/m².day ❖ TS: 191.67 – 1000.57 g/cm² ❖ SI: 407.38 – 453.28 %	➤ Greater GF leads higher crosslinking of the polymer chains

S. No.	Author	Polymer	Additives	Concentration range	Property range	Critical findings
6.	Wu et al. (2017)	PVA and St	Gl and CA	PVA: 7.39 – 9.92 w/w% St: 3.31 – 7.64 w/w% Gl: 5.08 – 6.61 w/w% CA: 2.63 – 2.64 w/w%	❖ GF: 49.11 – 75.32 % ❖ S: 6.2 – 45.6 % ❖ WVP: 0.21 – 1.95 ($\times 10^{-9}$) g/m ² .Pa.s. ❖ TS: 19.58 – 45.54 MPa & elastic modulus: 21 – 74 %	➤ Higher polymer concentrations increases crosslinking. Thereby decreased porosity and SL.

films. For the composite films, it was analyzed that with increasing Cs content, DS (1.5 – 2.1) and WVTR (600 – 1350 g/m².day) enhanced. However, along with porosity, these parameters reduced (DS: 0.9 – 1.5, WVTR: 500 – 850 g/m².day) with the inclusion of nZnO content. On the other hand, it was also analyzed that the TS (5.6 – 3 kgf) reduced with inclusion Cs content and the addition of nZnO reversed (6.5 – 3.4 kgf) the detrimental trend (Baghaie et al., 2017).

Fan et al. (2016) fabricated Cs-Gel-PVA hydrogels using gamma irradiation crosslinking method by varying Cs to gelatine ratio (1:3, 1:2, 1:1, 2:1 & 3:1). For a simultaneous enhancement in Cs and reduction in Gel, the authors analyzed that the gel content (84 – 57 %), TS (2.2 – 0.9 MPa) and %E (260 – 160 %) reduced but swelling (20.5 – 40 %, after 50 h) increased. However, the water loss index increased for the same case (Fan et al., 2016).

Fahmy et al. (2015) synthesized PVA-HA hydrogel membranes. Using a F-T cycle method and 0.3 % (m/v) ascorbic acid plasticizer, the authors targeted crosslinking of 5 % (m/v) PVA and variant HA (0, 10, 20, 30, 40 and 50 %, m/v). The experimental investigations affirmed that with an enhancement in HA content, the gel fraction (65 – 30 %) and mechanical stability (TS: 30 – 25 MPa and %E: 275 – 95 %) reduced. On the other hand, with increasing HA content, swelling behavior (200 – 1700 %), protein adsorption (0.75 – 2.075 mg/mL) and hydrolytic degradation (32 – 75 %) increased (Fahmy et al., 2015).

Hajian et al. (2017) developed PVA-AV hydrogel films (110 µm thick) with variant concentrations of AV content (10, 20, 30, 40 and 50 wt%). The authors evaluated that for increasing AV content, characteristics such as porosity, surface roughness, and hydrolytic film degradation enhanced (22 – 77 %). An enhancement in AV content up to 30 wt% enhanced swelling ratio (3.2 – 4.1), TS (22.99 – 40.85 MPa) and %E (11.59 – 20.33 %) (Hajian et al., 2017).

Ahmed et al. (2017) synthesized PVA-PEG hydrogel by following constrictive cycles of F-T process. The formulations were designed using design expert by targeting a variation in PVA concentration (6 – 10 %), PEG concentration (1 – 5 %) and number of F-T cycles (3 – 5). The systematic investigations affirmed that while WVTR increased (735.37 – 2508.88 g/m².day) with increasing PEG concentration and number of F-T cycles, the parameter reduced with increasing PVA concentration. However, for an enhancement in both PVA concentration and number of F-T cycles, the TS increased (191.67 – 1000.57 g/cm²). Also, with increasing PVA concentration and decreasing PEG concentration, the GF increased (49.11 – 75.32 %) but swelling index reduced (453.28 – 407.38 %) (Ahmed et al., 2017).

For a fixed CA concentration, Wu et al. (2017) targeted maximum variation in the concentrations of PVA (base polymer) and St (additive) along with a marginal variation in Gl additive during fabrication of PVA-Gl-St-CA composite films. Accordingly, the authors varied components in the range of 7.39 – 9.92 w/w% for PVA, 3.31 – 7.64 w/w% for St and 5.08 – 6.61 w/w% for Gl by choosing a fixed CA concentration of 2.63 – 2.64 w/w%. The authors considered TS (19.58 – 45.54 MPa), elastic modulus (21 – 74 %) and WVP (0.21 – 1.95 (x10⁻⁹) g.m/m².Pa.s) as important response variables in due course of film characterization (Wu et al., 2017).

1.5.2.2 Summary

The available prior art affirms that few literature emphasize upon the influence of constituent concentrations on the film characteristics of PVA composite films for wound dressing applications. Till date, no investigation has been reported that targeted the simultaneous variation of PVA, St, CA and Gl constituents in the PVA based composite films for wound dressing applications. Such investigations could provide useful insights into the film characteristics and especially in terms of their mechanical properties. Such experimental investigations are going to

be highly cumbersome due to larger number of experiments that are to be conducted with confidence insisting trials for the determination of desired characteristics of wound dressing films (Yoon et al., 2006b, Ahmed et al., 2017). A trial and error based approach is very likely to make the investigations highly tedious. Therefore, to reduce the number of such experiments, the successful methodologies adopted by Ahmed et al. (2017) are highly relevant due to the potential of the approach to facilitate greater insights with minimum experiments designed using the response surface methodology approach (Ahmed et al., 2017). Thereby, interesting research can be suitably targeted to evaluate upon the competence of constituent concentrations associated to PVA-St composite films using the RSM experimental design approach.

1.5.3 Response surface methodology based optimality of complex quaternary combinations

To further augment real world applications of PVA-St composite films towards wound dressing applications, experimental and theoretical optimality of PVA composite films is verily required for the maximization of desired characteristics. The available literature summary (Table 1.4) does not elaborate upon such methodologies.

1.5.3.1 Efficacy of polymers or additives for the improvisation of the properties of PVA composite films

Baghaie et al. (2017) achieved optimal PVA-St hydrogel membrane using 10 % Cs and appropriate content of nZnO particles. With a cell viability of 87 %, The hydrogel membrane exhibited antibacterial effectiveness against *E. coli*, *S. aureus* and *P. aeruginosa*. On the 14th day of experimentation, the wound healing efficacy was evaluated to be 95, 96 and 79 % for samples without nZnO, with nZnO and control samples respectively (Baghaie et al., 2017).

Table 1.4: Optimality of composite film constituents and their qualitative efficacy.

S. No.	Author	Polymer	Additives	Optimal composition	Qualitative analysis
1.	Baghaie et al. (2017)	PVA (15 w/v%), St (2.5 w/v%) and Cs (4, 7 and 10 w/v% dry weight of PVA-St)	nZnO (0.1 w/v% dry weight of PVA-St-Cs)	15 w/v% PVA, 2.5 w/v% St and 10 w/v% Cs with nZnO inclusion	❖ With increasing Cs content antimicrobial activity increase, further more increment with addition of nZnO. ❖ Based on inhibition diameters more effective against <i>E. coli</i> > <i>S. aureus</i> > <i>P. aeruginosa</i>. ❖ For only variation of Cs; cell viability: 85 – 100 % for 4 – 7 w/v% Cs, then decreased to 90 % for further increment. With addition of nZnO; for all Cs variation cell viability is 100 %. ❖ On the 14th day, wound healing efficacy: 95, 96 and 79 % for samples without nZnO, with nZnO and control.
2.	Fan et al. (2016)	PVA (5 %), Cs and Gel (Cs to Gel 1:3, 1:2, 1:1, 2:1 & 3:1)	-	5 % PVA and CS:Gel 1:1	❖ BCI: 0.032 within 5 min; best coagulation effect.
3.	Fahmy et al. (2015)	PVA (5 m/v%)	HA (0 – 50 m/v%)	5 m/v% PVA, 20 m/v% HA and ampicillin	❖ Cell viability of HepG2 cells: 100 – 75 % ❖ Presence of only HA; antimicrobial activity against <i>Candida albicans</i>. ❖ <i>E. coli</i> is resistant against compositions with or without ampicillin. ❖ Ampicillin effectively constrain <i>S. aureus</i>.
4.	Hajian et al. (2017)	PVA (100 – 50 wt%)	AV (0 – 50 wt%)	PA 30: 70 wt% PVA and 30 wt% AV	❖ Cell viability of optimized film: 225 % for L929 fibroblast cells.
5.	Ahmed et al. (2017)	PVA (6 – 10 %) and PEG 400 (1 – 5 %)	-	8 % PVA, 5 % PEG and 5 F-T cycles possessing <i>Centella asiatica</i>	❖ SI: 453.28 %, WVTR: 2278.51 g/m²-day, TS: 1000.57 g/cm², GF: 63.7 % ❖ Cell viability: 97.83 % for HDF and 98.74 % for HaCaT cell. ❖ Antimicrobial limit against <i>P. aeruginosa</i> and <i>S. aureus</i>.

S. No.	Author	Polymer	Additives	Optimal composition	Qualitative analysis
6.	Wu et al. (2017)	PVA (7.39 – 9.92 w/w%) and St (3.31 – 7.64 w/w%)	Gl (5.08 – 6.61 w/w%) and CA (0 – 2.64 w/w%)	7.39 w/w% PVA, 7.39 w/w% St and 5.55 w/w% Gl with CA inclusion	❖ Combinations with inclusion of CA showed strong antimicrobial activity to <i>L. monocytogenes</i> and <i>E. coli</i> .
7.	Shi et al. (2008)	PVA-St (3:1 w/w) and Gl (20 wt%)	CA (5 – 30 wt%)	PVA-St (3:1 w/w), Gl (20 wt%) & CA (20 wt%)	❖ Relative growth rate of L929 cells: 90 – 80 %; for CA 5 – 20 wt%. ❖ No significant negative effects of CA on the cell proliferation with CA content less than 20 wt%.

Fan et al. (2016) targeted optimality of Cs-Gel-PVA hydrogel membranes. The authors identified that an optimal Cs to Gelatine ratio of 1:1 provided best coagulation effect in terms of 0.032 BCI value within 5 min. time period (Fan et al., 2016).

Hajian et al. (2017) identified that the PVA composite films with 30 % AV content performed optimally in terms of good relative cell viability (225 % for L929 fibroblast cells) and enhanced mechanical properties (TS 40.85 MPa and %E 20.33 %). However, the optimally performing film did not possess significantly higher WVTR value (1250 g/mm².h). To this effect, the films prepared with 50 % AV content possessed highest WVTR value (1500 g/mm².h) (Hajian et al., 2017).

Fahmy et al. (2015) observed that the films prepared with HA content less than 20 % possessed higher cell viability % and were non-toxic. Further, the PVA-HA composite films impregnated with apicillin antibiotic exhibited excellent antimicrobial activity against *Candida albicans* (Fahmy et al., 2015).

In due course of PVA composite film fabrication, Ahmed et al. (2017) adopted analysis of variance methodology to optimize fabrication process and composition parameters as 8 % PVA, 5 % PEG and 5 F-T cycles. Thereby, the optimally fabricated film possessing *Centella asiatica* antibiotic has been analyzed to possess promising characteristics such as 453.28 % SI, 2278.51 g/m².day WVTR, 1000.57 g/cm² TS, 63.7 % GF, 97.83 % cell viability for human dermal fibroblasts and 98.74 % for HaCaT cell and enhanced antimicrobial limit against *P. aeruginosa* and *S. aureus*. The authors as well concluded that a similar steady enhancement in drug loss is apparent in the sample for up to 24 h. This is attributed to the greater WL characteristics of the polymer composite film (Ahmed et al., 2017).

Based on trial and error based optimization, Wu et al. (2017) inferred that for a fixed choice of CA concentration (2.64 w/w%), the optimal PVA, St and Gl composition refers to 7.39, 7.39 and 5.55 w/w% respectively. The optimized film exhibited enhanced antibacterial effect for both Gram-positive bacteria (*Listeria monocytogenes*) and Gram-negative bacteria (*Escherichia coli*) and possessed 54.31 times higher water holding capacity than its own weight (Wu et al., 2017).

Adopting widely varied CA concentrations (5 – 30 wt%), Shi et al. (2008) prepared PVA composite films using fixed concentration of PVA-St blend concentration (3:1 w/w) and Gl concentration (20 wt%). The authors reported that the optimized film prepared with 20 wt% CA concentration performed best in terms of cytotoxicity related best cell growth (Shi et al., 2008).

1.5.3.2 Summary

From the perspective of optimal wound exudate leaching characteristics, a typical wound healing film is expected to have good absorption efficacy, moderate permeability (for wound exudate penetration through the surface without accumulating on the surface and enable the surface to remain dry), good bio-degradability, good antibacterial ability (to avoid infection), appropriate strength and flexibility (to support as epidermis and stimulate cell growth) (Kamoun et al., 2017). The PVA-St films are highly promising for wound dressing applications. This is due to the reason that both PVA and St offer higher WS and hence higher water uptake characteristics (Kamoun et al., 2017, Torres et al., 2013). Higher water absorption capacity or SI of a bio-composite film translates into superior absorption of wound exudates. In the field of wound dressing materials, materials with a SI of 260 % and above have been regarded to be super absorbent materials (Ahmed et al., 2017). Also, Hajian et al. (2017) confirmed 50 to 60 WL% for PVA-AV (30 %) hydrogel film and Fahmy et al. (2015) conveyed 28 – 48 % and 29 – 58 % WL for PVA-HA (20 & 30 %) hydrogel membranes respectively during 27 days in-vitro degradation

study (Hajian et al., 2017, Fahmy et al., 2015). These values can be regarded to infer good degradation behavior for wound dressing applications. Considering all these, research thrust and emphasis continues in the field of cost effective fabrication of PVA-St composite films for wound dressing applications.

Till date, the available prior art in the chosen field of PVA-St composite films for wound dressing applications refers to additives like CA and Gl which facilitate better molecular dynamism and thereby promote the flexibility, plasticity, physicochemical and mechanical properties of PVA-St bio-composite films. Both CA and Gl are well known plasticizers with –COOH and –OH groups. Also, along with antibacterial effect, CA is a reputed organic acid to combinatorially induce both crosslinking and plasticizing effects (Shi et al., 2008). Thus, PVA-St blended films with better functional characteristics can be obtained with the addition of CA and Gl. In other words, PVA-St based composite hydrogels are promising for wound dressing applications and need to be further fine tuned to improvise upon their characteristics for wound dressing applications.

The available prior art indicates only one literature (Ahmed et al., 2017) with respect to software based design of experiments and subsequent statistical optimization. However, wider compositional variation is needed for all constituents (PVA 6 – 10 % and PEG 400 1 – 5 %) to evaluate upon the efficacy of PVA composite films using RSM design based statistical optimization approaches.

1.5.4 Influence of alternate organic acids on the characteristics of PVA composite films

Targeting variant additive (CA or Gl) concentrations, Yoon et al. (2006) considered fixed choice of PVA-St blend constitution to determine the effect of variant concentrations on the characteristics of PVA composite films. The authors targeted DS, TS, %E and Solubility as key

response variables. Based on their observations, the authors hypothesized that except for solubility, the multiple -COOH groups prevalent in CA facilitate achievement of better properties than those obtained with the GI (Yoon et al., 2006b). This is also in agreement with the observation that while CA possesses three -COOH groups and one -OH per monomer, the GI possesses only three -OH groups.

Based on extensive investigations associated to PVA-St-GI-CA composite film fabrication, Shi et al. (2008) inferred that with increasing CA concentration, swelling and TS characteristics mostly reduced and %E always enhanced to reach higher values. This is due to the plasticizing effect of the residual free CA. Due to the existence of three -COOH groups in its chemical structure, the CA can effectively enhance the formation of hydrogen bonds through esterification with -OH groups of PVA-St. Thereby, desired composite film characteristics such as TS, modulus of elasticity, water resistance, and swelling behavior are verily enhanced due to the CA effect (Shi et al., 2008). In a good agreement with this hypothesis for the CA, it is expected that hypothesized that other organic acids such as malic (with one -OH and two -COOH groups), tartaric (with two -OH and two -COOH groups) and succinic acids (with two -COOH groups) will have similar effect to enhance extent of esterification (grafting) and trans-esterification (crosslinking) between the polymer chains. These reactions enhance compatibility between hydrophilic polymer chains and thereby induce plasticizing effect into the composite blend. Consequently, the polycarboxylic acids promisingly influence and enhance the mechanical, structural and barrier properties of the base polymers through the principle of reactive extrusion. The effect of these polycarboxylic acids on the PVA based films have been tabulated (Table 1.5) and have been presented in the paragraph.

Table 1.5: Influence of alternate polycarboxylic and polyhydroxylic additives on the properties of PVA composite films.

S. No.	Author	Additives	Concentration range	Property range	Critical findings
1.	Yoon et al. (2006)	Gl or MA or TA or SA	For each; 0, 10.86, 21.72, 32.57, 43.43 and 54.29 mmol	❖ DS: 3.1 – 1.65 for Gl, 3.1 – 2 for MA, 3.1 – 2.2 for TA, 3.1 – 1.4 for SA & 3.1 – 1 for GISA. ❖ S: 0.19 – 0.35 for Gl, 0.19 – 0.51 for MA, 0.19 – 0.52 for TA, 0.19 – 0.39 for SA & 0.19 – 0.51 for GISA. ❖ TS: 56 – 2 MPa for Gl, 56 – 10 MPa for MA, 56 – 18 MPa for TA & 56 – 80 MPa for SA. ❖ %E: 20 – 150 % for Gl, 20 – 300 % for MA, 20 – 400 % for TA, 20 – 1 % for SA & 20 – 48 % for GISA.	➤ –OH and –COOH groups increased flexibility and strength of the films. ➤ MA and TA are highly hydrophilic, having –OH and –COOH groups. Hence, DS and S of the MA and TA added films were higher than those of the GL and SA added films.
2.	Yun et al. (2006)	CA or MA or TA or SA or Gl or SO	For each; 0, 10, 20, 30, 40, and 50 %	❖ DS: 2.8 – 1.95 for CA, 2.8 – 1.55 for MA, 2.8 – 1.85 for TA, 2.8 – 1 for SA, 2.8 – 0.8 for Gl, 2.8 – 1 for SO, 2.8 – 1.2 for GISA & 2.8 – 1.6 for SOSA. ❖ TS: 56 – 19 MPa for CA, 56 – 10 MPa for MA, 56 – 13 MPa for TA, 56 – 76 MPa for SA, 56 – 4 MPa for Gl, 56 – 6 MPa for SO, 56 – 22 MPa for GISA & 56 – 30 MPa for SOSA. ❖ %E: 20 – 220 % for CA, 20 – 190 % for MA, 20 – 200 % for TA, 20 – 1 % for SA, 20 – 155 % for Gl, 20 – 165 % for SO, 20 – 45 % for GISA & 20 – 65 % for SOSA.	-
3.	Park et al. (2005)	CA or Gl or SO	For each; 0, 10, 20, 30, 40, and 50 %	❖ DS: 2.4 – 1.85 for CA, 2.4 – 0.8 for Gl & 2.4 – 1.2 for SO. ❖ S: 0.19 – 0.37 for CA, 0.19 – 0.55 for Gl & 0.19 – 0.46 for SO. ❖ TS: 20 – 12 MPa for CA, 20 – 4.5 MPa for Gl & 20 – 10.5 MPa for SO. ❖ %E: 36 – 150 % for CA, 36 – 120 % for Gl & 36 – 140 % for SO.	➤ DS value of the CA added film was higher than Gl or SO added films. Because –OH and –COOH groups of CA, was superior in compared to the –OH groups of Gl or SO.

Yoon et al. (2006) evaluated the influence of alternate organic acids as additives to the St and PVA blend composite films. The authors focused upon organic acids and other additives such as SA, MA, TA and Gl. For a fixed choice of PVA-St blend (5 % each), the authors varied concentrations of the organic acids in the range of 0 – 54.28 mmol. It was analyzed that, except for SA, with increasing additive concentration, both DS (3.1 – 1.65, 3.1 – 2, 3.1 – 2.2 and 3.1 – 1 for Gl, MA, TA and GlSA, respectively) and TS (56 – 2, 56 – 10 and 56 – 18 MPa for Gl, MA and TA, respectively) reduced along with a simultaneous enhancement in solubility (0.19 – 0.35, 0.19 – 0.51, 0.19 – 0.52 and 0.19 – 0.51 for Gl, MA, TA and GlSA) and %E (20 – 150, 20 – 300 and 20 – 400 % for Gl, MA and TA). For the SA case, it was observed that with increasing organic acid content, the TS (56 – 80 MPa) and solubility (0.19 – 0.39) increased, DS (3.1 – 1.4) reduced significantly and %E (20 – 1 %) reduced negligibly. It is well known that the optimal PVA-St composite films possess higher combinations of TS, %E, DS and S values. Considering this as the subjective bias, the obtained results of the authors can be analyzed to infer upon a hierarchy for various parameters. Accordingly, films with higher TS have been obtained in the order of SA>TA>MA>Gl. Similarly, higher %E and DS was evaluated for films prepared with organic acids in the order of TA>MA>Gl>SA. Also, higher S was obtained for films prepared with the order of organic acids as TA>MA>SA>Gl. Considering all these, it can be analyzed that lower TS values can be significantly enhanced by considering the inclusion of SA in the Gl integrated PVA composite film constitution. Further, the authors observed that %E enhanced (20 – 48 %) marginally for an enhanced constitution of both SA and Gl in the composite polymer matrix (Yoon et al., 2006a).

The influence of varied concentrations (0 – 50 %) of CA, SA, MA, TA, Gl and SO have been investigated by Yun et al. (2006) for PVA-St blend based composite films. Except for SA, the

authors observed that with increasing additive content, the TS reduced 56 – 19, 56 – 10, 56 – 13, 56 – 76, 56 – 4 and 56 – 6 MPa, %E increases 20 – 220, 20 – 190, 20 – 200, 20 – 1, 20 – 155 and 20 – 165 % and DS reduced 2.8 – 1.95, 2.8 – 1.55, 2.8 – 1.85, 2.8 – 1, 2.8 – 0.8 and 2.8 – 1 for CA, MA, TA, SA, Gl and SO, respectively. Based on desired PVA composite film characteristics, the hierarchical performance of alternate organic acids is as follows: SA>CA>TA>MA>SO>Gl for higher TS; CA>TA>MA>SO>Gl>SA for higher %E and CA>TA>MA>SO>SA>Gl for higher DS (Yun et al., 2006).

PVA-St composite films possessing either Gl or SO have been analyzed to improve their film characteristics (TS: 56 – 22 and 56 – 30 MPa; %E: 20 – 45 and 20 – 65 %; DS: 2.8 – 1.2 and 2.8 – 1.6 for GlSA and SOSA, respectively) through the addition of SA with either Gl or SO in the polymer matrix. For lesser TS decrement, the hierarchy has been analyzed to follow the order of SOSA>GlSA>SO>Gl. Similarly, effective blending of Gl or SO with SA enhanced %E values. The property enhancement for the variable followed the hierarchy of SO>Gl>SOSA>GlSA (Yun et al., 2006). Similar investigations were conducted by Park et al. (2005). The authors considered variation of CA, Gl and SO and obtained trends similar (DS: 2.4 – 1.85, 2.4 – 0.8 and 2.4 – 1.2; S: 0.19 – 0.37, 0.19 – 0.55 and 0.19 – 0.46; TS: 20 – 12, 20 – 4.5 and 20 – 10.5 MPa; %E: 36 – 150, 36 – 120 and 36 – 140 % for CA, Gl and SO, respectively) to those reported by Yun et al. (2006) (Park et al., 2005).

1.5.5 Efficacy of alternate organic acids for the improvisation of the characteristics of PVA composite films

Very few literatures (Table 1.6) has elaborated upon the applicability of the organic acid based PVA composite film. Hence, limited study concentrate upon the qualitative study towards the effect of various organic additives and thereby their optimization.

Table 1.6: Qualitative effectiveness of alternate organic acid based PVA composite films.

S. No.	Author	Additives	Concentration range	Property range	Critical findings
1.	Mitra et al. (2013)	SA	0.05, 0.1, 0.2, 0.3, 0.4 and 0.5 w/v%	❖ For Cs: TS = 0.37 MPa, %E = 8.33 % ❖ For 0.2 % SACs: TS = 0.92 MPa, %E = 8.67 % ❖ For collagen: TS = 0.13 MPa, %E = 6.17 % ❖ For 0.2 % SACC: TS = 2.25 MPa, %E = 22 %	➤ Optimized formulation: 0.2 w/v% SA, 1.0 w/v% Cs and 0.5 w/v% collagen. ➤ Cell viability: Both SACs and SACC possessed more than 85 and 95 % after 24 and 72 h respectively.
2.	Olivato et al. (2012)	CA or MA or TA or Gl	CA or MA or TA: 0, 0.375, 0.75 and 1.5 wt% Gl: 10, 9.625, 9.25 and 8.5 wt%	❖ WVP: 1.8 – 3.5 and 1.8 – 3.8 ($\times 10^{-10}$) g/m.s.Pa, for TA and CA each up to 0.75 wt%, respectively. Then decreased to 1.8 and 1.9 ($\times 10^{-10}$) g/m.s.Pa. For MA, 1.8 – 4.6 ($\times 10^{-10}$) g/m.s.Pa. ❖ TS: 4 – 6.5 and 4.75 – 6.7 MPa for CA and TA, respectively. For MA up to 0.75 wt%, TS: 4.3 – 6.75 MPa; then decreased to 5.25 MPa. ❖ %E: 325 – 550 and 450 – 300 % for MA and TA, respectively. For CA up to 0.75 wt%, %E: 300 – 400 %, then decreased to 30 %. ❖ WL%: 12 – 21, 13 – 18 and 12 – 27 % for TA, MA and CA, respectively.	➤ Organic acids improved crosslinks, generating more resistant and less permeable films. ➤ Decreases material viscosity, improving the processing properties and producing a more homogeneous matrix.
3.	Cadogan et al. (2014)	Acetic acid, ascorbic acid, CA, glycolic acid, maleic acid, oxalic acid and TA	Acetic acid (1 %), ascorbic acid (2 %), CA (0.5 %), glycolic acid (2 %), maleic acid (2 %), oxalic acid (2 %) and TA (0.5 %)	❖ Acetic acid: TS = 62 Nmm⁻², %E = 22 % & swelling = 1100 % ❖ Ascorbic acid: TS = 78 Nmm⁻², %E = 36 % & swelling = 1700 % ❖ CA: TS = 15 Nmm⁻², %E = 56 % & swelling = 1400 % ❖ Glycolic acid: TS = 51 Nmm⁻², %E = 70 % & swelling = 2200 % ❖ Maleic acid: TS = 28 Nmm⁻², %E = 19 % & swelling = 1200 % ❖ Oxalic acid: TS = 21 Nmm⁻², %E = 19 % & swelling = 1050 % ❖ TA: TS = 59 Nmm⁻², %E = 34 % & swelling = 1200 %	➤ On best choice of higher bacterial inhibition against <i>E. coli</i> after 48 h, the organic acids hierarchy has been identified as follows: acetic>ascorbic>TA>maleic>oxalic>glycolic>CA

Mitra et al. (2013) prepared 3D scaffolds using varied SA concentrations (0.05 – 0.5 w/v%). The authors further optimized their formulations with SA (0.2 w/v%), Cs (1.0 w/v%) and collagen (0.5 w/v%). Based on extensive experimental investigations, the authors concluded that both Cs and CC crosslinked polymer matrix with SA possessed more than 87.5 and 95 % cell viability after 24 and 72 h respectively (Mitra et al., 2013).

Targeting an alternative to synthetic packaging film, Olivato et al. (2012) targeted the fabrication of biodegradable polymeric films using organic acid modified thermoplastic St-polyester blown films. For a fixed GI concentration, the authors varied CA or TA or MA concentration (0.375 – 1.5 wt%). The composite films prepared with higher acid constitution possessed higher combinations of WL and WVP values. For 1.5 wt% constitution of the organic acid, the hierarchy of highest WL of alternate organic acids is as per the order of CA>TA>MA (12 – 21, 13 – 18 and 12 – 27 % for TA, MA and CA, respectively). Compared to TA or CA increment (1.8 – 3.5 and 1.8 – 3.8 ($\times 10^{-10}$) g/m.s.Pa, for TA and CA each up to 0.75 wt%, respectively), an increment in MA up to 1.5 wt% affirmed highest WVP values (1.8 – 4.6 ($\times 10^{-10}$) g/m.s.Pa). However, for an increment of TA and CA beyond 0.75 wt%, the WVP reduced (to 1.8 and 1.9 ($\times 10^{-10}$) g/m.s.Pa, respectively). Also, for a simultaneous increment in TA and CA, an enhancement in TS was observed (4 – 6.5 MPa and 4.75 – 6.7 MPa for CA and TA, respectively). On the other hand, for an increment in MA concentration, both %E (325 – 550 %) and TS enhanced (4.3 – 6.75 MPa, for MA up to 0.75 wt%). However, TS was detrimentally (5.25 MPa) influenced with MA concentration greater than 0.75 wt% concentration. For the TA case, an increase in concentration reduced %E (450 – 300 %). For the CA case, up to 0.75 wt% of the additive concentration, the %E values increased (300 – 400 %) and thereafter reduced (30 %) sharply (Olivato et al., 2012).

Cadogan et al. (2014) fabricated chitosan membranes through subsequent dissolution of chitosan into alternate aqueous organic acid systems (acetic acid (1 %), ascorbic acid (2 %), CA (0.5 %), glycolic acid (2 %), maleic (2 %), oxalic acid (2 %) and TA (0.5 %)). Thereafter, the acid solvated Cs membranes were subjected to static swelling studies in deionized water at 25 °C. Based on best choice of individual characterization parameters, the organic acids hierarchy has been identified as follows: ascorbic>acetic>TA>glycolic>maleic>oxalic>CA for higher TS; glycolic>CA> ascorbic>TA>acetic>maleic=oxalic for higher %E; glycolic>ascorbic>CA>TA= maleic>acetic=oxalic for higher DS and acetic>ascorbic>TA>maleic>oxalic>glycolic>CA for higher bacterial inhibition against *E. coli* after 48 h (Cadogan et al., 2014).

Giglio et al. (2015) synthesized Gl/TA based copolymer containing ciprofloxacin for wound healing applications. MTT test performed at 48 h and 7 days indicated a good cell viability for HaCaT cells respective to the synthesized copolymer. However, skin mesenchymal stem cells affirmed a lower MTT value at 48 h. No differences were detected after 7 days of culture, with values higher than 100 % for both samples (De Giglio et al., 2015).

1.6 Lacunae

1.6.1 Influence of CA content on the low temperature fabrication of PVA composite films

The carried out research requires further insights from the following perspectives. Firstly, Birck et al. (2014) used high temperature processing and did not optimize the crosslinking temperature. However, the study focused upon the variation of drying time and observed its effect on film properties. Also, characterization parameters have not been extensively investigated (Birck et al., 2014). Shi et al. (2008) also used high temperature crosslinking process and did an elaborate study for PVA-St-CA composite films. However, the authors did not consider degradation, water

vapor transmission rate and antibacterial effectiveness study to evaluate the competence of wound dressing materials (Shi et al., 2008). The optimality of film constituents and the process parameters was also not considered by Yoon et al. (2006) during PVA-St-CA or Gl film fabrication at a lower crosslinking temperature (Yoon et al., 2006b).

Wu et al. (2017) considered fixed concentration of CA but optimized PVA, St and Gl precursors during composite film fabrication probably at higher fabrication temperature. The fabrication process has not been clearly mentioned by the authors as they have not mentioned about the crosslinking or baking temperature used but allude upon the optimized baking time which is 270 min (Wu et al., 2017). In this regard, it is well known that while CA contributes towards mechanical strength and antibacterial effectiveness, Gl contributes towards flexibility and swelling properties of the composite films. In addition, CA serves as both crosslinking and plasticizing agent. Therefore, the variation of CA in the PVA composite film is a very important issue in the context of the film characteristics for wound dressing applications. Further, low temperature fabrication of the composite films is to be targeted to reduce fabrication costs associated to energy consumption. Thus, based on available limited prior art, in the field of low temperature fabrication of PVA-St-CA-Gl films, the effect of CA concentration and drying process parameters (drying temperature and time) on the wound dressing material characterization parameters has not been examined and shall be regarded as a foremost objective of this Ph.D. thesis.

1.6.2 Wider compositional synergy of PVA, St, Gl and CA

A critical observation from the mentioned prior art in the confined research theme of PVA-St blended films for wound dressing applications indicates that all constituents have not been varied simultaneously and widely. Therefore, insights gained from such analysis are not generic and

could not be extended to further improvise upon desirable set of combinatorial characteristics.

Further details with respect to the methodology followed in the literature are as follows:

Baghaie et al. (2017) and Birck et al. (2014) varied either of PVA or St or Cs to evaluate their effect on film properties. Thereby, the investigations did not deliberate upon the effectiveness of St to improvise PVA composite film properties (Baghaie et al., 2017, Birck et al., 2014). Hence, it is apparent that while combinatorial synergy may be indicated, a systematic methodology is missing to serve as an effective guideline to further enhance film properties.

Further, few research groups targeted the inclusion of few additives or nanoparticles in those films to improvise upon the film characteristics (Baghaie et al., 2017, Yoon et al., 2006b, Shi et al., 2008). Despite conducting a vast study for PVA-St composite films, Wu et al. (2017) considered two levels of concentrations for PVA, St and Gl in the packaging film and the effect of CA was considered after optimizing the PVA-St-Gl film characteristics. Such a methodology may have been considered by the authors to potentially reduce the number of experiments (Wu et al., 2017). On the other hand, Ahmed et al. (2017) deployed RSM statistical tool for the design and fabrication of PVA-PEG composite films. Thereby, the authors enabled investigations in the wider concentration range of film ingredients (PVA 6 – 10 w/w% and PEG 1 – 5 w/w%). Through a carefully designed experimental set of runs that indicated minimal number of experiments, the authors achieved very good insights by considering constituent concentrations and preparation cycles as important process parameters for the evaluation of response characteristics (Ahmed et al., 2017). A similar approach is necessary but missing for the efficacy of PVA-St-CA-Gl composite films for wound dressing applications. This shall be set as the second objective of the Ph.D. thesis.

1.6.3 Rigorous mathematical optimality of complex quaternary combinations

Deeper introspection into the available prior art for PVA composite films towards wound dressing applications indicates several challenges and associated lacunae. While the literature elaborates upon the effect of a key constituent or additives to the PVA composite film matrix to critically influence characterization parameters, compositional optimality and synergy have not been effectively targeted by considering systematic variations in the compositions of all constituents. A detailed account of the gaps in the relevant literature is mentioned as follows.

The optimality of CA in the PVA-St-GI-CA composite film was primarily addressed by Shi et al. (2008) in terms of cytotoxicity (Shi et al., 2008). On the other hand, Ahmed et al. (2017) adopted ANOVA statistical tool and optimized PVA-PEG hydrogel film to evaluate antibacterial effectiveness, cytotoxicity and drug release as fitness parameters. Various characterization parameters have been considered for optimization of the PVA-PEG hydrogel film, such as GF, SI, WVTR, TS. Some of these parameters themselves have synergy or combinational influence and hence associated similarities do exist. Such insights are highly useful to improve the viability of PVA composite films for wound dressing applications.

Fahmy et al. (2015) hypothesized that with a decrement in GF, hydrogel strength reduced but flexibility increased (Fahmy et al., 2015). However, these trends have not been correlated with GF and TS trends. While Ahmed et al. (2017) evaluated the optimality of the composite based on both GF and TS, the authors did not elaborate upon the associated similarities and dissimilarities (Ahmed et al., 2017). Also, the literature reported in-vitro degradation was mostly based on solubility and the methodology lacks deployment of mathematical tools to understand the intricacies and complexities associated with the similarity of various characterization parameters.

Finally, considering CA increment, using Fourier Transform Infrared Spectroscopy, Shi et al. (2008) analyzed that the peak intensities varied due to variation in chemical group content (Shi et al., 2008). However, the effect of other components (PVA, St, Gl) in the composite was not targeted by the authors and the obtained results were not correlated in terms of all evaluated characterizing parameters. Thus, mathematical tool based optimization is the need of the hour to identify the synergy and optimality of most critical characterization parameters of wound dressing compatible PVA composite films. This is being set as the third objective of the Ph.D. thesis.

1.6.4 Influence of alternate organic acids on the characteristics of PVA composite films

The research works of Yoon et al. (2006), Yun et al. (2006) and Park et al. (2005) targeted upon the influence of alternate additive concentrations on the physical properties of the PVA-St blended films. However, the authors did not elaborate upon the optimality of such concentrations. Further, the applicability of such organic additive based PVA-St blended films towards any particular commercial sector has not been targeted by the authors. Thereby, the effectiveness of various organic acids to effectively plasticize and alter antimicrobial effects and in-vitro biocompatibility has not been explored (Yoon et al., 2006b, Yoon et al., 2006a, Yun et al., 2006, Park et al., 2005). However, among all the discussed organic acids, MA and TA are with most desirable characteristics in comparison with CA (Yun et al., 2006, Yoon et al., 2006a). In summary, the efficacy of alternate organic acids such as, MA and TA, to identify best organic acid for fabrication of functional PVA composite films shall be regarded as the fourth objective of the Ph.D. Thesis.

1.6.5 Optimization and qualitative analysis of alternate organic acid based PVA composite films

Targeting a qualitative analytical approach, Olivato et al. (2012) considered the development of alternative biodegradable polymer composite based synthetic packaging films. The authors elaborated upon the influence of variant bio-additives concentration on the physical properties of the films that are not fabricated using PVA (Olivato et al., 2012). A similar approach for the PVA composite film fabrication could be beneficial for wound dressing applications.

Through the desirable attribute of bacterial inhibition, Cadogan et al. (2014) carried out qualitative analysis of alternate organic acid modified chitosan membranes. The authors adopted variant non-similar concentrations to achieve dissolution of the chitosan and thereby prepare the said membranes. Therefore, the observed trends in the characteristics of the composite membranes in terms of antibacterial activity are very likely to have variant trends due to variation in the organic acid constitution. Also, the authors did not elaborate upon cost efficacy and combinatorial property improvisation of the said membranes. In other words, a useful and detailed methodology is lacking in the fabricated membranes by simultaneously considering alternate additive cost and film characteristics.

Giglio et al. (2015) found a novel route to improvise wound healing characteristics of organic acid (TA) incorporated copolymer films. The authors conducted suitable film characterization and biocompatibility assessment studies. But their work did not emphasized upon the influence of TA concentration on physical characteristics and biocompatibility assessment of PVA composite films. Thus, the investigations of Giglio et. al (2015) did not elaborate upon the influence of variant TA concentrations on the cell viability, given the fact that the authors only deployed a fixed concentration of 73.33 mmol of L-tartaric acid (De Giglio et al., 2015). Hence,

along with CA, TA have been considered as potential additives on PVA bio-composite films for wound healing characteristics (De Giglio et al., 2015). In summary, optimality of alternate organic acid based PVA composite films for combinatorial characteristics needs to be addressed and shall be regarded as the fifth objective of the Ph.D. Thesis.

1.7 Thesis objectives

In accordance to the summarized research gaps in section 1.6 of the chapter, the Ph.D. thesis targets the following objectives in the field of PVA composite films for wound dressing applications:

- a) Optimality of CA concentration and drying temperature to maximize combinatorial PVA-St-CA-Gl composite film characteristics such as SI, in-vitro hydrolytic degradation or WL, solubility, GF, barrier properties (WVTR, antibacterial effectiveness) and mechanical properties (TS, %E).
- b) Evaluation of synergistic influence of all constituents namely PVA, St, Gl and CA to further improvise upon the maximization of SI, WL and TS characteristics of PVA composite hydrogel films.
- c) RSM based optimality of PVA-St-CA-Gl composite films for the maximization of SI, WL, TS and %E and qualitative evaluation of the optimized film constitution using antibacterial effectiveness and in-vitro biocompatibility studies.
- d) Identification of best organic acid among TA or MA or CA to achieve best combinations of characterization parameters such as SI, WL, TS, %E, antibacterial effectiveness and in-vitro biocompatibility of PVA-St-Gl-organic acid film.

- e) Evaluation of optimal film constituents such as PVA, St and MA for a fixed GI concentration choice to achieve best combinatorial composite film characterizes such as SI, WL, TS and %E. Thereby, evaluation efficacy of best performing film in terms of qualitative assessment methods such as antibacterial effectiveness and in-vitro biocompatibility studies.

1.8 Organization of the thesis

Fulfilling the achievement of the above mentioned objectives, the Ph.D. thesis has been organized in eight chapters. A brief account of the chapters has been elaborated as follows:

Chapter 1 devotes towards the necessity towards furthering research in the field of PVA-St-CA-GI composite films to meet the requirements associated to low cost bio-degradable polymer composite films. The efficacy of all ingredients has been established prior to elaborating upon the available state of the art. Thereafter, customized research gaps have been identified to converge upon the objectives of the Ph.D. thesis. Finally, the organization of the thesis has been summarized.

Chapter 2 details upon the experimental and modelling approaches adopted for all investigations addressed in the thesis. These include procedures associated to (a) Fabrication of PVA-St composite films with variant constituent and additive concentrations using solution casting method, (b) RSM design based approaches for the design of experiments and statistical optimization of film compositions, c) Procedures and methodologies adopted for the evaluation of alternate characterization parameters of PVA-St composite films and (d) Qualitative assessment methods adopted to evaluate the characteristics of optimized PVA-St composite films through antibacterial effectiveness and cytotoxicity analysis.

Chapter 3 elaborates upon the findings associated to the fabrication of PVA-St-CA-Gl composite films by solution casting method. The findings indicate a prior evaluation of the appropriate optimality of fabrication time during low temperature drying associated to the solution casting method. Physical appearance and rheological studies have been considered to effectively converge upon the optimal fabrication time. Thereafter, for a fixed choice of PVA, St and Gl concentrations, the optimality of CA concentration and drying temperature have been addressed to evaluate the combinatorial optimal characteristics of PVA composite films. These include simultaneous maximization and achievement of acceptable combinations of SI, solubility, in-vitro degradation, GF, mechanical strength, antibacterial effectiveness and WVTR. Thereby, the experimental optimality of CA concentration and drying temperature have been evaluated for the fabrication of good quality PVA-St-CA-Gl films for wound dressing applications.

Chapter 4 addresses compatibility and synergy issues of PVA, St, CA and Gl for the fabrication of PVA-St composite film. Design expert (RSM) software was utilized to achieve data set associated to the design of experiments. Thereafter, the data set was meticulously followed to determine the non-mathematical (experimental data based) best constituent composition that indicated best combinations of evaluated combinatorial film characteristics. In other words, without addressing mathematical optimality, an insight into the complex interaction of the constituents and their concentrations has been addressed to indicate possible scope of improvisation in the film characteristics. Thereby, the obtained findings have been compared with those obtained from trial and error based approaches adopted in the relevant prior art.

Chapter 5 summarizes the optimality of the highly complex quaternary constituents (PVA, St, CA and Gl) by meticulously following the RSM design based statistical mathematical tool. The

statistical mathematical tool involved a two step strategy that initially involved fitness of the best fit mathematical model for various biases that also involved the consideration of %E as an additional response variable. Later, mathematical optimization was achieved through the design expert software environment. Thereby, coherence of alternate characterization parameters has been evaluated for mathematical optimization based improvisation in film constitution. The RSM statistical optimization based mathematical models provided greater insights into various similarities and dissimilarities associated to various characterization parameters. Finally, for the optimized film composition based PVA composite film, findings from antibacterial effectiveness and cytotoxicity studies have been presented. Along with other characterization parameters for the best film constitution, these parameters serve as a useful benchmark and guideline for the improvised research in the field of wound dressing compatible PVA composite films.

Chapter 6 elaborates upon the findings associated to the MA or TA organic acids in conjunction with those obtained with CA.

Chapter 7 delineates upon the optimal synergy of MA with PVA and St has been investigated using RSM design based methodology and subsequent statistical optimization. Finally, findings from antibacterial effectiveness and cytotoxicity analysis have been reported for the MA composite films fabricated with optimized film compositions. Thereby, the findings have been compared with available prior art to indicate upon the novelty and subjective enhancement in property characteristics and compositional know how of the other organic acid based PVA composite films.

Chapter 8 presents the most relevant deduced conclusions from the critical findings of the carried out research in the Ph.D. thesis. Thereafter, possible directions for future research have

been elaborated to accelerate the fabrication of bio-degradable polymer bio-composite films for bio-medical applications.

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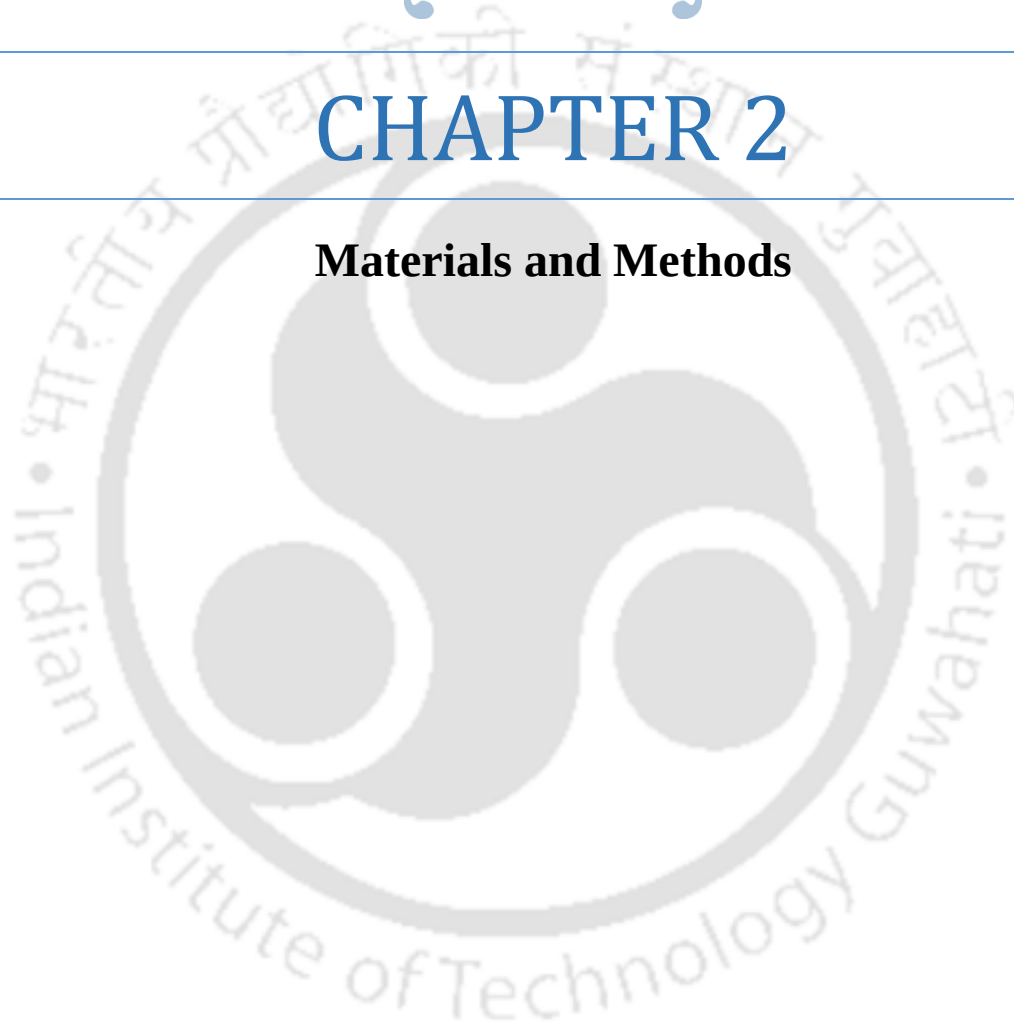






CHAPTER 2

Materials and Methods





Materials and Methods

In this chapter, relevant materials and adopted methods in the Ph.D. thesis have been elaborated. The content has been arranged as per the following hierarchy: (a) materials required to carry out the thesis work and fulfill the set objectives; (b) procedure associated with the fabrication of PVA composite films; (c) RSM based approaches to develop the design of experiments; (d) analytical methods adopted to evaluate the physicochemical properties of alternate PVA composite hydrogel films for their optimality and (e) finally qualitative characteristics of the optimal hydrogel films to serve as a benchmark for future research in wound dressing film preparation and application.

2.1 Materials

2.1.1 Casting solution precursors

Polyvinyl alcohol (average molecular weight 72,000 g/mol and 98 % hydrolyzed) was purchased from Merck Co. (Germany). Starch soluble (ACS) (reducing matter as maltose ≤ 0.7 %), anhydrous glycerol ($M = 92.09$ g/mol; purity (GC) ≥ 99.5 %), citric acid monohydrate (molar mass (M) = 211.14 g/mol; assay ≥ 99 %), malic acid and tartaric acid were procured from Merck Limited (Mumbai). Deionized water was collected from an indigenous setup installed at the analytical laboratory of the Chemical Engineering Department, IIT Guwahati, Assam, India.

2.1.2 Cell line and cell culture conditions

Human embryonic kidney cells (HEK-293) were procured from National Centre for Cell Science, (Pune, India). The cells were cultured in Dulbecco's modified Eagle's medium (purchased from Sigma Aldrich) supplemented with 10 % (v/v) fetal bovine serum, sodium bicarbonate, 1 % penicillin and streptomycin in humidified air condition containing 5 % CO₂ at 37 °C.

2.2 Crosslinking of PVA films

2.2.1 Fabrication of PVA-St-GI-CA films

2.2.1.1 Solution casting of composite films

The casting solution was prepared through the dissolution of PVA in 15 mL DI water at 95 °C for 1 h under vigorous stirring conditions. Following this, overnight stagnation was allowed to eliminate bubbles that might have formed during the vigorous stirring process. Thereafter, St solution was prepared separately in 15 mL DI water and was gelatinized at 90 °C for 45 min. Eventually, the gelatinized St solution along with GI and CA were mixed with the stagnated PVA solution at 80 °C for 30 min. CA was used as a chemical crosslinker. The CA concentration was varied in accordance with few trials and literature data (Table 2.1). Finally, the obtained homogeneous solution was subjected to casting on a glass plate with a casting knife (Digital Microm II Film Applicator; Model: 10574; Make: Gardco, US). To do so, the blended solution was casted through a path width of 25.4 cm that affirmed 635 µm thickness of the wet film. This was also set based on few trials to achieve defect free easily removable PVA composite hydrogel films. The film thickness of the casted film is a complex function of drying conditions and

compositional parameters. Thereby, the obtained dry film thickness values have been summarized in Appendix D.

Table 2.1: Constitution of aqueous solutions deployed for the fabrication of PVA-St-GI-CA composite films.

S. No.	PVA (g)/30 ml	PVA (wt%)	St (g)/30 ml	St (wt%)	GI (g)	GI (wt% on dry basis)	CA (g)	GI (wt% on dry basis)
1	2.81	9.36	2.81	9.36	2.11	37.54	0.5	8.89
2	2.81	9.36	2.81	9.36	2.11	37.54	1	17.79
3	2.81	9.36	2.81	9.36	2.11	37.54	2	35.58
4	2.81	9.36	2.81	9.36	2.11	37.54	3	53.38

2.2.1.2 Drying of casted films

The solution casted films were thermally and physically crosslinked using a hot air oven drying system (Model: Assembled; Make: Reico). The drying temperature and drying time of the oven have been varied to achieve a dried, transparent, smooth and easily removable film. This hydrogel film was subjected to further testing. Since the casting process followed the methodology outlined by Wu et al. (2017), drying experiments were carried out with the reported optimized drying time of 270 min (4.5 h) (Wu et al., 2017). Since low drying time required higher drying temperature for appropriately prepared dried films, the drying temperature value of 140 °C being mentioned by Shi et al. (140 °C) has been chosen as the maximum drying temperature (Shi et al., 2008). However, further reduction in drying temperature through an enhancement in drying time was targeted to gain useful insights with respect to their optimality. Thus, based on the literature reported values and further optimization of drying time and temperature, all dried films were subjected to physical optimization tests (ability to get separated from the glass plate while peeling

them with hand) to infer upon the optimality of drying temperature and time. Such data was missing in the literature and was necessary to further investigations in the said field of research.

2.2.2 Characterization

2.2.2.1 Physical optimization

The drying temperature and time were optimized based on various qualitative physical optimization parameters namely appropriate drying without any wet content in the film, lower stickiness, lower stretching and negligible brittleness of the films.

2.2.2.2 Reological measurement

Steady shear, storage modulus and loss modulus measurements of the blended PVA-St-GI-CA solution were measured out using a Reometer (Model: MCR 301; Make: Anton paar, Austria) being equipped with a 50 mm diameter plate geometry. The gap between the two plates was adjusted to 1 mm. For all measurements, dynamic strain and frequency were fixed at 5 % and 1 Hz, respectively. Further, the temperature of the system was enhanced from 5 to 140 °C (2 °C min⁻¹, heating rate) and for 1 h constant time duration.

2.2.2.3 Film thickness

The film thickness was measured using a digimatic micrometer (Model: APB-2D, Make: Mitutoyo Corporation, Japan; accuracy 0.001 mm).

2.2.2.4 ATR-FT-IR spectral analysis

The ATR-FT-IR spectra of the films were measured on a (Model: Spectrum two; Make: PerkinElmer, Singapore) FT-IR spectrometer using a diamond crystal. Four recordings were performed for each sample and an average spectrum was evaluated from such recordings. The measurements were carried out in the spectral wavelength range of 4000 to 400 cm^{-1} and with 4 cm^{-1} resolution.

2.2.2.5 Swelling index

The swelling index is defined as the capacity to absorb wound exudates. It is one of the most important properties of wound dressing materials. The SI of the fabricated PVA hydrogel film was evaluated using the following procedure (Ahmed et al., 2017). Firstly, the PVA composite film sample pieces ($1 \times 1 \text{ cm}^2$) were dried for 6 h at 37 °C in an incubator. Thereby, the samples were soaked in phosphate buffer saline solution (7.4 pH at 37 °C) in an incubator for 24 h. As reported in the literature, the water absorption reached an equilibrium state in the PBS system after a duration of 12 h (Zou et al., 2007). Hence, 24 h time period has been set to ensure the definite achievement of the equilibrium. Thereafter, using weights that were measured before and after soaking the samples, the SI (%) was evaluated using the expression:

$$SI = \frac{W_s}{W_0} \times 100\% \quad (1)$$

where, W_0 represents the dry weight of the samples and W_s represents the weight of the sample soaked in PBS for 24 h.

2.2.2.6 Solubility

After 6 h of drying time at 37 °C, the film samples were kept immersed in distilled water for 24 h. Thereafter, the samples were again dried for 6 h at 37 °C. The weights of the samples were appropriately measured before immersion in water and after drying for 6 h. Thereby, PVA composite film solubility was evaluated using the equation (Yoon et al., 2006):

$$S = \frac{(W_0 - W_d)}{W_0} \quad (2)$$

where, W_0 and W_d represents the dry weight and water-soaked sample weight measured after drying at 37 °C and 6 h respectively.

The available prior art indicates variant drying time durations (6 to 24 h) and drying temperatures (25 to 60 °C) for the initial drying phase. This was carried out for the conditioning of the sample pieces ($1 \times 1 \text{ cm}^2$) in terms of dried initial weight (W_0) (Ahmed et al., 2018, Yoon et al., 2006, Hajian et al., 2017). As mentioned in section 2.2.1.2, the primary objective was to investigate upon the influence of drying temperature on the physical crosslinking characteristics on the film samples. Conditioning at higher temperatures and for a long time may simultaneously dry the surface and enhance the extent of crosslinking in the polymer network. Hence, room temperature (37 °C) conditioning for a 6 h drying time duration was chosen for all the samples to reach an equilibrium state of the initial dried sample and its weight. Also, for the similar dried samples obtained after the water soaking process, the same drying temperature was considered to maintain a similar environment.

2.2.2.7 Water vapor permeability

An ideal wound dressing material should have an appropriate range of water vapor transmission rate to ensure a local moist environment for proper wound healing. A high WVTR value of the film enables effective shrinkage of films and skin dryness. On the other hand, films with lower WVTR do not function well due to the undesired accumulation of wound exudates. WVTR measurements were carried out by adopting the standard ASTM E398 method and a water vapor permeability tester (Model: 1/50 Mocon; Make: PERMATRAN-W, USA). During these tests, samples with an area of 50 cm² were kept under 30 % relative humidity (RH) and 25 °C temperature for 24 h to reach equilibrium.

2.2.2.8 In-vitro hydrolytic degradation

During the wound healing process, the rate of release of drug molecules from the polymer matrix is dependent upon its in-vitro degradation characteristics. These have been determined by following the procedure summarized in the next few lines (Hajian et al., 2017, Fahmy et al., 2015). The in-vitro hydraulic degradation behavior of the prepared film samples (1 × 1 cm²) was evaluated based on the evaluated weight loss against immersion time for 14 days in PBS solution (at 37 °C and 7.4 pH). In order to ensure complete removal of degraded or dissolved portions of the samples on a daily basis, the PBS media was changed every day during prolonged experimentation for up to 14 days. On 1, 2, 3, 7 and 14 days, the media was drained and the soaked sample was completely dried in an incubator at 37 °C for 6 h. The WL% during degradation was determined using the expression:

$$WL\% = \frac{(W_0 - W_f)}{W_0} \times 100 \% \quad (3)$$

where, W_0 and W_f represents the initial dry sample weight and weight obtained after drying (37 °C and 6 h) of PBS soaked sample, respectively.

2.2.2.9 Gel fraction

The film samples ($1 \times 1 \text{ cm}^2$) were dried for 6 h at 37 °C in an incubator and were subsequently kept in distilled water for 24 h. Thereafter, the hydrogels were once again dried at 37 °C for 6 h. The gel fraction percentage was evaluated using the expression (Ahmed et al., 2017):

$$GF = \frac{W_d}{W_0} \times 100 \% \quad (4)$$

where, W_0 and W_d represents the initial dry weight and dry weight measured after drying (at 37 °C and 6 h) past soaking in distilled water for the PVA composite film samples respectively.

2.2.2.10 Mechanical strength

For appropriate handling and utility purposes, a wound dressing material should have good combinations of flexibility, durability and elasticity. Thus, mechanical characterizations of the prepared PVA composite films were conducted under ambient conditions, by duly following the standard ASTM method D 882-02. Instron Universal Testing Machine (Model: Instron 5944; Make: Instron Engineering Corporation, Norwood, MA, USA) equipped with a 100 N load cell (Musetti et al., 2014, Shi et al., 2008) was used for the purpose. During the test, each film was cut into a dog-bone size strip ($1 \times 3 \text{ cm}^2$) and was mounted in silicon carbide paper grips prior to placement in the equipment. Thereafter, the sample was subjected to a crosshead speed of 1 mm/min. Thereby, tensile strength, percentage elongation were measured and expressed in MPa and percentage, respectively. The Young's modulus was calculated using an offset-yield approach.

For this purpose, 22 A line was drawn parallel to the linear regression elastic region of the stress-strain curve with an offset of 0.5% to the initial sample gauge length. The Young's modulus for the samples was expressed by the intersection point, i.e., at the point at which the line met the stress-strain curve.

2.2.3 Qualitative assessment

2.2.3.1 Antibacterial activity

The antibacterial activities of PVA-St-GI-CA films were examined for the growth of both Gram-positive (*Listeria monocytogenes*) and Gram-negative bacteria (*Escherichia coli*). The tests involved aseptic inoculation of either of these microbes in 20 mL nutrient broth and subsequent incubation at 37 °C under continuous stirring conditions (120 rpm) in a shaking incubator. After every 1 h time interval, the pathogenic growth was evaluated by measuring the absorbance of the samples at 600 nm using a UV-Vis spectrophotometer (Model No.: UV-2600, Make: Shimadzu, Singapore). After the pathogen growth reached an optical density of 0.6, the broth was spread on a petri-plate over nutrient agar media under a sterile environment. Thereafter, the films (with a specific circular area) were placed over the plate and were incubated for 6 h at 37 °C. Subsequently, the growth on the agar plate was verified through visual observation and the diameter of the inhibition was measured using a ruler.

2.2.4 Statistical analysis

Statistical analysis was carried out using analysis of variance (one-way ANOVA). The measured data were analyzed using a Tukey test with a significance level of $p < 0.05$, $p < 0.01$ and $p < 0.001$.

All measured and evaluated data have been reported as the mean (average of three replicates) $\pm$ SD in the Ph. D. thesis.

2.3 Compositional synergy of PVA, St, Gl and CA precursors

2.3.1 Preparation of PVA-St-Gl-CA films

The concentrations of all four constituents (PVA, St, Gl and CA) have been varied in the blended solution by following the approach of the statistical design of experiments (Ahmed et al., 2017). The casting solution was prepared following the procedure being mentioned in section 2.2.1.1. Only the dissolution of PVA and St has been done in 20 mL DI water, separately. The solution casting procedure was similar to that being mentioned in section 2.2.1.1. Finally, casted PVA composite films were dried in a hot air oven at an optimal drying temperature and time that was evaluated in due course of investigations associated with the first objective.

2.3.2 Experimental design

Response surface methodology characterized with central composite design was applied for the statistical design of experiments. These experiments were conducted by considering PVA concentration (A), St concentration (B), CA concentration (C) and Gl concentration (D) as independent variables and SI, in-vitro degradation or WL, TS and %E (optional) as dependent variables.

2.3.2.1 Statistical design of experimental formulations

The value range for independent variables was chosen based on the trial and error approach and insights available in a few prior arts. Accordingly, the PVA and St concentration was varied from

5 to 10 w/w%. This is due to the fact that a pertinent concentration lower than 2.5 w/w% from the RSM would foster the solution to become too light and enhance difficulties associated with the achievement of appropriate thickness. On the other hand, concentrations above 12.5 w/w% of both PVA and St in the RSM assisted formulation fostered the solution to become highly dense and enhance ductility, brittleness and associated difficulties to peel the film from the glass plate after drying. Other additives (Gl and CA) in the PVA composite film constitution have been varied to be about 10 – 50 wt% (dry basis of the total polymer amount). These are in accordance with the range indicated in the relevant literature (Ahmed et al., 2017). Therefore, 15 – 40 wt% variation has been allowed for these constituents to achieve a maximum and minimum concentrations of 52.5 wt% and 2.5 wt% respectively for each of the said constituents. With such compositional variations and six points at the central level, 30 different combinations of the independent variables have been suggested by the CCD. Table 2.2 summarizes the CCD-RSM based design of experimental data sets for 30 alternate formulations.

Table 2.2: CCD-RSM based compositional data sets being deployed for the fabrication of PVA-St-Gl-CA composite films.

S. no.	A: PVA concentration (w/w%)	B: St concentration (w/w%)	C: CA concentration (wt%)	D: Gl concentration (wt%)
1	7.5	7.5	27.5	27.5
2	7.5	7.5	27.5	27.5
3	10	5	15	40
4	7.5	7.5	27.5	2.5
5	7.5	7.5	2.5	27.5
6	5	5	15	40
7	7.5	7.5	27.5	27.5
8	5	10	40	40
9	5	10	15	15

S. no.	A: PVA concentration (w/w%)	B: St concentration (w/w%)	C: CA concentration (wt%)	D: GI concentration (wt%)
10	7.5	12.5	27.5	27.5
11	10	10	15	15
12	10	10	40	15
13	10	10	15	40
14	10	5	15	15
15	5	5	15	15
16	2.5	7.5	27.5	27.5
17	5	10	15	40
18	10	5	40	15
19	12.5	7.5	27.5	27.5
20	7.5	7.5	52.5	27.5
21	7.5	7.5	27.5	52.5
22	7.5	2.5	27.5	27.5
23	7.5	7.5	27.5	27.5
24	5	10	40	15
25	5	5	40	40
26	7.5	7.5	27.5	27.5
27	5	5	40	15
28	10	5	40	40
29	7.5	7.5	27.5	27.5
30	10	10	40	40

2.3.2.2. Experimental and numerical optimization

Initially, the RSM based approach was chosen to reduce the number of experiments by targeting a design based variation in concentrations of all film constituents. The 30 different formulations indicated in the table yielded in alternate PVA composite films which have been characterized for various parameters like, SI, WL, solubility, GF, TS and %E. The SI represents the absorption capacity of the dressing materials. A maximum value of SI is required to avoid an accumulation of wound fluid that enhances contamination. On the other hand, the in-vitro degradation represents the weight loss of the film driven by the leaching of the components from the composite film

matrix to prevent infection and enable localized antibacterial effect. Moreover, easy handling and appropriate usage of wound dressing materials on the surface of the wound requires minimum shape stabilities of the film in fluids and hence moderate mechanical strength. Considering these issues as the most appropriate and relevant for wound dressing applications, the said responses have been considered for the fabricated PVA composite films. These refer to considering either SI, in-vitro degradation and TS as response variables or considering %E as an additional response variable.

Two alternate approaches have been considered for compositional optimality. These refer to either experimental data based optimization or ANOVA based optimization. For the experimental data optimization case, the response variable optimality was based on a manual approach and hence RSM can be regarded to only generate design formulation data sets. On the other hand, the optimization of the obtained data sets for different response variables can undergo ANOVA statistical analysis. These software based optimizations are expected to provide more useful and precise formulations for the four constituent concentrations during PVA composite film fabrication. The bias for RSM optimization is in terms of the best possible combinations of SI, in-vitro degradation, TS and %E (optional) values.

2.3.2.3. ANOVA based statistical analysis

The ANOVA analysis enabled appropriate model development for each dependent variable using multiple regression analysis. The coefficients of the significant terms were provided by the ANOVA in design expert 7.0 in which the F value of the regressed data affirms towards the significance of the terms. The p -value for each term ensures towards null hypothesis and hence the coefficient has no effect. A low p -value (<0.05) indicates that one can reject the null hypothesis. For a model to be considered suitable among alternate models, the F value should be as high as

possible and the p -value should be as low as possible. Adequacy in judging model fitness was also based on the R^2 value or coefficient of determination, which is a statistical measure to indicate the closeness of the measured data with the best fit regression model data.

For the obtained responses, a second-order polynomial equation (Myers et al., 2016) was used to represent the dependent variables as appropriate functions of four independent variables using the expression:

$$Y = \sum_{i=1}^4 a_i x_i + \sum_{i=1}^4 b_{ij} x_i y_j + \sum_{i=1}^4 c_i x_i^2 \quad (5)$$

where, x , y are independent and dependent variables respectively

a_i = linear coefficients

b_{ij} = bi-linear coefficients

c_i = quadratic coefficients

2.3.3 Characterizations

2.3.3.1 Alternate physical characterizations

The ATR-FT-IR spectra, SI, solubility, GF and in-vitro degradation characteristics have been evaluated by following procedures outlined in sections 2.2.2.4, 2.2.2.5, 2.2.2.6, 2.2.2.8 and 2.2.2.7, respectively.

2.3.3.2 Mechanical strength

The appropriate usage of PVA-St composite films for wound dressing applications needs certain characteristics such as the ability to stretch and fit on the wound surface and easy handling. These can be ensured by evaluating the film strength characteristics. For the PVA composite films,

mechanical characterizations were conducted to determine TS and %E. These experiments were conducted using a 5 kN electromechanical universal testing machine (Model: Z005TN; Make: Zwick Roell) (Shi et al., 2008, Musetti et al., 2014) by adopting the standard ASTM D790 method at ambient conditions. The sample preparation for the test involved cutting each film into an I-shaped strip ($1 \times 3 \text{ cm}^2$). The sample thickness was measured using the procedure outlined in section 2.2.2.3. Finally, the sample was mounted in pneumatic grips and was subjected to a crosshead speed of 10 mm/min to determine the mechanical strength characteristics of the PVA composite film.

2.3.4 Qualitative assessment of optimized PVA composite films

2.3.4.1 Antibacterial activity

The fabricated PVA-St-CA-GI composite films have been evaluated for the growth of both *L. monocytogenes* and *E. coli*. All procedures such as culturing of microorganisms, design of tests, incubation conditions and determination of the bacterial inhibition zone diameter have been duly followed as per the procedures elaborated in section 2.2.3.1.

2.3.4.2 In-vitro biocompatibility

Cytotoxicity of the optimized films with HEK cells

The relative growth rate of the HEK cells was determined by following the colorimetric MTT assay. This involved the catalytic conversion of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide to achieve insoluble MTT formazan crystals by deploying succinate dehydrogenase enzyme of healthy mitochondria. For the MTT assay, HEK cells were seeded in 96

well plates (4×10^3 cells/well cell density) and were allowed to attach for 24 h. Subsequently, the cells were treated with the extract solutions with increasing concentrations. These extract solutions were prepared by incubating the UV sterilized optimized films in FBS supplemented DMEM media for 24 and 48 h, respectively. Three dilutions (5, 10 and 100 % volume proportions, in 100 μ L of total treatment) of each extract were chosen for treatment and were tested in triplicate for 48 h of incubation. The relative growth rate was assessed by carefully adding 0.5 mg/mL MTT to individual well(s) and subsequent incubation in humidified air containing 5 % CO₂ at 37 °C for 2 h. After carrying out the said treatment, the MTT was carefully removed. Thereafter, 150 μ L of dimethyl sulfoxide was added to each well for the dissolution of the insoluble formazan crystals. The absorbance of the dissolved formazan crystals was measured using a microplate reader (Model: M200 pro; Make: Tecan Infinite) at 570 nm wavelength. For these measurements, 630 nm wavelength is considered to obtain reference data sets.

The cytotoxicity test results of all the optimized PVA-St composite films was assessed according to the GB/T16175-1996 standard (Shi et al., 2008). According to the standard, the relative growth rate was evaluated using the following equation and the value of RGR% was classified to six grades as presented in Table 2.3 and these grades define the acceptance level of toxicity.

Table 2.3: Grade and cytotoxicity level quantification based on the relative growth rate (adapted from Shi, Bi et al. 2008).

Relative growth rate (%)	Grades	Cytotoxicity level
100	0	Qualified
75 – 99	1	Qualified
50 – 74	2	Combining with the cell's morphology
25 – 49	3	Combining with the cell's morphology
1 – 24	4	Unqualified
0	5	Unqualified

$$\text{Relative growth rate} = \frac{\text{Absorbance of the treated cells}}{\text{Absorbance of the control or untreated cells}} \times 100 \% \quad (6)$$

Statistical analysis

Using one-way ANOVA variance, statistical analysis was conducted with the Tukey test and for a significance level of $p < 0.05$.

Visual evaluation of relative growth rate using Annexin V-FITC and PI

Differential staining with Annexin V-Fluorescein Isothiocyanate and Propidium Iodide was performed to qualitatively evaluate the cytotoxicity potential of the optimized film compositions. The extracts of the films fabricated with optimal compositions were subjected to treatment with HEK cells. After treatment, the cells were washed with PBS. Subsequently, Annexin V-FITC and PI (BD Pharmingen) were added to the cell as per the protocol provided by the manufacturer (Becton Dickinson India Pvt. Ltd.). Thereafter, the plates were incubated in a dark environment for 30 min. Finally, the image of the cells was obtained using a Confocal microscope (Model: LSM 880; Make: Zeiss).

2.4 Efficacy of alternate organic acid based PVA composite films

2.4.1 Comparative assessment of CA, TA and MA

2.4.1.1 Preparation of PVA-St-Gl-TA or MA films

The PVA composite films have been prepared by using either TA or MA as additives and by the following procedure outlined in section 2.2.1.1. Needless to convey, CA has been conveniently replaced with TA or MA as a chemical crosslinker. Finally, the prepared PVA-St-Gl-TA or PVA-St-Gl-MA blended solutions were cast on a glass plate with a casting knife (thickness of 635 μm) and were dried in a hot air oven at an optimal drying temperature and time that was obtained after realization of the first objective. The formulations used for the preparation of PVA-St-Gl-TA or PVA-St-Gl-MA films have been summarized in Table 2.4. The optimized formulations associated with PVA-St-Gl-CA composite films during the accomplishment of the second and third objective was considered to be relevant for the alternate organic acid. Hence, substituting CA, with either TA or MA, four optimal compositions of PVA-St-Gl-CA films have been considered to generate 8 alternate data sets for TA and MA. Accordingly, a comparative preliminary assessment was targeted to evaluate upon the best organic acid among TA, MA and CA.

Table 2.4: A summary of deployed solution compositions for the fabrication of alternate organic acid based PVA composite films.

S. no.	PVA (w/w%)	St (w/w%)	Gl (wt%)	CA (wt%)	TA (wt%)	MA (wt%)
1	5	10	15	15	0	0
2	7.5	7.5	27.5	27.5	0	0
3	5.5	8.1	22.7	22.8	0	0
4	7.5	7	18.38	28.6	0	0

S. no.	PVA (w/w%)	St (w/w%)	Gl (wt%)	CA (wt%)	TA (wt%)	MA (wt%)
5	5	10	15	0	15	0
6	7.5	7.5	27.5	0	27.5	0
7	5.5	8.1	22.7	0	22.8	0
8	7.5	7	18.38	0	28.6	0
9	5	10	15	0	0	15
10	7.5	7.5	27.5	0	0	27.5
11	5.5	8.1	22.7	0	0	22.8
12	7.5	7	18.38	0	0	28.6

2.4.1.2 Characterizations

All physical properties such as SI, solubility, in-vitro degradation tests and GF have been evaluated for the fabricated films by adopting procedures respectively described in sections 2.2.2.5, 2.2.2.6, 2.2.2.7 and 2.2.2.8. TS, %E and ATR-FT-IR spectra of the TA or MA based PVA composite films have been evaluated using procedures outlined in sections 2.3.3.2 and 2.2.2.4.

2.4.1.3 Qualitative assessment

Antibacterial activity

The antibacterial activities of PVA-St-Gl-organic acid films were examined against *L. monocytogenes* and *E. coli*. The tests involved aseptic inoculation of both of the bacterias in 10 mL nutrient broth and subsequent incubation in a shaking incubator at 37 °C and 160 rpm. After every 2 h time interval, the bacterial growth was evaluated by measuring the absorbance at 600 nm using a UV-Vis spectrophotometer (Model No.: GENESYS 10S UV-Vis, Make: Thermo Scientific). After the bacterial growth reached an optical density of 0.6, the broth was spread on a petri-plate over nutrient agar media under a laminar hood. Thereafter, the circular films were

placed over the plate and were incubated for 12 h at 37 °C. Subsequently, the zone of inhibition on agar plate was evaluated through visual observation and diameter was measured using a ruler.

In-vitro biocompatibility

The in-vitro biocompatibility characteristics have been evaluated by following the procedure outlined in section 2.3.4.2.

2.4.2 Compositional synergy of Gl based PVA-St-MA composite films

RSM based design formation methodology was adopted for PVA-St-Gl-MA composite film. Thereby, synergy and compatibility of PVA, St and MA were targeted. Similar to the CA, the two other organic acids MA and TA do have multiple carboxylic groups. However, unlike CA and MA, TA is a strong organic acid. CA is a triprotic and weak acid. It has a pK_a value of 3.13, 4.76 and 6.40 with respect to its 1st, 2nd and 3rd carboxylic group, respectively. On the other hand, MA is a dicarboxylic acid. It has a pK_a value of 3.40, and 5.20 with respect to its 1st and 2nd carboxylic group, respectively. TA is the strongest organic acid and a diprotic acid also. The pK_a (at 25 °C) of TA has been reported to be 2.98 and 4.34 respectively for the dissociation of the first and the second carboxylic group (Ceciliani, 2017). The acidity of these organic acids is responsible to inhibit the propagation of bacteria cell and toxicity. Also, among all three acids, TA is the most expensive. Hence, MA has been chosen for compositional synergy studies.

2.4.2.1 Fabrication of TA or MA based PVA composite films

Adopting a statistical design of experiments approach, the concentrations of PVA, St and MA have been varied by choosing a fixed concentration of Gl in the PVA-St-Gl-CA blended solution. The casting solution procedures have been similar to those mentioned in section 2.2.1.1. Finally, the

PVA-St-Gl-MA blended solutions were cast onto glass plates and were dried in a hot air oven at 50 °C for 12 h.

2.4.2.2 Experimental design and statistical analysis

A set of experimental designs were obtained for a variation of PVA, St and MA concentration and fixed Gl concentration using design expert software (RSM methodology). Accordingly, experiments were conducted by considering PVA concentration (A), St concentration (B) and MA concentration (C) as independent variables and SI, in-vitro degradation, TS and %E as dependent variables. The value range for independent variables was chosen according to the methodology presented in section 2.3.2.1. For PVA and St, the solution concentrations were allowed to vary between 4.5 – 10.5 w/w% concentration. Thereby the CCD based model allowed a maximum and minimum range of concentrations of 12.55 w/w% and 2.45 w/w%, respectively. Similarly, 12 – 43 wt% variation was allowed for MA to achieve a maximum and minimum concentrations of 53.57 wt% and 1.43 wt%, respectively.

With such compositional variations and six points at the central level, 20 alternate combinations of the independent variables have been suggested by the CCD (Table 2.5). Accordingly, 20 alternate MA based PVA composite films have been fabricated.

Table 2.5: A summary of CCD-RSM based alternate solution formulations for MA based PVA composite films.

S. no.	A: PVA concentration (w/w%)	B: St concentration (w/w%)	C: CA concentration (wt%)	Gl concentration (wt%)
1	12.55	7.5	27.5	18.38
2	7.5	7.5	27.5	18.38
3	7.5	7.5	27.5	18.38

S. no.	A: PVA concentration (w/w%)	B: St concentration (w/w%)	C: CA concentration (wt%)	Gl concentration (wt%)
4	4.5	10.5	12	18.38
5	7.5	7.5	27.5	18.38
6	7.5	7.5	53.57	18.38
7	7.5	2.45	27.5	18.38
8	7.5	7.5	1.43	18.38
9	2.45	7.5	27.5	18.38
10	10.5	4.5	12	18.38
11	4.5	4.5	43	18.38
12	10.5	10.5	43	18.38
13	4.5	10.5	43	18.38
14	4.5	4.5	12	18.38
15	7.5	7.5	27.5	18.38
16	7.5	7.5	27.5	18.38
17	7.5	7.5	27.5	18.38
18	10.5	4.5	43	18.38
19	10.5	10.5	12	18.38
20	7.5	12.55	27.5	18.38

2.4.2.3 Characterizations

To evaluate upon the optimality of MA based PVA composite films, all dependent variables such as SI, in-vitro degradation tests, TS and %E have been determined respectively using procedures summarized in sections 2.2.2.5, 2.2.2.7 and 2.3.3.2. Also, the ATR-FT-IR spectra of the PVA-St-Gl-MA composite films have been obtained by adopting the procedure summarized in section 2.2.2.4.

2.4.2.4 Qualitative assessment

ANOVA based optimal film constitution has been evaluated for its efficacy in terms of antibacterial effectiveness and in-vitro biocompatibility. Methodology and procedures outlined in sections 2.4.1.3 and 2.3.4.2 have been respectively followed for such analysis and evaluation.

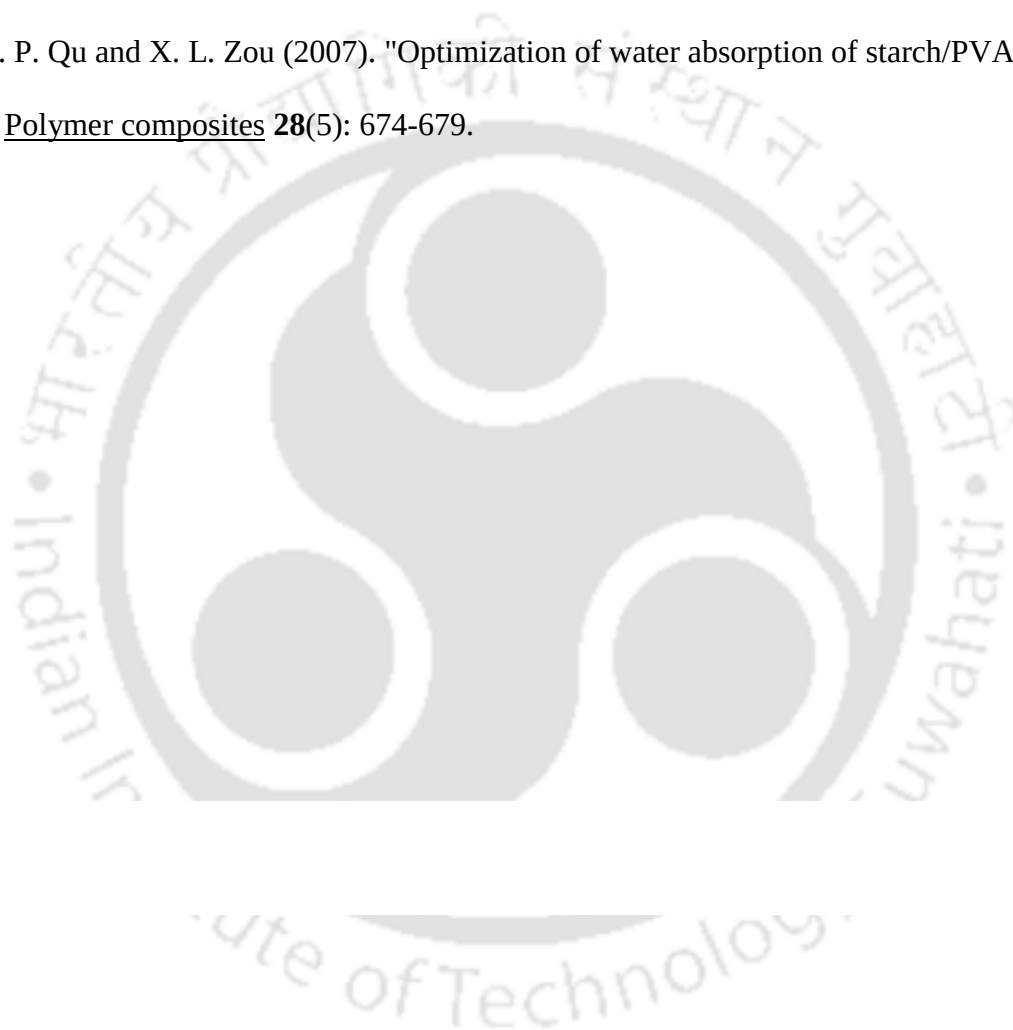
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CHAPTER 3

**Optimality of CA Concentration and Drying Temperature to
Maximize Combinatorial PVA-St-CA-GI Composite Film
Characteristics**



Optimality of CA Concentration and Drying Temperature to Maximize Combinatorial PVA-St-CA-Gl Composite Film Characteristics

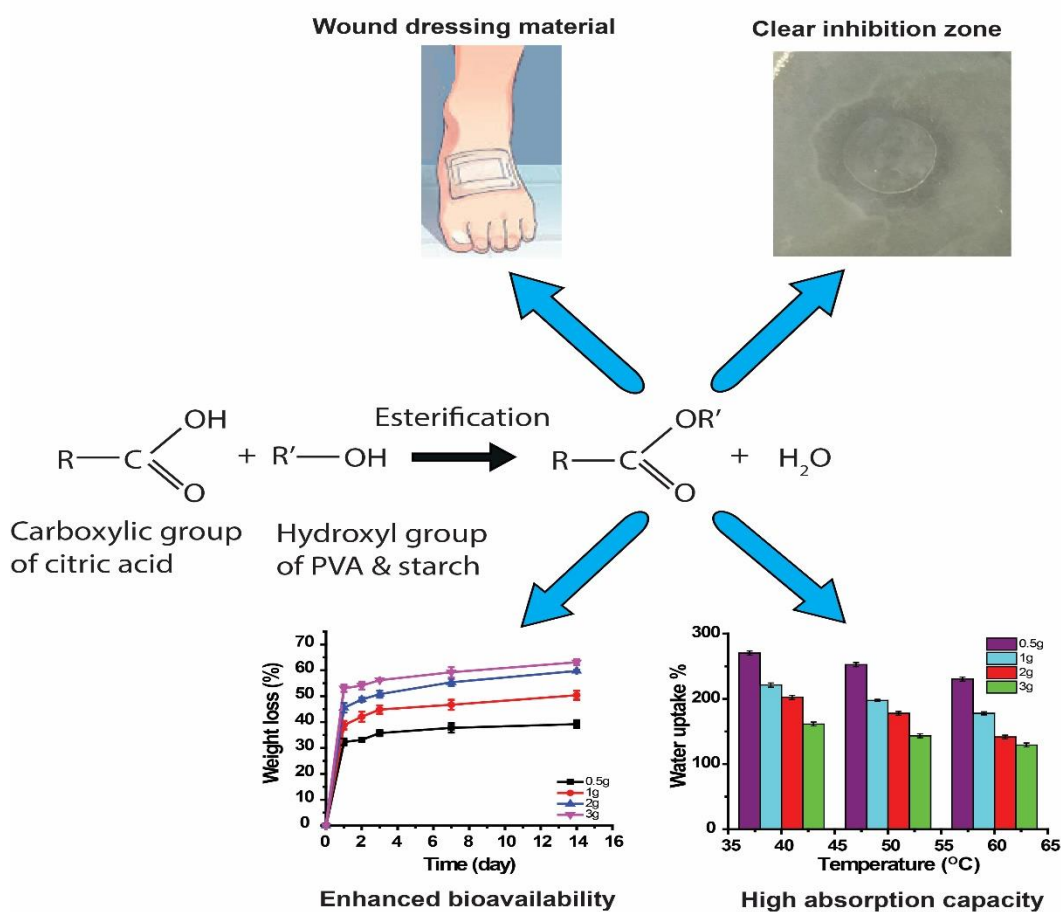
This chapter, presents the results and discussions associated to optimality of CA concentration and drying temperature during solution casting based fabrication of PVA-St-CA-Gl composite films. After a brief introduction in section 3.1, Sections 3.2 details upon the finding of the range of the fabrication parameters such as, drying temperature and time using literature database and trial and error method. For these cases of success range of fabrication process additional characterization studies have been conducted to validate the optimal compositions and summarizes in section 3.3. Section 3.4 and 3.5 present comparative assessment with literature data of cost analysis and characterization parameters, respectively for optimally prepared films followed by a summary of key findings in section 3.6[#].

Overview

The optimality of crosslinking temperature and CA concentration have been investigated during solution casting based composite film fabrication. The prepared polymer films have been characterized with SI, solubility based biodegradability, GF, mechanical strength, WVTR and antimicrobial effectiveness. The optimized film composition were analyzed to possess excellent SI and in-vitro degradation characteristics. Further, the film possesses enhanced combinations of

[#] Published article: Das, A., R. Uppaluri and C. Das (2019). "Feasibility of poly-vinyl alcohol/starch/glycerol/citric acid composite films for wound dressing applications." International Journal of Biological Macromolecules **131**: 998-1007.

WVTR and antibacterial activity with Gram-negative (*E. coli*) and Gram-positive (*L. monocytogenes*) bacteria.



3.1 Introduction

This chapter targets the optimality of the solution casting process parameters during the fabrication of PVA-St-CA-Gl composite films. The primary emphasis of the carried out investigations is to visualize upon the compatibility of PVA-St-CA-Gl composite films as ideal wound dressing materials. Given the contextual insights gained from available prior art, process optimization studies refer to optimality of CA concentration, fabrication time and drying temperature for a fixed choice of PVA, St and Gl concentrations during low temperature solution

casting fabrication process. The optimality of PVA and St compositions will be addressed in the future research after obtaining significant insights with respect to the optimality of CA and other parameters. All relevant characterization parameters such as SI, in vitro degradation or WL, mechanical strength, antibacterial effectiveness and WVTR will be considered to evaluate upon the efficacy of PVA-St-CA-Gl composite films as wound dressing materials.

3.2 Evaluation of critical process parametric range of PVA-St blended film fabrication process

3.2.1 Physical optimization

Since Wu et al. only targeted optimization of drying time (4.5 h) based on mechanical strength, water vapor permeability and solubility; but not considered physical optimization approaches (Wu et al., 2017). Thereby, trials are conducted for various cases by considering 4.5 h drying time and 140 °C crosslinking temperature as the starting parameter dataset (Shi et al., 2008, Birck et al., 2014). Thereafter, the drying time are varied up to 12 h and the crosslinking temperature is reduced to about 30 °C to evaluate the optimality of films based on physical optimization methods. For a drying time of 4.5 h, at 140 to 70 °C, the films being very dry, harden, brittle, cannot be collected without damaging the films from the glass plate. At 60 °C and for 4.5 h time, the drying is good and uniform. However, for 40 and 50 °C cases, the films do not get properly dried and do not get peeled from the glass plate. For these temperature cases, an enhancement in drying time from 6 to 12 h is apparent due to physical optimization based insights. For 1 g CA concentration and crosslinking temperature range of 60 to 40 °C, the PVA-St-CA-Gl composite film drying is highly effective for 10 to 12 h of drying time. However, for

higher CA concentration (2 and 3 g cases), 12 h drying time has been the most efficient. Table 3.1 presents a summary of the physical observations associated with all trials conducted for various combinations of crosslinking temperature and time.

Table 3.1: A summary of physical observation based optimality of crosslinking temperature and time during PVA-St blended film fabrication.

Drying temperature (°C)	Drying Time (h)	Observations during film peeling from the glass plate (after drying)
140	4.5	Unable to peel
70	4.5	Peeling or collection possible but very brittle film
60	4.5	Less sticky and collectable
50, 40	4.5	Very sticky and difficult to peel
50, 40	6	Very sticky and difficult to peel
40	8	Very sticky and difficult to peel
40	10	Less sticky and collectable. However, the film gets stretched during peeling from the glass plate
50, 40	12	Properly dried, transparent, smooth and easily peelable
60	12	Properly dried, transparent, smooth and easily peelable
70	12	Unable to peel
30	12	Very sticky and gets stretched while peeling from the glass plate

Thus, based on physical optimization studies, appropriately dried, transparent, smooth and easily removable PVA composite films (less sticky and not stretched while removing from glass plate) have been prepared for 12 h drying time and 60, 50 and 40 °C crosslinking temperature. Such insights have not been concluded in the appropriate literature (Shi et al., 2008, Wu et al., 2017). Also, the chosen crosslinking temperature range is supported by the rheological study for PVA-St-CA-Gl blend solution conducted and discussed in the next section. Accordingly, PVA composite films have been prepared for variant crosslinking temperature (40 to 60 °C) and CA concentrations, fixed choice of PVA, St and Gl concentration and fixed drying time (12 h). A

summary of these has been presented in Table 3.2. Only for these cases, additional characterization studies have been conducted due to the success of the fabrication process for these cases only.

Table 3.2: Constituent composition of solution casting systems deployed for the fabrication of PVA-St-CA-Gl composite films.

S. No.	PVA (g)	St (g)	Gl (g)	CA (g)	Drying Time (h)	Crosslinking temperature (°C)
1	2.81	2.81	2.11	0.5	12	40, 50, 60
2	2.81	2.81	2.11	1	12	40, 50, 60
3	2.81	2.81	2.11	2	12	40, 50, 60
4	2.81	2.81	2.11	3	12	40, 50, 60

3.2.2 Rheological measurements

Figure 3.1 presents the viscoelastic behaviors (storage modulus and loss modulus) of PVA composite film precursors as a function of temperature, for a variation of CA concentration from 0.5 to 3 g, respectively. The rheological behavior of the mixture indicates the onset of significant storage energy thus elastic response of the mixture in comparison to viscous response after 48.6 °C for 0.5 and 1 g CA concentration cases. On the other hand, the temperature increases to 53.2 °C and 62.4 °C respective for CA concentration 2 and 3 g, respectively. Crosslinking increases the storage modulus because with crosslinking the interconnection between different long backbone chains increase the elastic energy (stress applied and strain) of the polymer. These indicate that the onset of crosslinking temperature of the PVA composite film is within 48.6 to 60 °C for all cases. In summary, the rheological studies have confirmed that the crosslinking temperature of 40 to 60 °C is justified. Also, for each sample, it is observed, mostly after 100 °C temperature the solution became very unstable. So, higher crosslinking temperatures reported in

the literature are unreasonable (Shi et al., 2008, Birck et al., 2014) and can be avoided for energy consumption.

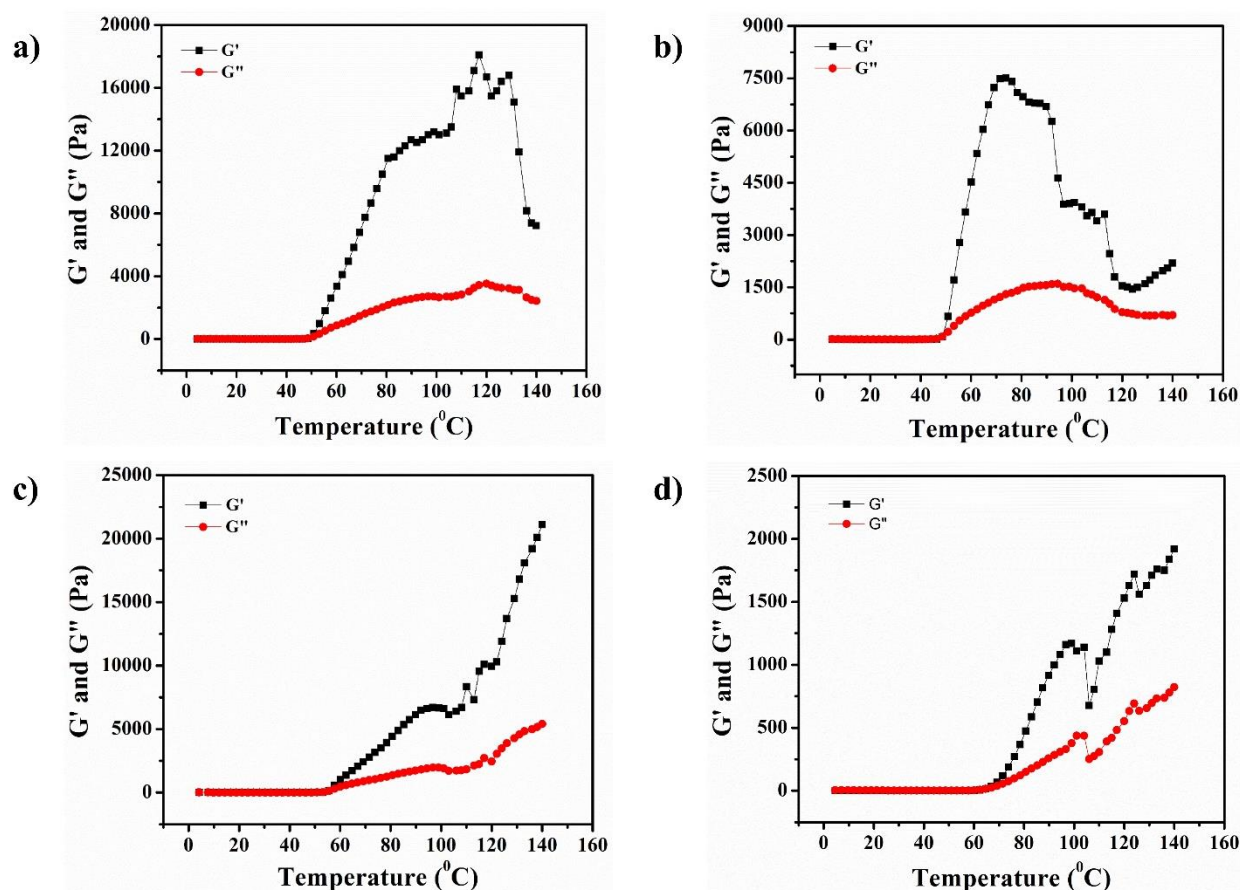


Figure 3.1: Temperature dependent storage and loss modulus characteristics of PVA-St-CA-Gl blends for various CA concentrations (a) 0.5g, (b) 1g, (c) 2g and (d) 3g.

3.3 Characterization of PVA-St blend films

3.3.1 ATR-FT-IR spectra based structural analysis

Figure 3.2 (a) and (b) represents the ATR-FT-IR spectra of PVA, St, PVA-St-Gl and PVA-St-Gl-CA blended films which is prepared at different temperature (40, 50 and 60 °C), at 3700 – 2700 and 900 – 1800 cm^{-1} spectral region, respectively. All samples indicated peaks in the wavelength

range of 3270 – 3315, 2920 – 2941, 1709 – 1722, 1634 – 1657, 1543 – 1557, 1408 – 1420, 1364 – 1375, 1327 – 1341 and 1016 – 1040, 1202 – 1244, 1142 – 1152, 1076 – 1088, 995 – 1003 and 916 – 932 cm^{-1} which corresponds to stretching vibration of –OH groups, symmetric and asymmetric stretching vibration of C–H from methyl or methylene groups, coalescence peak (C=O stretching vibration peak) caused by the ester bond and carboxyl C=O groups in CA, deformation vibration of the –OH groups from the bound water, bending vibration of C–O–H groups, symmetric bending mode of CH₂ groups, the mixed bending modes of C–H and –OH groups and attributed to the associated alcohols, deformation vibration of the C–O groups, wagging vibration of C–H groups, symmetric stretching vibration of the C–C groups and related to the regular repeating of the trans-configuration of the zigzag chain in the crystalline region, stretching vibration of C–C and C–O groups, syndiotactic structure and assigned to rocking vibration of CH₂ groups respectively (Popescu et al., 2018). Other notable variations in the composite structures are as follows:

- In 1709 – 1722 cm^{-1} spectrum region (Figure 3.2 (b)) St and PVA-St-Gl does not reflect any peak. As here the peak demonstrate the ester bond and –COOH groups presents due to CA; PVA, St and PVA-St-Gl should not reflect peak in this region. But a small peak was observed for PVA spectra which may be caused by some impurity. Whereas PVA-St-Gl-CA 0.5 g film show peak with very small intensity (almost negligible) for 40 and 60 °C temperature. This may be due to presence of less CA amount or less esterification reaction.
- At 1634 – 1657 cm^{-1} spectrum region pure PVA 60 °C film and PVA-St-Gl-CA 3 g 50 °C film did not reflect peak for bound water. This may be caused due higher degree of crosslinking at high temperature and CA concentration.

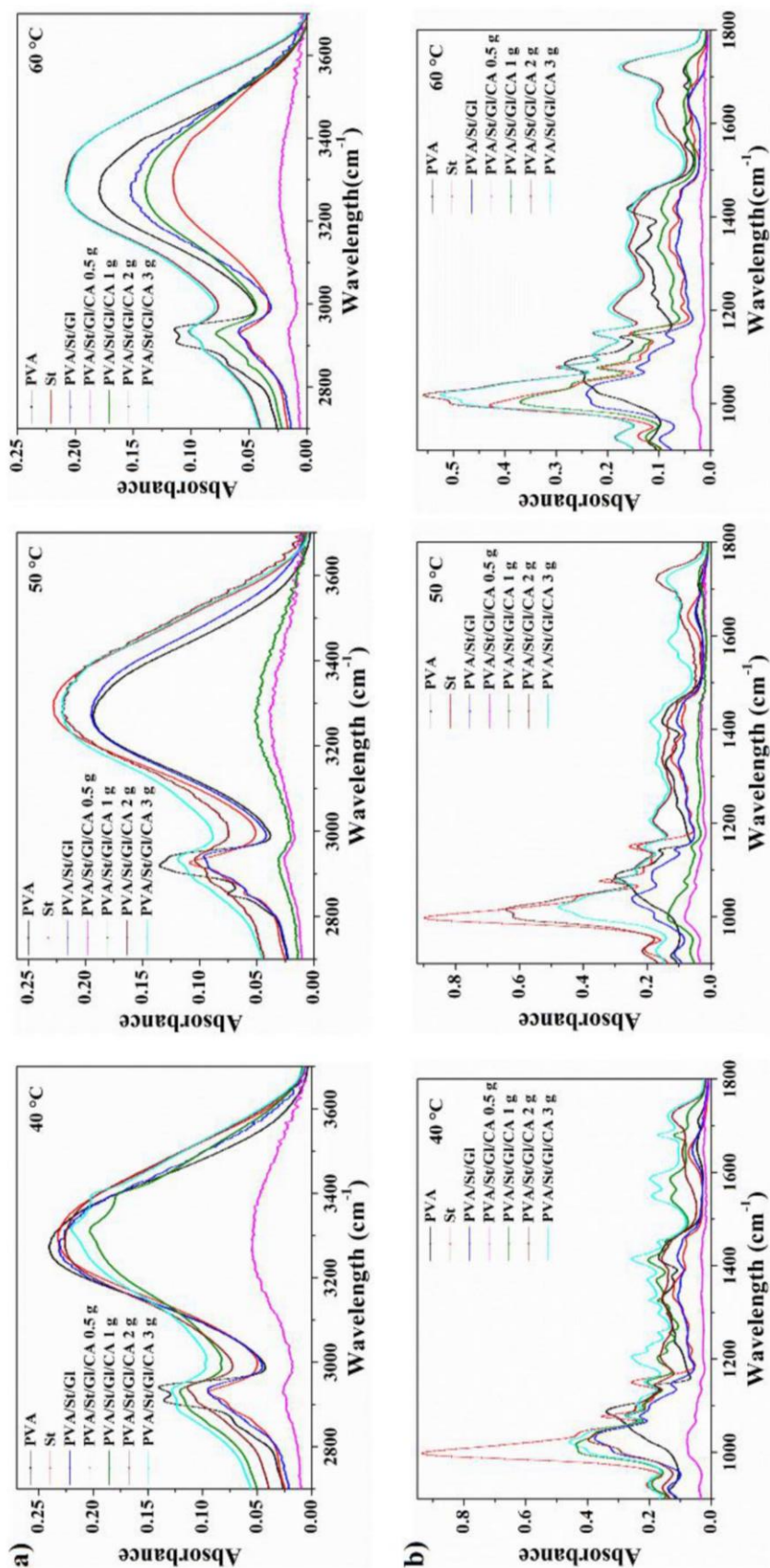


Figure 3.2: ATR-FT-IR spectra of PVA, St, PVA-St-GI and PVA-St-GI-CA composite films prepared at 40, 50 and 60 °C and (a) 3700 – 2700 and (b) 2700 – 1800 cm^{-1} wavelength range.

- In Figure 3.2 (a), the peak around $3270 - 3315 \text{ cm}^{-1}$ is assigned to the stretching vibration of $-\text{OH}$ groups, where the peak's height increased as the CA concentration increased. The increased peak intensity attributed due to excellent esterification degree and number of increased $-\text{OH}$ and $-\text{COOH}$ groups. It was also reflected, PVA-St-Gl (37.54 wt%) resulted higher peak intensity than PVA-St-Gl (37.54 wt%)-CA (8.89, 17.79, 35.58 and 53.38 wt% or 0.5, 1, 2 and 3 g). This is due to, Gl, having three $-\text{OH}$ groups, forms hydrogen bonds with St which does not reduce the $-\text{OH}$ groups in the PVA-St-Gl blend. Whereas CA, having one $-\text{OH}$ and three $-\text{COOH}$ groups, involves in esterification reaction with PVA and St which produce esters and reduce $-\text{OH}$ groups. But it is unlikely that all the present CA performed esterification reaction. Thus the excess CA left as free CA provide the available $-\text{OH}$ group and act as plasticizer. Also a part of CA was reacted with Gl to obtain some glycerol citrates which diffused into the water in the process of immersion. The presence of Gl prevented the reaction between CA and St or PVA (Shi et al., 2008).
- Further, in this region indicated in the Figure (3.2 (a)), all the films prepared at 60°C temperature represents lower peak intensity than the 40°C and 50°C films. After physical cross linking reaction number of $-\text{OH}$ group decreased, resulting decrease in peak intensity (Mittal et al., 2016). At 40°C temperature PVA, St and PVA-St-Gl has highest peak intensity, more than PVA-St-Gl-CA films. Whereas at 50°C PVA and PVA-St-Gl spectra intensity reduced below PVA-St-Gl-CA 2 and 3 g spectra and at 60°C PVA, St and PVA-St-Gl both spectra intensity comes below PVA-St-Gl-CA 2 and 3 g spectra. So, it also been observed the physical crosslinking effect is much prominent for pure PVA

and St film. The same phenomenon was attributed by St film in 990 – 1100 cm^{-1} spectrum region (Figure 3.2 (b)).

3.3.2 Swelling index and solubility measurements

The effect of CA concentration and crosslinking temperature on the SI is depicted in Figure 3.3 (a). For a constant crosslinking temperature of 40 °C and for a variation in CA concentration from 0.5 to 3 g, the SI has been reduced from 408.02 ± 4.96 to 223.78 ± 5.31 % respectively. The corresponding values varies from 383.78 ± 3.67 to 219.14 ± 4.76 % and 355.78 ± 4.63 to 205.95 ± 5.52 % for crosslinking temperature values of 50 and 60 °C respectively. This indicates that SI reduces significantly with increasing CA concentration but does not vary much with crosslinking temperature for higher CA concentration. Higher CA concentration and crosslinking temperature facilitate greater crosslinking and due to this reason, the SI gets reduced. Typically wound dressing materials shall possess significantly higher swelling capacity to effectively absorb wound exudates. It's been inferred that the hydrogel films with a SI of ≥ 260 % can be regarded as super absorbent (Ahmed et al., 2018). In this regard, the PVA composite films prepared with 2 g CA concentration and 50 °C crosslinking temperature possesses 260.5 % SI and can be therefore classified as a super absorbent and compatible for wound dressing applications.

Further, since greater crosslinking facilitates enhanced mechanical strength, the PVA composite films are also having an additional advantage in this perspective. From statistical analysis, it is observed that for each particular temperature and at different concentrations, the variations in the SI are significant (significant level $p \leq 0.001$). However, it is interesting to evaluate that the SI values vary rapidly with a variation in crosslinking temperature for CA concentrations up to 1 g (for 0.5 g CA concentration, $p \leq 0.001$ and for 1 g CA concentration, $p \leq 0.01$). For a further

enhancement in CA concentration, the change in SI is not that significant for increasing crosslinking temperature (both 2 and 3 g CA concentration, $p \leq 0.05$).

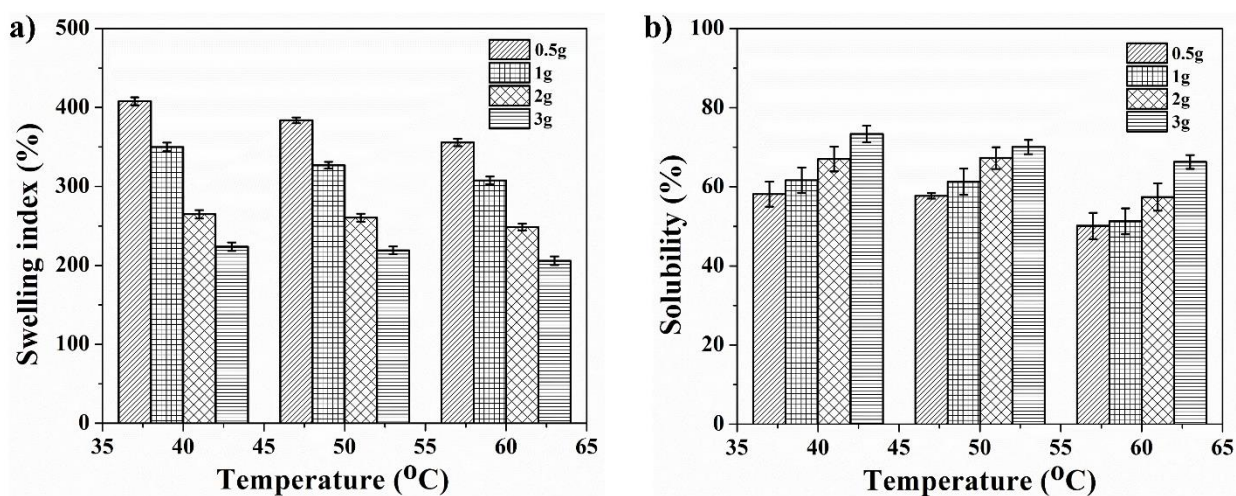


Figure 3.3: Effect of CA concentration and crosslinking temperature on the (a) swelling index and (b) solubility characteristics of PVA-St composite films.

Figure 3.3 (b) depicts the variation in solubility of PVA composite films with respect to variant CA concentration and crosslinking temperature. As shown, the solubility increases from 58.18 ± 3.16 to 73.33 ± 2.09 % for an increase in CA concentration from 0.5 to 3 g and at crosslinking temperature of 40 °C. The corresponding values varies from 57.72 ± 0.69 to 70.05 ± 1.8 % and 50.12 ± 3.29 to 66.29 ± 1.74 % for crosslinking temperature values of 50 and 60 °C respectively. Thus, for enhanced CA concentration and reduced crosslinking temperature, solubility increases and this is due to associated tradeoffs due to crosslinking and plasticizing effects. It is well known that polymer solubility reduces with increasing crosslinking effect as crosslinking minimizes interaction between polymer chains and solvent molecules and reduces their transport into the polymer matrix. However, the residual CA in the polymer film facilitates the plasticizing effect that enhances the solubility. With increasing CA concentration, free residual CA content

increases and hence solubility increases with increasing CA concentration. However, higher crosslinking temperature enhances cross-linking significantly and facilitates reduction in solubility. These mixed effects contribute to tradeoffs between diverse effects and the net effect is apparent in the observed trends. As CA serves both as a crosslinker and plasticizer in the PVA composites, it has been reported in the literature that the function of CA as either a plasticizer or crosslinker is strongly dependent on the CA concentration (Shi et al., 2008). Also, at 60 °C crosslinking temperature, solubility reduction is very high in comparison with those values obtained at 40 and 50 °C. In other words, the dominant effect of crosslinking is apparent at a highest crosslinking temperature (60 °C).

3.3.3 In-vitro degradation

The surface area of Figure 3.4 represents the hydrolytic degradation or in-vitro degradation (measured in terms of WL during 14 days of incubation in PBS (7.4 pH, at 37 °C)) of PVA composite film with time. For fixed choice of crosslinking temperature, the WL based in-vitro degradation increases with increasing CA concentration and time. For fabricated PVA composite films at 40 °C, the WL% varies 33.4 ± 3.24 to 65.63 ± 0.54 % for a variation in CA concentration from 0.5 to 3 g. Corresponding WL characteristics refer to 32.33 ± 2.21 to 63.14 ± 1.83 % and 28.14 ± 3.34 to 60.03 ± 3.3 % at 50 and 60 °C respectively. Therefore, it is apparent that higher crosslinking temperature facilitates reduction in WL due to greater crosslinking effect. Thereby, it also affirms that CA concentration has significant effect on WL in comparison with the crosslinking temperature. The time dependent trends indicate significant WL on the first day followed by gradual WL up to 3 to 4 days followed with steady WL up to 14 days for all cases. The hydrogel films that are in contact with the wound exudates partly dissolve and form soluble hydrophilic gel that protects the wound and stimulates granulation and epithelialization

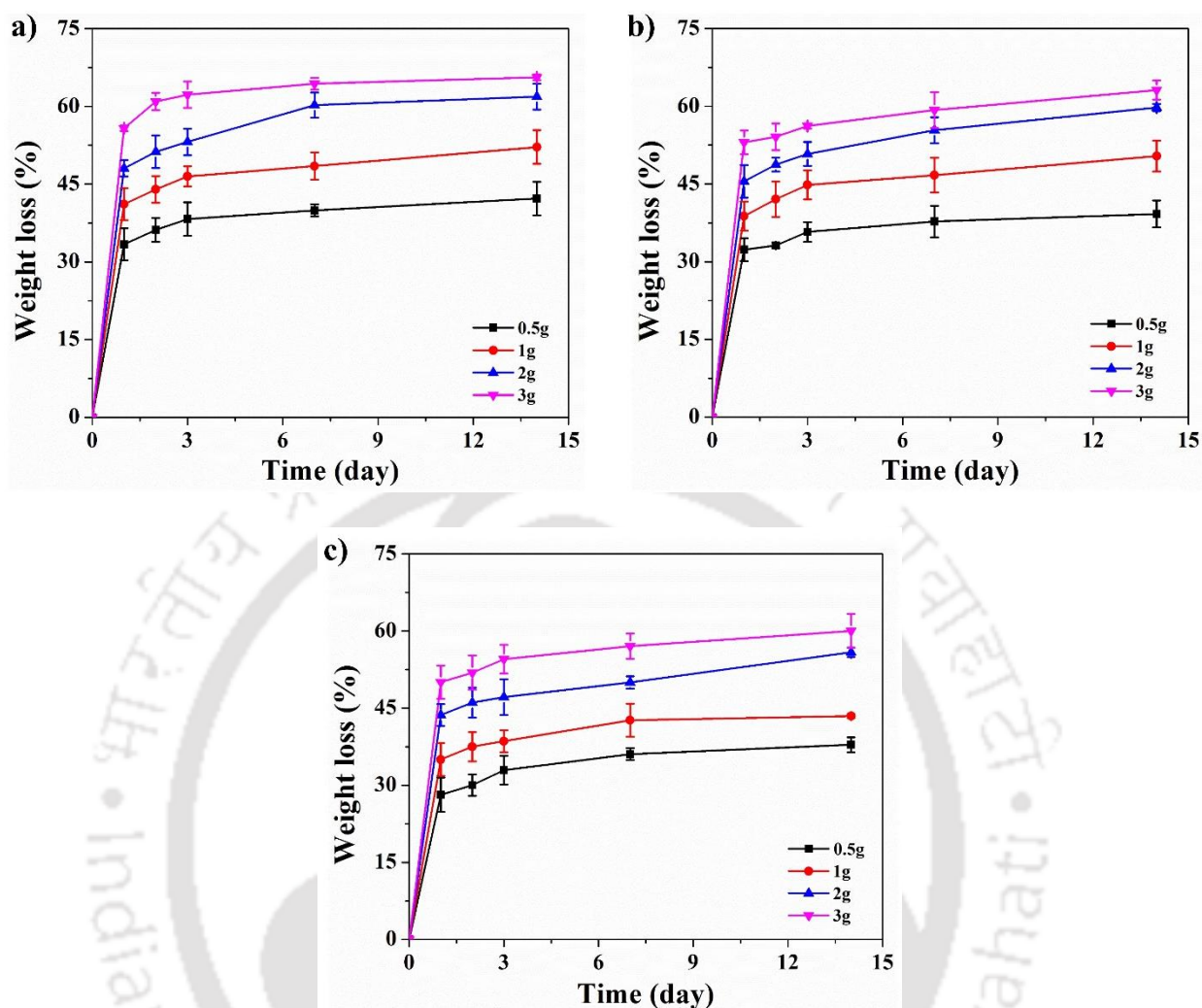


Figure 3.4: Time and CA concentration dependent in-vitro degradation characteristics of PVA-St blend films casted at (a) 40 °C, (b) 50 °C and (c) 60 °C crosslinking temperature.

(Guo and DiPietro, 2010). Subsequently, with increasing CA concentration, the structure may become porous and facilitates higher water penetration and thereby enhance the rate of hydrolytic degradation. Therefore, appropriate in-vitro degradation characteristics need to be established for wound dressing materials to ensure their technical feasibility. The obtained in-vitro degradation characteristics of PVA composite films are in good agreement with those reported in the literature. Hajian et al. (2017) confirms 50 to 60 % WL for PVA-AV (30 %)

hydrogel film and Fahmy et al. (2015) affirms an enhancement in the WL values (27 days data) from 28 to 48 % and 29 to 58 % WL for PVA-HA (20 & 30 %) and hydrogel membranes respectively (Hajian et al., 2017, Fahmy et al., 2015). This is attributed to an enhancement in the AV and HA concentration, respectively.

To a large extent, the in-vitro degradation characteristics with respect to variation in CA concentration and crosslinking temperature agrees with the trends obtained from the solubility data, as solubility refers to bioavailability/biodegradability enhancement of biocomposite materials. It is well known that biodegradability trends of wound dressing material are strongly influenced by swelling and solubility.

3.3.4 Gel fraction and mechanical strength

The variation of percentage GF with CA concentration and crosslinking temperature has been depicted in Figure 3.5. As shown, for crosslinking temperature of 40 °C, the GF reduces from 41.82 ± 3.16 to 26.67 ± 2.09 % for an increase in CA concentration from 0.5 to 3 g. Corresponding values for higher temperatures are 42.28 ± 0.69 to 29.95 ± 1.8 and 49.88 ± 3.29 to 33.71 ± 1.74 % for 50 and 60 °C, respectively. In summary, the GF value increases with crosslinking temperature and decreases with CA concentration. Also, it has been analyzed that for each CA concentration, the variation in the GF is not significant between 40 and 50 °C, but does enhance significantly at 60 °C ($p \leq 0.05$). As being elaborated in the section 3.3.2, both crosslinking and plasticizing effect are contrary to one another and since CA is both plasticizer and crosslinker, it behaves as plasticizer under certain conditions and vice-versa in certain other conditions. Due to this reason, pure crosslinking cannot be achieved with CA. In general, an enhancement in crosslinking (due to higher crosslinking agent concentration) would enhance the GF of the hydrogel films as the parameter determines the extent of crosslinking between the

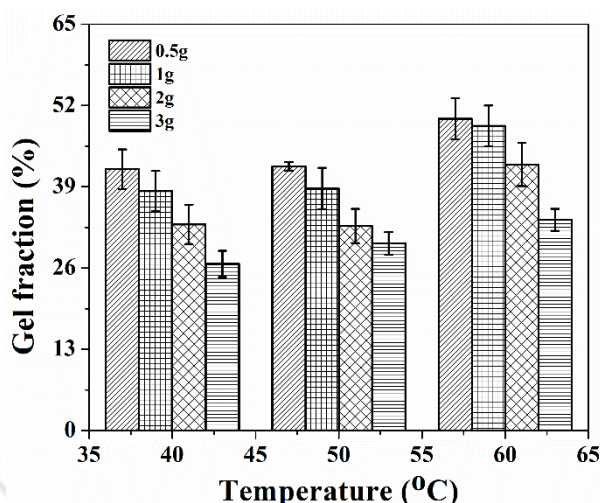


Figure 3.5: Effect of CA concentration and crosslinking temperature on the gel fraction characteristics of PVA-St films.

polymer chains. Therefore, for scenarios involving enhanced CA concentration, the GF gets reduced and thereby weakens hydrogel strength and enhances its flexibility (Ahmed et al., 2018, Fahmy et al., 2015). On the contrary, the higher drying temperature established higher extent of crosslinking in the hydrogel film matrix. Hence, the highest drying temperature (60 °C) enabled the realization of highest GF value.

The GF data of polymer films is significantly correlated with the mechanical strength of these

Table 3.3: A summary of mechanical strength characteristics of PVA-St-CA-Gl composite films.

Sample Name	A40	A50	A60	B40	B50	B60	C40	C50	C60
Tensile strength @ yield (Mpa) ¹	2.22	2.59	4.12	2.08	2.65	3.48	2.07	2.54	3.52
Tensile strain @yield (%) ²	13.29	10.43	11.04	11.61	14.43	49.69	19.34	38.55	27.12
Elastic modulus (Mpa) ²	34.62	52.47	74.44	42.54	35.03	66.35	25.21	22.13	32.41

¹ All samples were examined thrice and the overall SD value were $\leq \pm 1$

² All samples were examined thrice and the overall SD value were $\leq \pm 5$

films. Hence, the measurement of such data is relevant to gain useful insights into the optimality of the films. Table 3.3 summarizes the mechanical strength related parameters of the PVA-St-GI-CA films. As conveyed in the relevant literature, with increasing CA concentration TS at yield doesn't vary much for a fixed crosslinking temperature (Shi et al., 2008), but decreases mostly by following GF trends. This is due to the plasticizing effect of CA. It is found, at the highest crosslinking temperature as 60 °C, (rather than 40 and 50 °C) the TS at yield increases significantly, to result in an enhancement in the modulus of elasticity. This is possibly due to crosslinking through heat treatment.

3.3.5 Water vapor transmission rate

Figure 3.6 depicts the WVTR data of PVA composite films for various combinations of CA concentration and crosslinking temperature. With respect to all three crosslinking temperature (40 to 60 °C), the WVTR data trends can be analyzed to decrease initially drastically with increasing CA concentration and moderate enhancement thereafter. For crosslinking temperature of 40 °C, the WVTR reduces from 88.62 to 30.66 g/m².day initially, for an increase in CA concentration from 0.5 to 1 g; then it increases to 44.66 g/m².day for an increase in CA concentration (2 g). For an increase in CA concentration by 0.5, 1 and 2 g, resulted WVTR's are 46.56, 24.57 and 40.87 g/m².day, at 50 °C and it is 32.9, 18.55 and 33.81 g/m².day, at 60 °C. This is due to predominance of crosslinking effect in the lower to intermediate CA concentration range and greater availability of free residuals of CA in the intermediate to higher concentration range. Further, WVTR can be analyzed to reduce significantly with increasing temperature for each CA concentration case and this is due to the crosslinking effect. For the 2 g CA case, due to the availability of higher amount of residual CA, the dominance of plasticizing effect with respect to crosslinking effect is apparent to obtain a WVTR value range of 44.66 to 33.81

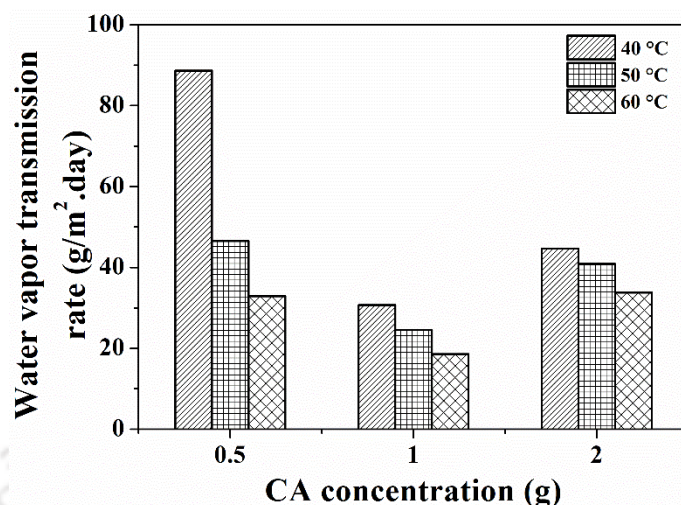


Figure 3.6: Influence of CA concentration and crosslinking temperature on the water vapor transmission rate characteristics of PVA-St films.

g/m².day.

From wound dressing applications perspective, it is well known that a favorable moisture transmission rate is required for any material to qualify as ideal wound dressing material. With high WVTR, wound dressing materials can facilitate quick transfer of water and leave the wound surface dry and under gel dehydration conditions. This leads to shrinkage of dressing material and facilitates reduction of bacterial contamination of wounds. On the other hand, materials with lower WVTR cause greater build-up of exudates and delay the healing due to the enhanced risk of bacterial growth. Ideally, WVTR has been suggested to be in the range of 2000–2500 g/m².day and more preferably close to the lower value (Ahmed et al., 2018, Queen et al., 1987). In this regard, it can be observed that the obtained WVTR values of prepared PVA composite films are significantly lower. This is due to the very reason that the data was obtained at 30 % RH and 25 °C temperature, which is not the case for literature reported measurement parameters (70 % RH and 30 °C). Due to high swelling of the PVA-St-GI-CA films and inability to achieve equilibrium value, the WVTR could not be obtained under these conditions. However, it is well

known that WVTR of PVA films increase exponentially with RH and temperature (Mo et al., 2014). Hence, the prepared PVA composite films are regarded to be appropriate for wound dressing applications due to excellent swelling propensity.

3.3.6 Antibacterial efficacy

The antibacterial activity of PVA-St-CA films with variant CA content and a control (kanamycin, 0.125 $\mu\text{g/mL}$) has been tested using *Escherichia coli* (Gram-negative bacteria) and *Listeria monocytogenes* (Gram-positive microbe). All PVA-St-CA films exhibits positive antimicrobial activity and negative bacterial growth against both Gram-positive and Gram-negative bacteria. Among all samples, the PVA composite film prepared with 2 and 3 g CA

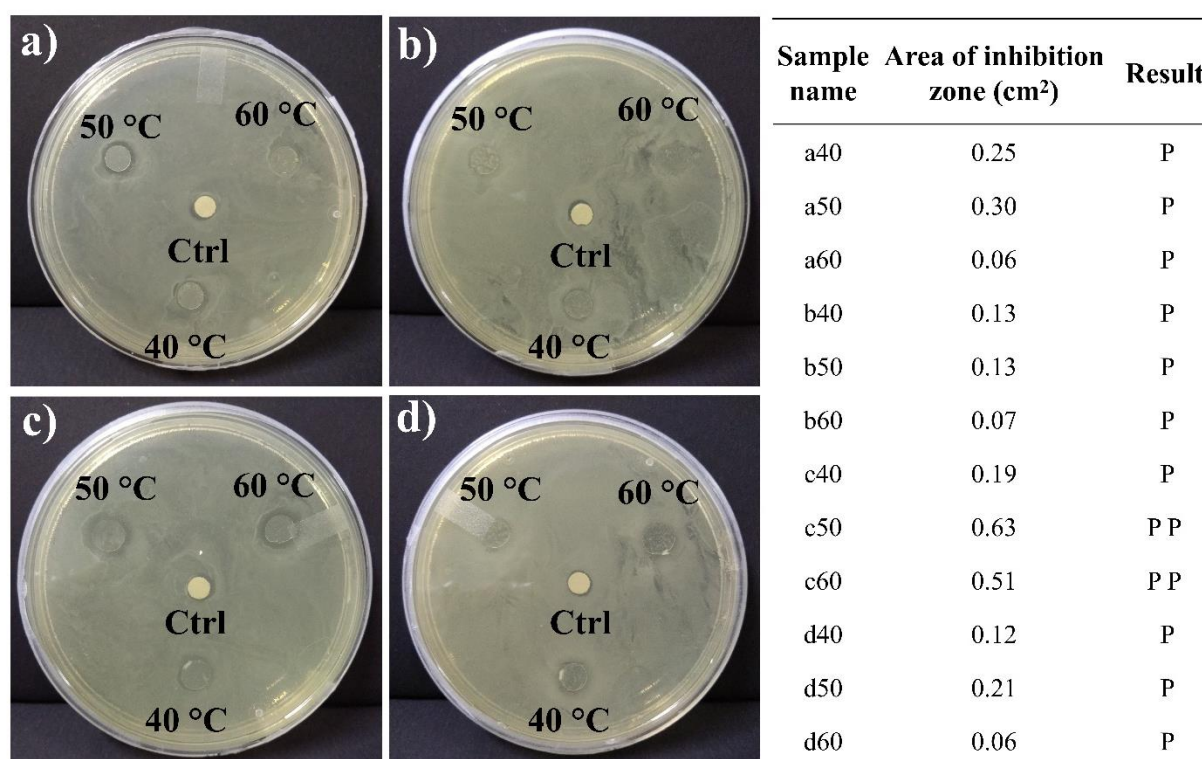


Figure 3.7: Images depicting antibacterial activity against *E. coli* of PVA-St films being casted at alternate crosslinking temperatures and with (a) 0.5 g, (b) 1 g, (c) 2 g and (d) 3 g CA solution concentration.

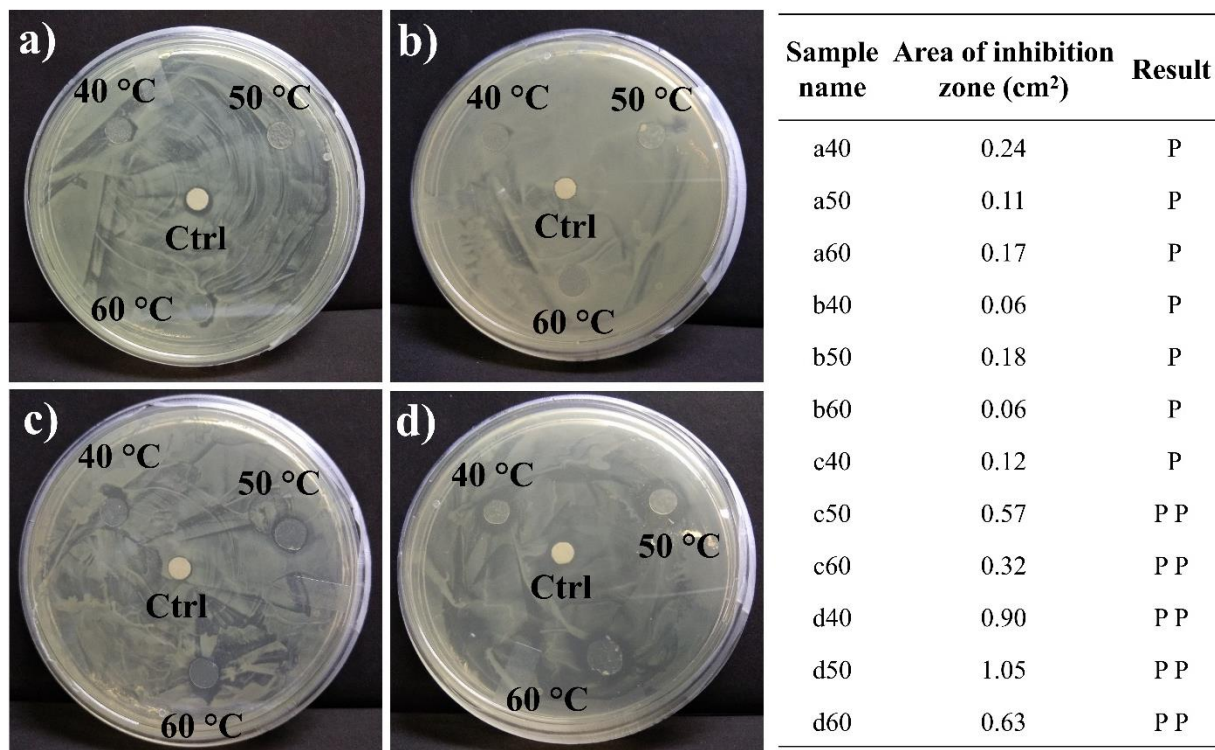


Figure 3.8: Images depicting antibacterial activity against *L. monocytogenes* of PVA-St films being casted at alternate crosslinking temperatures and with (a) 0.5 g, (b) 1 g, (c) 2 g and (d) 3 g CA solution concentration.

exhibits highly promising results as conveyed by the antimicrobial limit test. However, it is interesting to know that the PVA composite film prepared with 2 and 3 g CA facilitates upon the creation of a circular clear zone of inhibition against *L. monocytogenes* (Figure 3.8) but only with PVA film prepared with 2 g CA concentration against *E. coli* (Figure 3.7). The PVA composite film prepared with 50 °C crosslinking temperature and 2 g CA concentration proves to be the best with *E. coli*. The hypothesis behind such antibacterial activity of the film has been conveyed by Wu et al. (2017). The authors convey that the antibacterial mechanism of CA is due to its effective linkage with the cell membrane of bacteria to break down the synthesis

system of protein and cell membrane to thereby inhibit the propagation of bacteria (Wu et al., 2017).

3.4 Conceptual cost analysis

The optimally fabricated PVA-St-CA-GI composite films (225 cm² area, 50 °C drying temperature, 12 h fabrication time) have been compared in terms of retail materials and energy costs with the best available literature (Wu et al., 2017) whose composition has been followed except for the CA. The authors did not mention about the high temperature adopted during fabrication but it has been assumed to be similar to that reported by other researchers (140 °C) (Shi et al., 2008, Birck et al., 2014). Considering these, the materials cost enhances by 10 % (to 0.002 Rs./cm²) and the energy costs reduces by 35 %. The energy costs reduces significantly due to long term fabrication at lower temperature (50 °C at 12 h) compared to literature reported optimal fabrication parameters (140 °C and 4.5 h).

3.5 Comparative analysis with prior art

A comparative assessment of the best PVA composite film (obtained with 2 g CA concentration) has been conducted with the best PVA composite films available in the literature. Table 3.4 summarizes the same. It can be observed that the work of Shi et al. considered few characterization parameters. However, the authors affirms >80 % cell growth after 7 days of incubation using PVA-St-GI-CA film with 20 % CA concentration (Shi et al., 2008). For instance, Khoswanto et al. have been evaluated the effect of different concentrations of CA (40, 50 and 60 %) on cell viability using MTT and reported 95.14, 93.42 and 93.14 % cell viability, respectively (Khoswanto et al., 2008). The findings of our work indicate optimality of film

Table 3.4: A comparative summary of fabricated PVA composite film optimal characteristics and literature reported data.

S. No.	Work	Material & composition	Optimized film	Swelling	Solubility	In-vitro Degradation	Mechanical strength	WVTR	Antibacterial effectiveness
1.	This work	PVA & St each (7.88 w/w%) + Gl (37.54 wt%) + CA (8.89, 17.79, 35.58, 53.38 wt%)	PVA & St Gl-CA 35.58 wt%, 50 °C oven drying, 12 h drying time	SI: 260.5 %	67.26 %	45.51 – 59.77 %, during 14 day	Elastic modulus: 28.47 Mpa TS: 2.54 Mpa & %E: 38.55 %	40.87 g/m ² /day, at 30 % RH and 25 °C temperature	Against both gram negative and positive bacteria
2.	Wu et al. (2017)	PVA (7.88 & 10.71 w/w%)-St (3.57 & 7.88 w/w%)-CA (17.79 wt%)-Gl (33.27, 37.54 & 44.55 wt%)	PVA (7.88 w/w%)-St (7.88 w/w%)-Gl (37.54 wt%)-CA (17.79 wt%); 270 min baking	Water holding capacity: 54.31 times	45.6 %	-	Elastic modulus: 74 %, TS: 45.54 Mpa	-	Against both gram negative and positive bacteria
3.	Shi et al. (2008)	PVA + St (3:1 w/w) + Gl (20 wt%) + CA (5 – 30 wt%)	PVA-St-Gl-CA (20 wt%)	DS: 19.6 %	-	-	TS: 44 MPa and %E: 178.9 %	-	-
4.	Yoon et al. (2005)	PVA & St (5 w/w%) + Gl (10 – 50 wt%) + CA (10 – 50 wt%)	PVA & St (5%, w/w) + Gl (40 wt%)/CA (40 wt%)	DS: 200 % with CA/ 110 % with Gl	52 % with CA/34 % with Gl	-	TS: 14 MPa with CA/6 MPa with Gl; %E: 130 % with CA/100 % with Gl	-	-

characteristics for 35.58 wt% CA concentration and given the said findings on the above two literatures (Shi et al., 2008, Khoswanto et al., 2008), it is hypothesized that the prepared films are very likely to meet the cytotoxicity or cell viability assay test specifications. However, the same needs to be addressed in the near future. On the other hand, Wu et al. (2017) have been prepared PVA-St-Gl-CA composite films that are subjected to many characterization studies but not in-vitro degradation, as the authors targeted packaging applications (Wu et al., 2017). The authors concludes that fixed choice of 17.79 wt% of CA and 270 min baking time and non-specified crosslinking temperature used to yield PVA composite films with water holding capacity of 54.31 times, 45.6 % solubility, 74 MPa elastic modulus and good antibacterial activity. Compared to their work, the optimized PVA composite film from this work refers to 35.58 wt% CA, 50 °C crosslinking temperature and 12 h drying time as optimal parameters to achieve 260.5 % SI, 67.26 % solubility, 45.51 to 59.77 % in-vitro degradation (during 14 days), 40.87 g/m².day WVTR and good antibacterial activity. Further Yoon et al. (2006) fabricates PVA-St composite film (different composition) using CA or Gl (10 – 50 wt%) separately and refer to important characterization parameters (swelling, strength, solubility) for physical optimization but didn't infer applicability; thus characterization like in-vitro degradation, WVTR, antimicrobial efficiency analysis are missing (Yoon et al., 2006). With optimal (40 wt%) CA or Gl, they have achieved 200 and 110 % degree of swelling, 52 and 34 % solubility, 14 and 6 MPa TS, 130 and 100 % elongation, respectively. In comparison with their work our prepared PVA composite films provides better solubility thus better in-vitro degradation and biodegradability. Hence, the prepared PVA composite films are comparable with those in the literature and better in terms of solubility and SI.

3.6 Summary

This chapter addressed the optimality of CA concentration, crosslinking temperature and drying time during solution casting based laboratory fabrication of PVA-St-GI-CA films and thereby following points can be summarized.

- Optimality of process conditions indicates 35.58 wt% CA, 50 °C crosslinking temperature and 12 h drying time.
- Fabricated PVA composite films possessed significantly higher SI (260.5 ± 5.04 %), higher solubility (67.26 ± 2.74 %), lower elastic modulus, very good in-vitro degradation (59.77 ± 0.71 % after 14 days), good antibacterial activity and are excellent materials to serve for wound dressing applications.
- Compared to the best available prior art (Shi et al., 2008, Wu et al., 2017, Birck et al., 2014), the optimized formulation demonstrated the possibility to reduce energy costs by 35 % to achieve 10 % expensive films towards wound dressing applications.

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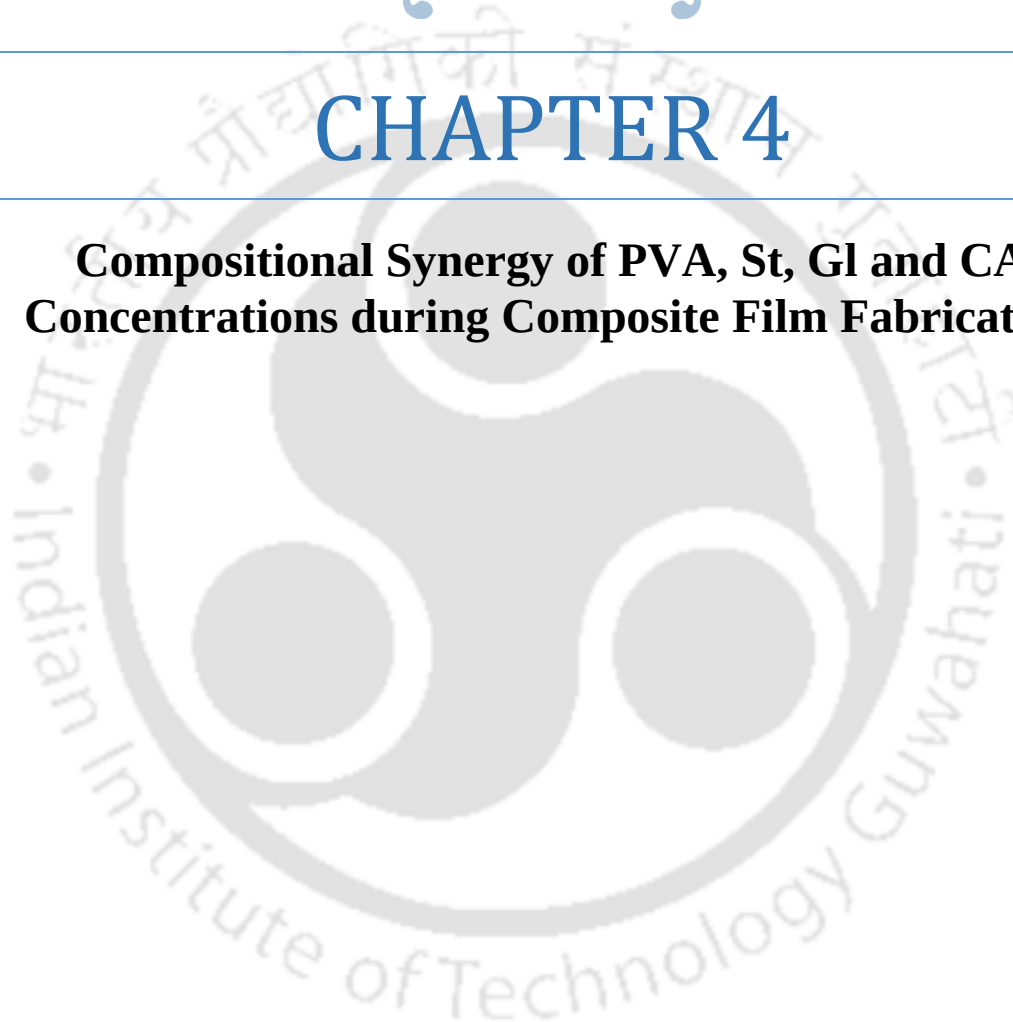






CHAPTER 4

**Compositional Synergy of PVA, St, Gl and CA
Concentrations during Composite Film Fabrication**





Compositional Synergy of PVA, St, Gl and CA

Concentrations during Composite Film Fabrication

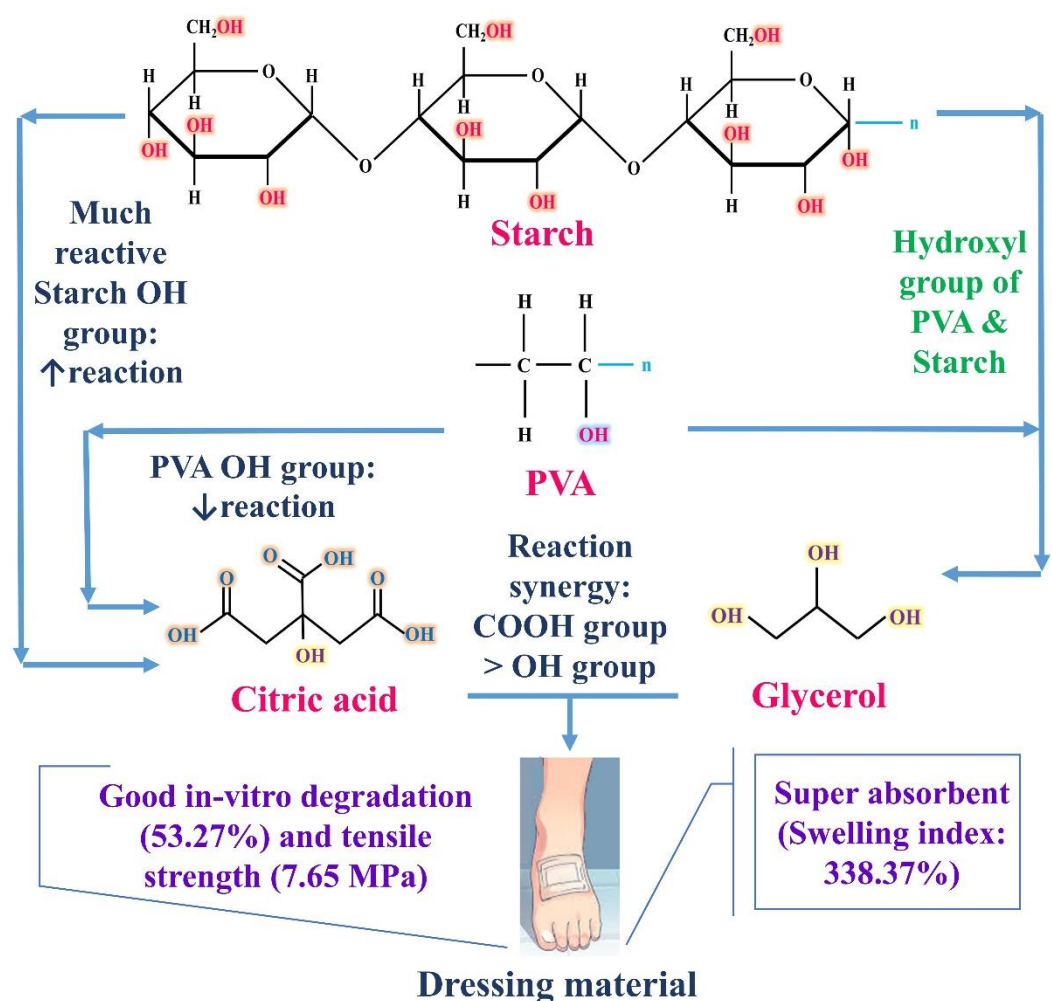
This chapter elaborates upon the findings and discussion related to the performance of statistical design approach based alternate quaternary formulations and thereby achieves optimally fabricated wound dressing compatible PVA composite films. Adequate variations in PVA, St, Gl and CA constitution indicated highly complex interactions for the desired set of response characteristics. After presenting a brief introduction in section 4.1, the chapter devotes towards criticality of response characteristics associated with the SI, in-vitro degradation and TS in sections 4.2, 4.3 and 4.4 respectively. Each of these sections delineates upon best fit model being identified to represent response characteristics and associated response surface plots to visualize upon the sensitivity and criticality of the constituent compositions. Thereafter, sections 4.5 and 4.6 respectively delineate upon experimental validation of optimal quaternary formulations and comparative analysis with best reported literature data. Finally, section 4.7 summarizes the key findings associated with the mentioned objective in the thesis[#].

Overview

RSM design based investigations has been done to obtain useful insights on the compatibility and synergy of quaternary formulations [variant concentrations of St, PVA, CA and Gl]. The design studies inferred that while SI and in-vitro degradation followed quadratic expressions with respect

[#] Published article: Das, A., R. Uppaluri and C. Das (2020). "Compositional synergy of poly-vinyl alcohol, starch, glycerol and citric acid concentrations during wound dressing films fabrication." International Journal of Biological Macromolecules **146**: 70-79.

to variations in the quaternary compositions, the TS was relatively less complex with bilinear and linear terms of the independent variables. Experimentally investigated optimized film (composition: 5 w/w% PVA, 10 w/w% St, 15 wt% CA and 15 wt% Gl) exhibited excellent SI (338.37 %), acceptable in-vitro degradation (53.27 %) and higher TS (7.65 MPa).



4.1 Introduction

This chapter targets compatibility and synergy issues of PVA, St, CA and Gl in due course of the fabrication of solution casting based PVA-St composite films. Thereby, the central objective of such experimental investigations was to improvise upon the composite film characteristics through

a rigorous optimization of the constituent concentrations. The specific novelty of such an approach is with respect to the complex interaction of these constituents that facilitate substantial improvement in the optimality of the film constitution in comparison with the non-mathematical and trial and error based approaches adopted in the most relevant literature. A critical insight with respect to such literature reported methodologies can be found in section 1.5.2.1. Design expert software was deployed to design the experimental runs using the CCD approach and thereby achieve statistically tuned PVA composite films with the variant constitution of PVA, St, Gl and CA. Thus, the CCD based statistical design involved the variation of four mentioned macromolecular constituents as independent variables. Consequently, wound dressing compatible composite film characteristics such as SI, in-vitro degradation or WL, and TS have been chosen as the dependent variables. For each desired response, best fit models were being identified using the ANOVA approach. Thereafter, surface response plots have been prepared for each response as a function of any two constituent concentrations. Thereby, the plots were being analyzed to gain useful insights into possible directions for compositional optimality in the highly complex quaternary formulation based alternate PVA composite films. Finally, the software assisted optimal PVA composition was identified based on relevant bias being set as follows: excellent SI (260 %) (Ahmed et al., 2018), acceptable in-vitro degradation (48 % WL after 27 days) (Fahmy et al., 2015) and improved TS value (Basiak et al., 2018). The bias corresponds to a minimum desired set of wound dressing film characteristics. These have been delineated in the available prior art.

4.2 Influence of macromolecular constitution on the swelling index

4.2.1 Best fit model

A second order polynomial equation was found to best represent the obtained average SI data and for given constraints. The relevant expression is presented as follows:

$$SI = +260.78 - 20.97A - 26.65B - 46.10C - 51.93D + 22.12AB + 29.70AC + 1.84AD + 10.29BC + 5.82BD + 9.78CD - 13.95A^2 - 13.84B^2 + 9.83C^2 - 0.14D^2 \quad (7)$$

where, A , B , C and D correspond to the independent variables namely PVA, St, CA and Gl concentration, respectively and SI is the swelling index response variable. In the above expression, positive parametric values indicate that the dependent variable enhances SI and negative values indicate a vice-versa trend. From Table 4.1, it can also be analyzed that the data set numbered 15 refers to the best set among experimental investigations. This is due to maximum SI (PVA 5 w/w%, St 5 w/w%, features namely insignificant p -value (<0.0001) and high F value (35.33)) (Table 4.2 b). The corresponding regression coefficient value was high and affirms 97.06% response variability.

4.2.2 Response surface plots

From experimental investigations, the maximum and minimum SI have been obtained as 473.93 and 114.15 %, respectively (Table 4.1) for the independent variable range of PVA, St, CA and Gl concentration as 2.5 – 12.5 w/w%, 2.5 – 12.5 w/w%, 2.5 – 52.5 wt% and 2.5 – 52.5 wt%. These values are in agreement with the available guidelines from the literature that affirmed the SI value to be over and above 260 % for a wound dressing film with superior fluid sorbing characteristics (Ahmed et al., 2018). Also, while all four constituents reduced SI, the effect was significant for CA and Gl. Among binary interactions, PVA-St and PVA-CA interactions had a predominant

Table 4.1: A summary of central composite design based experimental data of PVA-St-CA-Gl composite films.

S. no.	A: PVA concentration (w/w%)	B: St concentration (w/w%)	C: CA concentration (wt%)	D: Gl concentration (wt%)	Response 1: Swelling index (%)	Response 2: In-vitro degradation (%)	Response 3: Tensile strength (MPa) ¹
1	7.5	7.5	27.5	27.5	261.48±4.83	53.04±1.39	5.12
2	7.5	7.5	27.5	27.5	218.74±4.96	49.89±2.92	4.61
3	10	5	15	40	184.63±4.63	34.65±1.06	8.5
4	7.5	7.5	27.5	2.5	371.11±4.3	44.41±2.19	5
5	7.5	7.5	2.5	27.5	398.45±4.84	31.09±0.48	9.35
6	5	5	15	40	338.94±4.82	38.61±3.9	6.55
7	7.5	7.5	27.5	27.5	297.88±4.79	53.14±4.43	5.31
8	5	10	40	40	114.15±4.69	71.72±2.79	2.14
9	5	10	15	15	338.37±4.95	53.27±2.77	7.65
10	7.5	12.5	27.5	27.5	170.14±4.43	58.07±1.31	3
11	10	10	15	15	258.95±4.98	34.19±2.07	6.53
12	10	10	40	15	237.64±4.76	52.3±1.3	1.77
13	10	10	15	40	167.89±4.7	43.7±4.43	5.01
14	10	5	15	15	317.58±4.93	29.34±1.03	9.93
15	5	5	15	15	473.93±4.23	32.43±2.68	6.41
16	2.5	7.5	27.5	27.5	229.32±4.85	65.79±4.79	3.38
17	5	10	15	40	218.06±4.61	60.14±4.99	8.01
18	10	5	40	15	248.69±4.19	45.15±4.9	4.77
19	12.5	7.5	27.5	27.5	180.39±4.78	39.28±4.92	5.73
20	7.5	7.5	52.5	27.5	201.497±3.94	59.69±0.93	0.54

¹ All samples were examined thrice and the overall SD value were $\leq \pm 1$

S. no.	A: PVA concentration (w/w%)	B: St concentration (w/w%)	C: CA concentration (wt%)	D: Gl concentration (wt%)	Response 1: Swelling index (%)	Response 2: In-vitro degradation (%)	Response 3: Tensile strength (MPa) ¹
21	7.5	7.5	27.5	52.5	149.01±4.95	48.11±1.34	3.81
22	7.5	2.5	27.5	27.5	240.4±4.82	37.13±1.86	7.85
23	7.5	7.5	27.5	27.5	260.09±3.8	56.33±0.87	4.04
24	5	10	40	15	188.59±4.79	68.74±1.34	1.1
25	5	5	40	40	193.34±4.93	55.55±3.76	2.17
26	7.5	7.5	27.5	27.5	260.52±4.94	55.9±2.33	4.51
27	5	5	40	15	279.39±4.95	53.93±6.54	2.78
28	10	5	40	40	155.08±4.78	45.6±1.18	4.37
29	7.5	7.5	27.5	27.5	265.99±4.95	56.38±2.8	3.47
30	10	10	40	40	168.93±4.93	51.55±4.92	1.35



Table 4.2: (a) Alternate model fitness and (b) ANOVA parameters of all responses associated to the CCD-RSM based PVA-St-CA-Gl composite film fabrication.

(a)

Source	Swelling index					In-vitro degradation					Tensile strength				
	Sum of squares	df	Mean square	F value	p-value prob > F	Sum of squares	df	Mean square	F value	p-value prob > F	Sum of squares	df	Mean square	F value	p-value prob > F
Mean vs Total	1.82E+06	1	1.82E+06			72929.68	1	72929.68			698.18	1	698.18		
Linear vs Mean	1.43E+05	4	35831.19	19.59	< 0.0001	3139.29	4	784.82	40.38	< 0.0001	154.42	4	38.6	40.53	< 0.0001
2FI vs Linear	25755	6	4292.5	4.08	0.0085	190.6	6	31.77	2.04	0.1094	15.15	6	2.52	5.54	0.0018
Quadratic vs 2FI	14401.58	4	3600.4	9.71	0.0004	234.61	4	58.65	14.48	< 0.0001	1.99	4	0.5	1.12	0.3839
Cubic vs Quadratic	2379.69	8	297.46	0.65	0.7186	24.99	8	3.12	0.61	0.7477	4.17	8	0.52	1.46	0.3151
Residual	3184.64	7	454.95			35.76	7	5.11			2.5	7	0.36		
Total	2.01E+06	30	66968.38			76554.93	30	2551.83			876.41	30	29.21		

(b)

Swelling index						In-vitro degradation					Tensile strength				
Source	Sum of squares	df	Mean square	F value	p-value > F	Sum of squares	df	Mean square	F value	p-value > F	Sum of squares	df	Mean square	F value	p-value > F
Model	1.84E+05	14	13105.81	35.33	< 0.0001	3564.5	14	254.61	62.87	< 0.0001	169.57	10	16.96	37.2	< 0.0001
A-PVA	10552.04	1	10552.04	28.45	< 0.0001	949.22	1	949.22	234.39	< 0.0001	4.27	1	4.27	9.37	0.0064
B-St	17042.31	1	17042.31	45.94	< 0.0001	842.99	1	842.99	208.16	< 0.0001	19.45	1	19.45	42.68	< 0.0001
C-CA	51010.57	1	51010.57	137.51	< 0.0001	1281.86	1	1281.86	316.53	< 0.0001	129.55	1	129.55	284.24	< 0.0001
D-GI	64719.84	1	64719.84	174.47	< 0.0001	65.22	1	65.22	16.11	0.0011	1.14	1	1.14	2.5	0.13
AB	7825.8	1	7825.8	21.1	0.0004	134.15	1	134.15	33.12	< 0.0001	12.08	1	12.08	26.5	< 0.0001
AC	14109.47	1	14109.47	38.04	< 0.0001	10.21	1	10.21	2.52	0.1332	0.46	1	0.46	1	0.3289
AD	54.29	1	54.29	0.15	0.7074	0.61	1	0.61	0.15	0.7025	1.37	1	1.37	3.02	0.0986
BC	1693.26	1	1693.26	4.56	0.0495	9.28	1	9.28	2.29	0.1509	0.78	1	0.78	1.72	0.2052
BD	541.62	1	541.62	1.46	0.2456	1.59	1	1.59	0.39	0.5409	0.19	1	0.19	0.42	0.5225
CD	1530.57	1	1530.57	4.13	0.0603	34.77	1	34.77	8.58	0.0103	0.26	1	0.26	0.57	0.4586
A ²	5334.54	1	5334.54	14.38	0.0018	3.47	1	3.47	0.86	0.369					
B ²	5254.87	1	5254.87	14.17	0.0019	69.32	1	69.32	17.12	0.0009					
C ²	2652.53	1	2652.53	7.15	0.0173	125.88	1	125.88	31.08	< 0.0001					
D ²	0.57	1	0.57	1.53E-03	0.9693	101.56	1	101.56	25.08	0.0002					
Residual	5564.33	15	370.96			60.75	15	4.05			8.66	19	0.46		

Source	Swelling index					In-vitro degradation					Tensile strength				
	Sum of squares	df	Mean square	F value	p-value > F	Sum of squares	df	Mean square	F value	p-value > F	Sum of squares	df	Mean square	F value	p-value > F
Lack of fit	2392.07	10	239.21	0.38	0.9112	27.57	10	2.76	0.42	0.8889	6.34	14	0.45	0.97	0.5607
Pure error	3172.25	5	634.45			33.18	5	6.64	62.87		2.32	5	0.46	37.2	
Cor total	1.89E+05	29				3625.25	29				178.23	29			not significant

effect. Among squared terms of independent variables, except for CA, the three other terms affirmed a similar negative effect as indicated by the individual variable terms. The square term of CA had a positive effect and this is due to the suppression of crosslinking characteristic with its plasticizing characteristic.

The three dimensional response surface graphs of SI with respect to any two independent variables (PVA, CA, St and Gl) have been depicted in Figure 4.1. The second order quadratic expression presented for the case refers to the elliptical response in the entire region for associated interactions of PVA-St, PVA-CA, PVA-Gl, St-CA, St-Gl, and CA-Gl. From the response surface plots, the following can be analyzed to be key observations with respect to the binary interactions:

- a) For the PVA-St case, the SI reduced significantly with an enhancement in both PVA and St. This is due to an increment in polymer concentration in the polymer matrix that enhances crosslinking and reduces porosity and SI (Ahmed et al., 2018). Also, for the case of either the highest PVA content or St content case, a marginal reduction in SI can be observed for a corresponding increase in St or PVA content.

For the PVA-CA interaction case, the PVA concentration did not influence the SI at higher CA concentration. However, for lower CA concentrations, an enhancement in the PVA concentration indicated a detrimental influence upon the SI. Also, for both lower and higher PVA concentrations, an increment in CA content indicated detrimental influence upon the SI. In this regard, a more prominent reduction can be observed for lower PVA concentration cases. Similar trends are applicable for the St-CA interaction case. However, for both lower and higher CA concentrations, the SI reduces with an increase in St content. This is due to the reason that the St participates more easily in the esterification reaction than the PVA (Shi et al., 2008). Thereby, CA's crosslinking effect with PVA or St has a

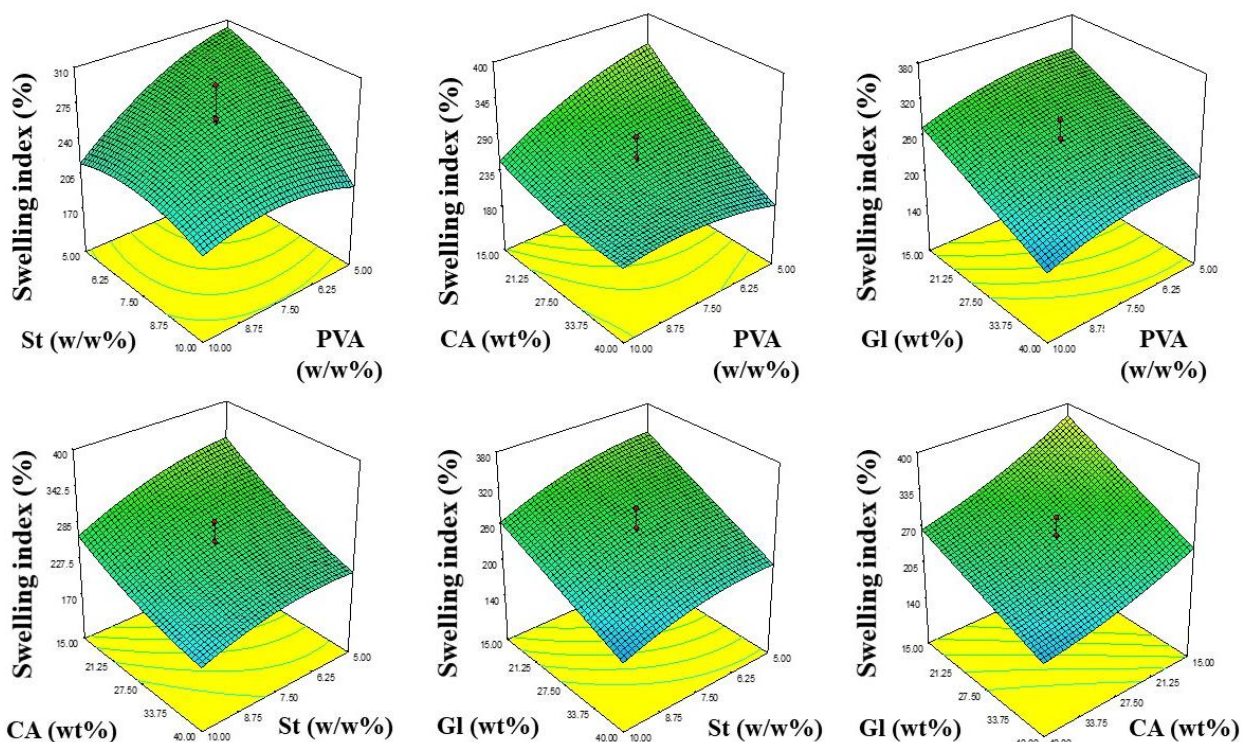


Figure 4.1: Response surface plots of swelling index characteristics of CCD based PVA-St-CA-Gl composite films.

role in the esterification reaction.

- b) For both scenarios of PVA-Gl and St-Gl interactions, Gl enables a significant reduction in the SI. Also, for the CA-Gl interaction case, the SI reduction sharp with respect to Gl concentration and thereby affirms that in comparison with the CA, Gl prominently influences the SI. In this regard, the relevant prior art confirms that for an increase in CA in the chosen case, the SI remains fairly constant in due course of reaching a specific intermediate concentration (Yoon et al., 2006). Three (multiple) –COOH groups prevalent in the CA facilitate its key functionality as a crosslinking agent. On the other hand, it shall be noted that the three –OH groups in the Gl facilitate the formation of hydrogen bonds with the corresponding –OH groups of the PVA and St. Compared to the –OH groups of

GI, the –COOH groups enable the formation of much stronger hydrogen bonds with the PVA-St –OH groups. However, with increasing CA concentration, the free CA content available in the polymer matrix increases. Thereby, the additional free –COOH groups have a strong tendency to bond along with the water molecules. For the case of excess CA, due to possessing three –COOH and one –OH group in its molecule, the macromolecule will have the edge and strong affinity to bond along with the water molecule. This is not the case for the GI due to it possessing only three –OH groups. Thus, the cases corresponding to enhanced CA concentration (i.e., points located after the central point) will facilitate significant plasticization effect. This is due to its reduced crosslinking ability under mentioned circumstances (Shi et al., 2008).

4.3 Influence of macromolecular constitution on the in-vitro degradation

4.3.1 Best fit model

Based on CCD design and model fitness studies, the in-vitro degradation or WL response variable has been found to follow a quadratic expression with respect to variations in the independent variables. The relevant expression is presented as follows:

$$WL = +54.11 - 6.28A + 5.93B + 7.30C + 1.64D - 2.88AB - 0.81AC - 0.21AD - 0.77BC + 0.30BD - 1.46CD - 0.35A^2 - 1.59B^2 - 2.14C^2 - 1.92D^2 \quad (8)$$

Among all measured data sets, the data set numbered 8 indicated the highest weight loss percentage. The set corresponds to 5 w/w% PVA, 10 w/w% St, 40 wt% CA and 40 wt% GI (Table 4.1). Model fitness parameters refer to high significance (p -value <0.0001), high F value (62.87) (Table 4.2 b) along with an R^2 value being close to 1 (0.9832). All these affirm a very good degree of fitness of the quadratic model.

4.3.2 Response surface plots

Figure 4.2 depicts the contour plots and corresponding response surface plots for in-vitro degradation or WL with respect to variations in any two independent variable concentrations (PVA, St, CA, and Gl). In general, the variable varied from 31.09 – 71.72 % during 27 days of in-vitro degradation period (Table 4.1) for a change in PVA, St, CA and Gl concentration as 2.5 – 12.5 w/w%, 2.5 – 12.5 w/w%, 2.5 – 52.5 wt% and 2.5 – 52.5 wt%. In this regard, for 27 days duration, Hajian et al. (2017) and Fahmy et al. (2015) indicated that the in-vitro degradation variable varied as 50 – 60 % for 30 % AV-PVA and 28 – 58 % for 20 & 30 % HA-PVA composite membranes, respectively (Hajian et al., 2017, Fahmy et al., 2015). Such WL characteristics have been considered to be a promising value range for a wound dressing material. Compared to such literature reported data for relevant wound dressing materials, the fabricated PVA composite films exhibited comparable and better WL values. Henceforth, the optimized films reported in this chapter applicable towards wound dressing applications.

The WL enhanced significantly with increasing CA and St concentrations. However, the variable increased normally with increasing Gl concentration. On the contrary, PVA had a significantly negative effect on the WL characteristics. Excluding St-Gl interaction, the interactions of any among four independent variables indicated a detrimental effect on the WL. Among these, the PVA-St interaction indicated the most prominent effect. Among squared terms of the independent variables, a negative effect exists for all cases. Except for PVA, the binary interactions of all other constituents had a comparatively negative effect in comparison with their single terms on the WL. The St square term indicates higher polymer concentration which in turn facilitates higher crosslinking (Ahmed et al., 2018) and hence detrimental WL characteristics of the composite film. The elliptical response surface graphs for variation in WL (in-vitro degradation) with respect to

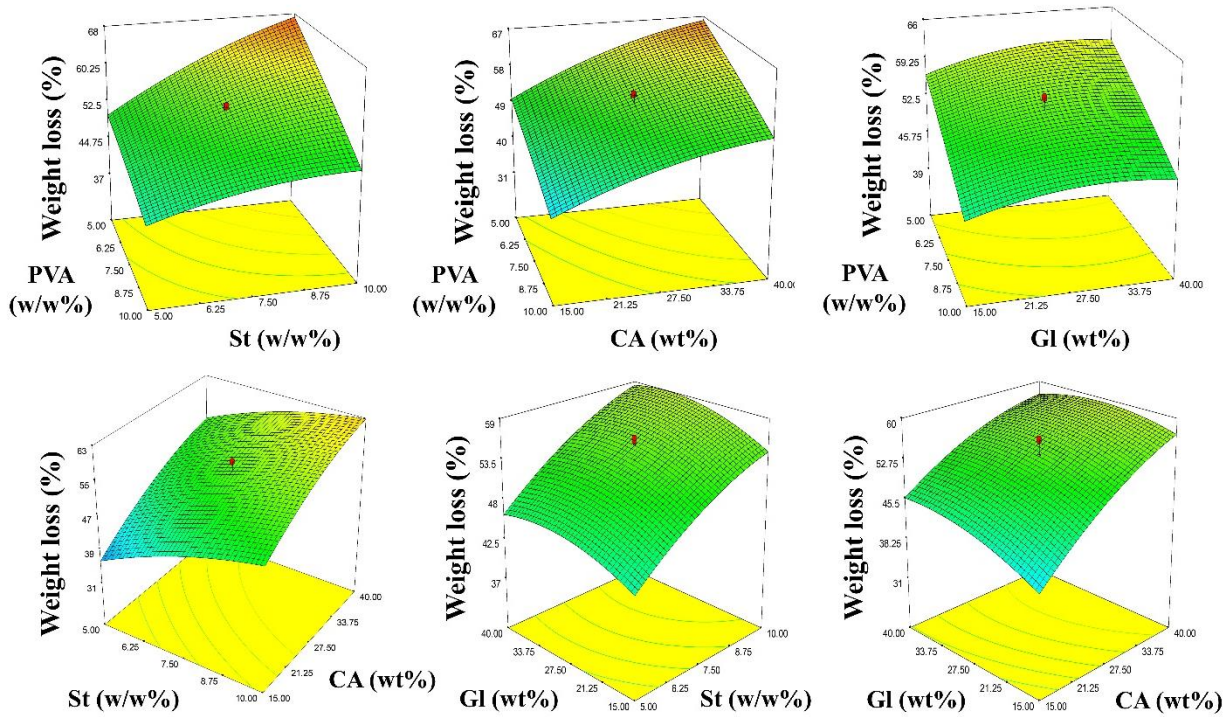


Figure 4.2: Response surface plots of in-vitro degradation characteristics of CCD based PVA-St-CA-Gl composite films.

any two independent variables (PVA-St, PVA-CA, PVA-Gl, St-CA, St-Gl, and CA-Gl) have been depicted in Figure 4.2. Relevant key findings with respect to these binary interactions can be summarized as follows:

- For the PVA-St case, WL reduced with an increase in the PVA concentration. This is due to an enhancement in the solid content of the polymer matrix. On the other hand, since St is a degradable natural polymer, its enhancement in the film matrix enhanced the WL (Raj and Somashekar, 2004). Also, the WL increment was higher for the cases corresponding to higher St and lower PVA concentrations.
- For PVA-CA and PVA-Gl interaction cases and for both lower and higher PVA concentrations, an increment in CA or Gl content fostered WL to increase. However, for

lower PVA concentrations, significantly higher WL occurred. This is due to the presence of lesser PVA content. Moreover, for higher PVA concentration and lower additives (Gl or CA) concentration, lower WL were achieved for CA but not Gl. This is due to the reason that the -COOH groups of CA form stronger hydrogen bonds and thereby facilitate better intermolecular interaction rather than the -OH group of Gl (Yoon et al., 2006, Shi et al., 2008). Also, for lower PVA concentrations, it can be analyzed that the enhancement in the WL was significant for the CA but not the Gl increment case. This is because higher CA concentration fosters enhancement in the residual free CA (Shi et al., 2008). Thereby, such films possess a higher constitution of groups having affinity towards water. In other words, both solubility and plasticizing effects enhance for such cases.

- c) For St-CA and St-Gl interaction cases, it can be analyzed that the WL enhances with increasing concentrations of both St and CA. Similar trends exist with respect to St-Gl interaction. However, among both cases, value increment is highest for higher combinations of CA and St. For the St-CA interaction case, the WL increment is significant for CA but not St. However, for the St-Gl interaction case, the WL profile for St increment resides better than the corresponding profile being obtained with respect to Gl. The regressed expression is in good agreement with the hypothesis that for WL, the contributing factors follow the order of $\text{CA} > \text{St} > \text{Gl}$. However, deeper analysis and comparison of PVA-CA and St-CA interaction cases indicates that the WL for both lower PVA and CA concentrations is higher than the WL value being obtained for lower St and the entire range of the CA concentration. In this regard, it can be analyzed that the CA reacts more easily with the St but not the PVA (Shi et al., 2008). Hence, crosslinked St has an improved networking structure to foster lower WL. Also, for lower PVA and higher CA

concentrations case, the WL was lower than the corresponding value being obtained for higher combinations of St and CA. This is possibly due to the greater availability of free residual CA (due to lesser PVA interaction and greater CA constitution) and hence fosters better WL.

- d) For the CA-Gl interaction case, both CA and Gl assist to enhance WL. However, among these two, CA has the edge to assist WL. For higher CA concentration, an enhancement in Gl fostered higher WL initially and subsequent reduction. This may be due to the formation of glycerol citrates at higher CA concentrations due to the partial reaction of CA with Gl (Shi et al., 2008).

4.4 Influence of macromolecular constitution on the tensile strength

4.4.1 Best fit model

For the obtained data, the RSM-CCD based model fitness studies affirmed the validity of the following expression to represent the tensile strength response variable of the fabricated PVA composite films:

$$TS = +4.82 + 0.42A - 0.90B - 2.32C - 0.22D - 0.87AB + 0.17AC - 0.29AD - 0.22BC + 0.11BD + 0.13CD \quad (9)$$

Thus, it is apparent that in contrast to SI and WL responses, the TS involved bilinear and linear terms of the independent variables *A*, *B*, *C* and *D*. Hence, the model complexity for the TS is relatively inferior in comparison with the best fit models representing SI and WL responses. Table 4.1 summarizes the relevant data. As per the mentioned data sets, the best set corresponds to a composite film (10 w/w% PVA, 5 w/w% St, 15 wt% CA and 15 wt% Gl) possessing 9.93 MPa TS. The best fit regression model corresponds to the two function interaction model with high

significance (p -value <0.0001) and high F value (37.2) indicates model adequacy in terms of appropriate fitness (Table 4.2 b). ANOVA indicated a determination coefficient of 0.9514 and thereby affirmed 95.14 % fitness of the response variable in terms of statistical analysis.

4.4.2 Response surface plots

Corresponding contour plots and response surface plots for the TS variation with any two independent variables have been depicted in Figure 4.3. From experimental investigations, the obtained value range of TS varied from 9.93 – 0.54 MPa (Table 4.1) for the independent variable range of PVA, St, CA and Gl concentration as 2.5 – 12.5 w/w%, 2.5 – 12.5 w/w%, 2.5 – 52.5 wt% and 2.5 – 52.5 wt%. From the response surface plots, it can be analyzed that the TS was enhanced due to PVA variations. However, the response reduced with an increment in St, CA and Gl and especially CA. The binary interactions indicated that the interactions between PVA-CA, St-Gl, and CA-Gl had a positive influence upon the TS. On the contrary, PVA-St, PVA-Gl and St-CA interactions indicated a negative influence on the TS. Among all binary interactions, the PVA-St interaction had a prominent influence on the TS of the PVA composite film. Notable inferences from the response surface plots of TS characteristics are as follows:

- a) With respect to PVA-St interactions and for lower St concentrations, an increase in PVA enhanced TS. However, for higher St concentrations and marginal enhancement in the PVA, the TS values were reduced. For higher PVA concentration cases, the TS reduced rapidly with St concentration (Raj and Somashekar, 2004). This is due to the reason that the St additive in the PVA matrix has been detrimental towards the physical properties (TS) of the blend. The poor compatibility of PVA and St are responsible for such characteristics. Thus, the best TS value has been obtained for the highest and lowest content of PVA and St respectively. Similar reasoning is applicable for higher and lower content of St and PVA

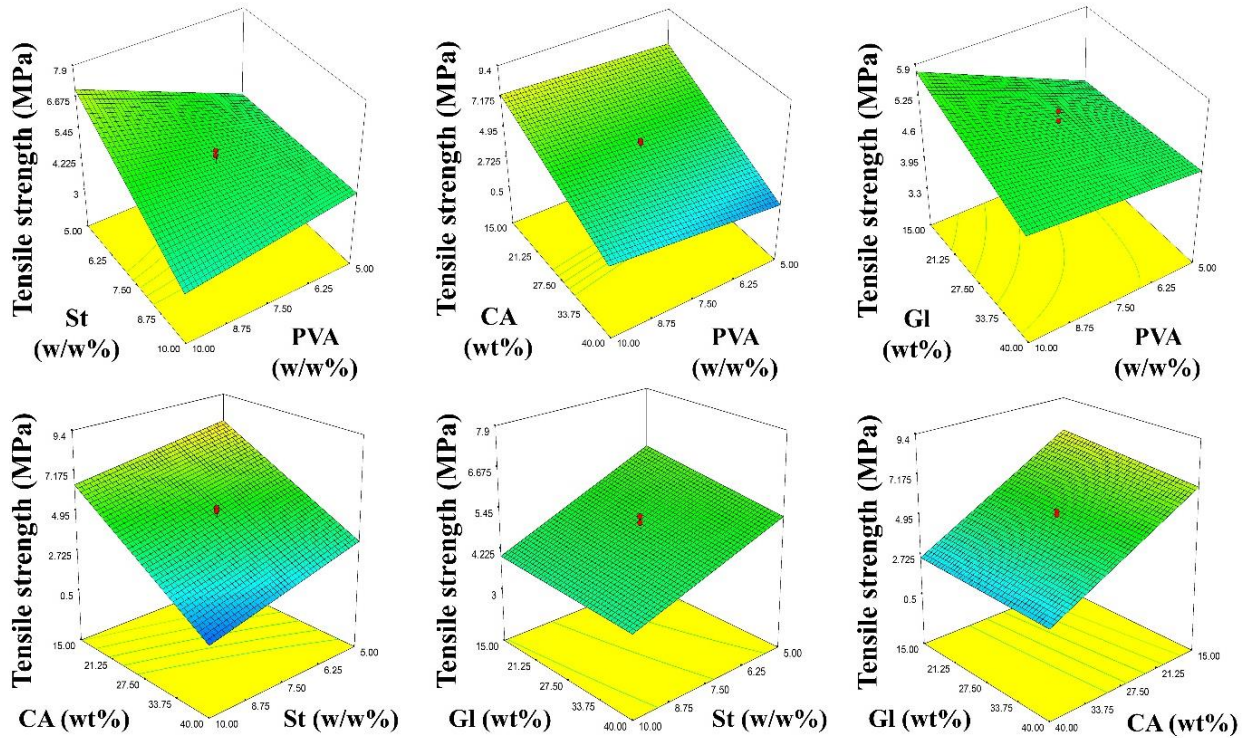


Figure 4.3: Response surface plots of tensile strength characteristics of CCD based PVA-St-CA-Gl composite films.

respectively.

- b) For PVA-CA interactions and both cases of lower and higher PVA concentrations, the TS was reduced with increasing CA content. On the other hand, for both lower and higher CA concentrations, the TS increased marginally with increasing PVA content.
- c) For the St-CA interactions and both cases of lower and higher CA concentrations, the TS reduced with increasing St concentrations. For lower combinations of St and CA concentrations, higher TS values have been obtained in comparison with lower combinations of PVA and CA concentrations. This has been hypothesized with the following statement. CA crosslinked St facilitates better networking structure and thereby

- improvises upon TS along with WL characteristics (Shi et al., 2008). However, with increasing CA, a rapid reduction in TS can be analyzed which was not the case for the Gl.
- d) For the PVA-Gl interaction case and lower Gl concentration, an enhancement in the PVA increased the TS characteristics. However, for the high Gl concentration case, the TS variation has been marginal. Similarly, for a high PVA concentration case, an increment in the Gl had a detrimental influence upon the TS. However, for low PVA content cases, such influence of Gl was marginal. Also, a comparative assessment of PVA-CA and PVA-Gl interactions affirmed that lower PVA and CA concentrations increased TS in comparison with the TS trend obtained for lower PVA and Gl combinations. This is in accordance with the available literature (Shi et al., 2008). Also, for the CA-Gl interaction case, the influence of Gl is marginal with CA concentration predominantly contributing towards the TS variation effect.
- e) For the St-Gl interaction case, the influence of Gl is marginal. Also, as observed in several cases, an increase in the St concentration reduced the TS. As St has a detrimental effect towards the mechanical properties of the PVA-St composite.

4.5 Experimental design based optimality of quaternary compositions

For each response (independent variables), data sets numbered 15, 8 and 14 refer to the highest values of SI, WL and TS respectively. However, in due course of consideration of the maximization of all three mentioned responses, it can be analyzed that the statistical design based experimental formulations are unable to indicate any data set. In this regard, it can be analyzed that the data set numbered 15 refers to the best SI value but acceptable TS and poor WL values. Similarly, for data set number 8, the highest WL characteristics were obtained but with poor

combinations of TS and SI. On the other hand, for data set numbered 14, while the highest TS was obtained, high SI and poor WL characteristics were evident. Based on an extensive literature search, the acceptable characteristics for a wound dressing film have been set as a 260 % SI (minimum) along with appropriate combinations of WL and TS (Ahmed et al., 2018). Basiak et al. (2018) reported a TS value of 3.5 MPa for packaging films and indicated the same as a good value (Basiak et al., 2018). Compared to the mentioned threshold TS value by Basiak et al. (2018), the carried out experimental investigations indicated good TS data for data sets numbered as 1-9, 11, 13-15, 17-19, 22, 23, 26, 28th formulation (Table 4.1). Also, a minimum SI of 260 was achieved for films obtained with data sets numbered 1, 4-7, 9, 11, 14, 15, 23, 26, 27, 29. Compared to available literature that affirmed good characteristics of wound dressing film WL characteristics (with a value of 48 % for 27 days (Fahmy et al., 2015)), the corresponding values indicated in data sets numbered 1, 2, 7-10, 12, 16, 17, 20, 21, 23-27, 29 and 30 are promising and acceptable. Among these data sets numbered 1, 2, 7, 23, 26 and 29 indicate center points and the same constitution. Among all data sets, it can be analyzed from Table 4.1 that the best data refers to a data set number 9. This corresponds to 338.37 % SI, 53.27 % WL and 7.65 MPa TS for a PVA composite film fabricated with 5 w/w% PVA, 10 w/w% St, 15 wt% CA and 15 wt% Gl concentration.

4.6 Comparative analysis with the prior art

A comparative assessment was carried out to compare and contrast experimentally obtained optimal formulation characteristics with the literature reported best data in the field of PVA composite films and applications. A summary of the key analysis parameters has been presented in Table 4.3. The table affirms that compared to the earlier work of our research group (chapter 3 of the thesis) the film SI and TS increased significantly for appropriate or similar WL

characteristics. A comparative assessment with other literature data was not considered, given the fact that WL was not addressed and other units have been used to evaluate SI. Further, it is interesting to note that films with very high TS have been achieved in comparison with films prepared from this work. Despite following similar experimental procedures, the obtained TS data in this work has been significantly lower than that being reported by Wu et al. (2017) (Wu et al., 2017). Hence, the obtained TS values of the literature need to be verified in the near future by adopting appropriate fabrication and evaluation methods and eventually confirm upon the very high TS values being reported in the mentioned literature.

4.7 Summary

This chapter provided useful insights into the possible extensions of organic precursors formulation based on available literature data and the perceptions are summarized as follows.

- The optimal data refers to 5 w/w% PVA, 10 w/w% St, 15 wt% CA and 15 wt% Gl to achieve polymer composite film with appropriate product characteristics. The optimal film exhibited very high SI (338.37 ± 4.95 %), acceptable in-vitro degradation (53.27 ± 2.77 %), good TS values (7.65 MPa) and positive antibacterial effect with respect to those reported in the literature.
- RSM based analysis indicated a highly non-linear dependence of SI and in-vitro degradation and moderate non-linear dependence of TS with variations in the organic constituent formulation.
- Among all binary interactions, the PVA-St combination influenced all dependent variables significantly. However, for the SI case, PVA-CA interaction was the best among all binary interactions. For the other two dependent variables, namely in-vitro degradation and TS,

Table 4.3: A comparative summary of optimal CCD based PVA composite film constitution and responses and most relevant prior art data.

S. No.	Work	Drying temperature (°C)	Drying time (h)	Optimized aqueous precursor constitution (w/w%)			Swelling index (%)	In-vitro degradation (%)	Tensile strength (MPa)
				PVA	St	CA			
1.	This work ¹	50	12	4.79	9.59	2.16	338.37	53.27	7.65
2.	Objective 1 ²	50	12	7.08	7.08	5.04	260.5	59.77	2.54
3.	Wu et al. (2017) ³	-	4.5	7.27	7.27	2.59	5431	-	45.54
4.	Shi et al. (2008) ⁴	140 (Ventilated)	-	3	1	0.8	119.6	-	44

¹ Concentrations widely varied from 2.38 – 11.29 w/w% for PVA and St and from 0.36 – 7.05 w/w% for CA and Gl

² Following Wu et al. (2017) optimized PVA-St-Gl film were taken; only CA concentration were varied from 1.33 – 7.49 w/w%; thus concentration varied from 7.02 – 7.49 w/w% for PVA and St and from 5.27 – 5.62 w/w% for Gl (variation for PVA, St and Gl is negligible)

³ Concentration moderately varied from 7.39 – 9.92 w/w% for PVA, 3.31 – 7.64 w/w% for St, 5.08 – 6.61 w/w% for Gl and 2.63 – 2.64 w/w% for CA (variation is negligible)

⁴ Concentrations varied from 0.2 – 1.2 w/w% for CA, for fixed concentrations of PVA (3 w/w%), St (1 w/w%) and Gl (0.8 w/w%)



CA-Gl and PVA-Gl interaction respectively are the second best binary interactions to influence the same.

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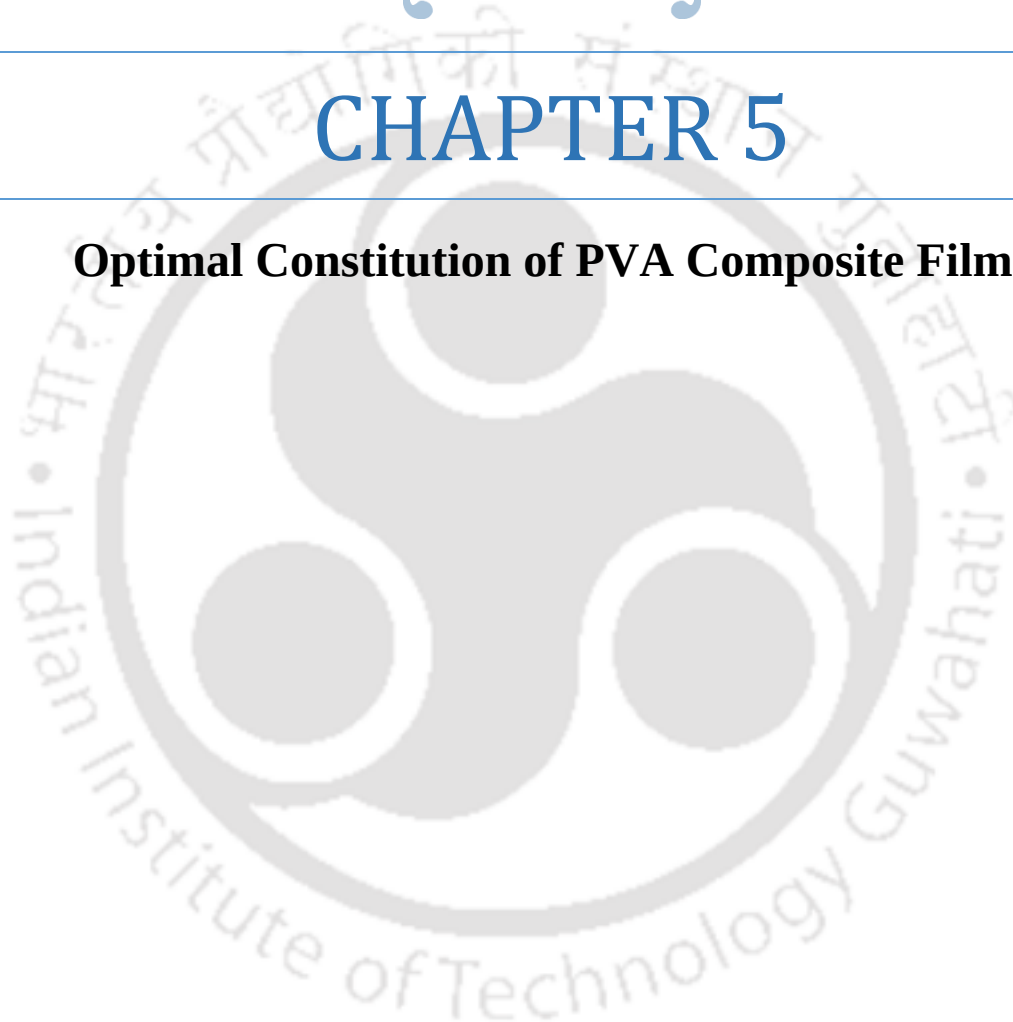
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CHAPTER 5

Optimal Constitution of PVA Composite Films





Optimal Constitution of PVA Composite Films

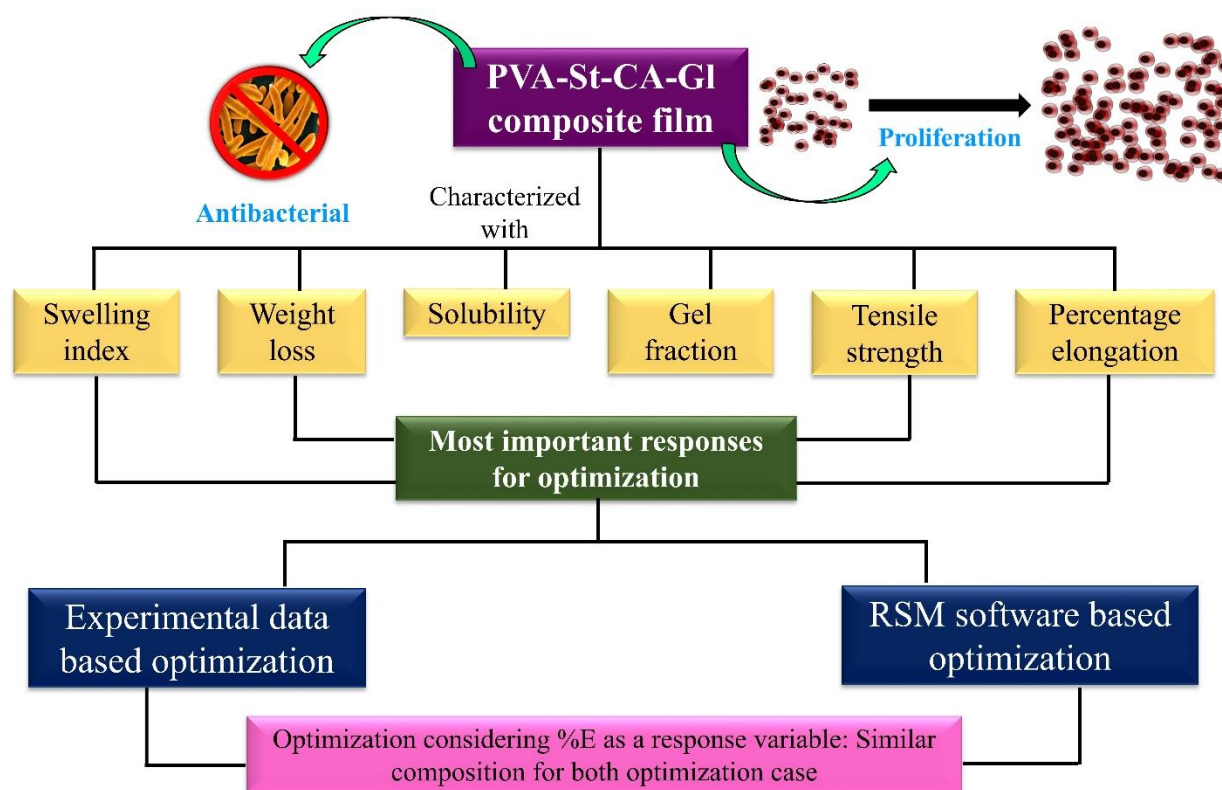
This chapter summarizes upon both experimental database and software base optimization of a quaternary formulation with variant concentrations of PVA, St, Gl and CA macromolecules. Section 5.1 presents the relevant background and the specific novelty of the work. Following this, section 5.2 addressed the structural analysis associated with the pertinent variations in the macromolecular concentrations. Sections 5.3 and 5.4 present findings associated with the best model fitness and associated compatibility of the macromolecules in terms of the physical characteristic such as GF, solubility and %E. Thereafter, section 5.5 devotes towards the findings associated with the interaction of various characterization parameters. Section 5.6 briefly describes the findings associated with the optimization of the blended formulation and a comparative assessment of the critical findings with the prior art data. Finally, section 5.7 and section 5.8 devote towards the qualitative evaluation of the optimized film and key findings summary respectively[#].

Overview

Optimality of quaternary formulations (PVA, St, CA and Gl) associated to wound dressing films was targeted and achieved through RSM design based optimization. The best formulation exhibited very good response characteristics in terms of water absorption (300.48 %) and flexibility (87.54 %E), and acceptable characteristics associated to in-vitro degradation (51.4 %

[#] Published article: Das, A., S. Bhattacharyya, R. Uppaluri and C. Das (2020). "Optimality of poly-vinyl alcohol/starch/glycerol/citric acid in wound dressing applicable composite films." International Journal of Biological Macromolecules 155: 260-272.

WL) and strength (4.98 MPa) responses. Further, the film constitution indicated amplified antibacterial effectiveness against both *L. monocytogenes* and *E. coli* and enhanced cell growth (145.48 %). These critical findings affirm upon their viability as a competent wound dressing applicable film.



5.1 Introduction

This chapter elaborates upon the findings associated with the optimality of the highly complex quaternary combinations of the ingredients (PVA, St, CA and Gl) during the fabrication of solution casting based PVA-St composite wound dressing film. In the previous chapter, the optimal formulation was achieved using the experimental data sets being generated using the design expert software and CCD based approach. Also, it shall be noted that the optimization procedures and methodology did not consider percentage elongation as a response variable.

Considering such additional response variable, response surface plots can be developed to gain useful insights with respect to associated similarities and dissimilarities of the alternate characterization parameter values. The specific novelty of the addressed work in this chapter of the thesis refers to the following issues:

- a) Coherence of characterization parameter values with respect to rigorous variations of quaternary ingredients (PVA, St, CA and Gl) and numerical optimization using a mathematical tool.
- b) Visualize upon the role of %E as an additional characterization variable to influence the combinatorial compositional optimality.
- c) Deeper insights into ATR-FT-IR based characterizations and their inferences with respect to compositional optimality and characterization parametric trends. In this regard, the effect of every component (PVA, St, CA and Gl) concentration needs to be considered for critical evaluation.
- d) Antibacterial effectiveness and cell growth studies using the film being prepared with the optimal composition of macromolecular constituents.

5.2 ATR-FT-IR spectral analysis of PVA-St-Gl-CA composite films

Figure 5.1 presents the ATR-FT-IR spectra in the spectral region of 3700 – 2700 and 900 – 1800 cm^{-1} and for various alternate formulations of the PVA-St-Gl-CA blended films (Table 5.1). For all samples, in the 3700 – 2700 cm^{-1} spectral region, a strong and broad absorbance band can be identified in the 3272 – 3310 cm^{-1} spectral region. This is due to the stretching vibration of the –OH groups on the composite matrix. Also, in the end region, a coalescence peak can be observed at 1713 – 1716 cm^{-1} . Such a peak corresponds to the ester bond and C=O groups in the CA.

Table 5.1: Nomenclature associated to CA, GI, St and PVA variant constitutions of composite hydrogel films.

CA variant samples				GI variant samples				St variant samples				PVA variant samples			
PVA (w/w) %	St (w/w) %	CA (wt) %	GI (wt) %	PVA (w/w) %	St (w/w) %	CA (wt) %	GI (wt) %	PVA (w/w) %	St (w/w) %	CA (wt) %	GI (wt) %	PVA (w/w) %	St (w/w) %	CA (wt) %	GI (wt) %
S1	7.5	7.5	2.5	27.5	S4	7.5	27.5	2.5	S7	7.5	27.5	2.5	7.5	27.5	27.5
S2	7.5	7.5	27.5	27.5	S5	7.5	27.5	27.5	S8	7.5	27.5	7.5	7.5	27.5	27.5
S3	7.5	7.5	52.5	27.5	S6	7.5	27.5	52.5	S9	7.5	27.5	12.5	7.5	27.5	27.5

The pertinent peak intensities in the spectral range of 3272 – 3310 and 1713 – 1716 cm^{-1} affirm that the peak intensities enhanced with increasing CA concentration. In the first region, the enhancement in the intensities is due to the increment of –OH and –COOH groups of CA. However, in the second region, the increment was due to the enhanced constitution of ester bonds and –COOH groups. Thus, the formulation with lowest CA (PVA 7.5 w/w%, St 7.5 w/w%, Gl 27.5 wt% and CA 2.5 wt%) corroborated with insignificant peak intensity in this spectral region. The same has been in good agreement with the relevant prior art (Shi et al., 2008).

In the spectral region of 3272 – 3310 cm^{-1} , the formulation with the highest Gl corroborated with the highest peak intensity (Figure 5.1(b)). Also, it can be analyzed that the formulation with lowest Gl has higher intensity (PVA 7.5 w/w%, St 7.5 w/w%, Gl 2.5 wt% and CA 27.5 wt%) in comparison with the intensity being indicated by the medium Gl content containing formulation (PVA 7.5 w/w%, St 7.5 w/w%, Gl 27.5 wt%, and CA 27.5 wt%). This is due to the fact that the lowest Gl content containing formulation (2.5 wt%) constitutes medium CA content (27.5 wt%). Since CA possesses three –COOH and one –OH group in comparison to the Gl that only constitutes three –OH groups, the formulation with a higher CA constitution will always corroborate with better peak intensity values. This is due to the reason that the multiple –OH groups prevalent in the CA form a better and stronger hydrogen bond with those being prevalent in the St and PVA. Needless to analyze, certain CA portions reacted with Gl to form glycerol citrates to potentially reduce hydrogen bonds being formed in the polymer matrix. The CA and Gl content increment (i.e., the formulation with both medium CA and Gl content) significantly facilitated the formation of glycerol citrates. Similar observations do exist in the spectral region of 1713 – 1716 cm^{-1} region. Thus, the formulation with lowest Gl and medium CA constitution

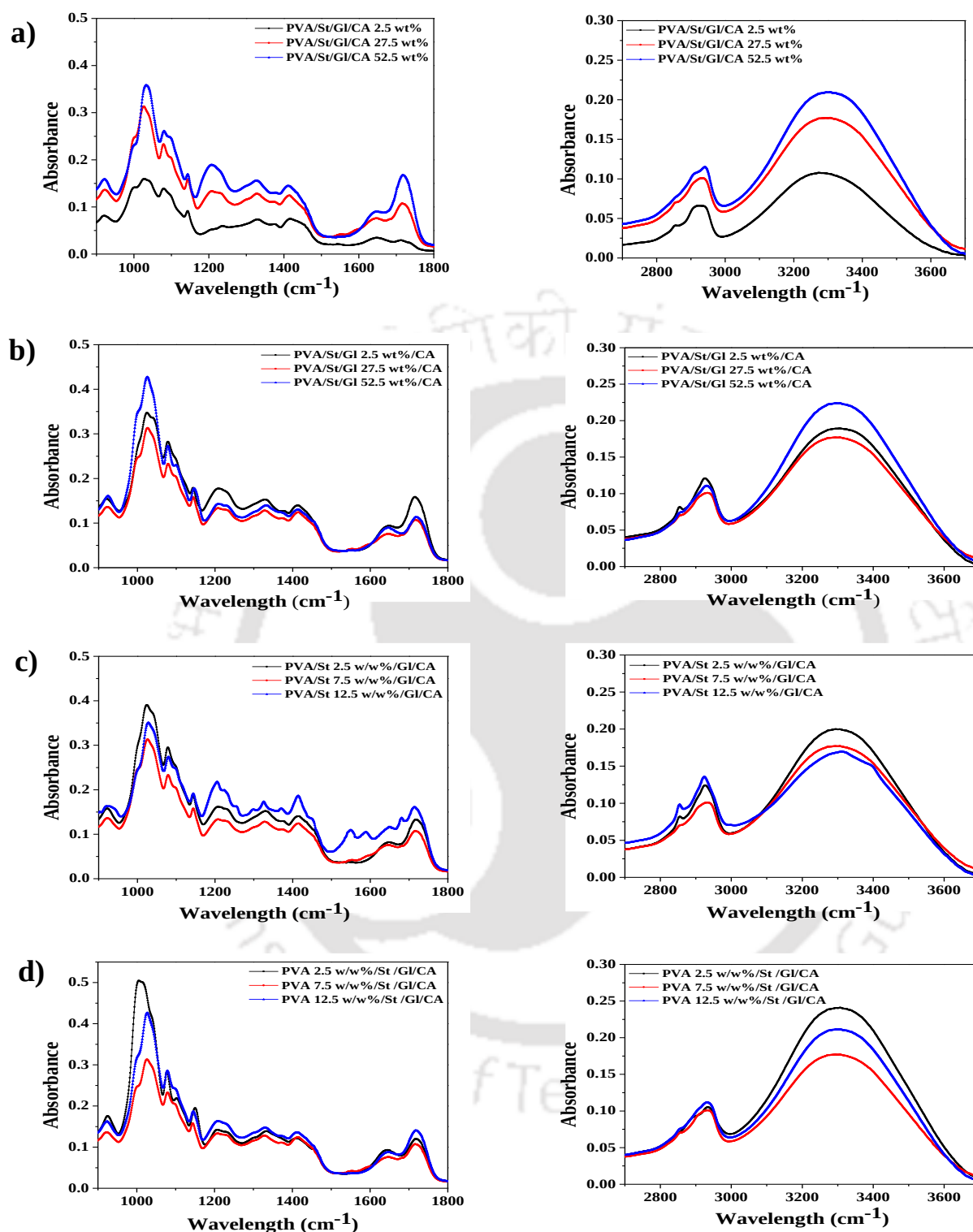


Figure 5.1: ATR-FT-IR spectra of composite films constituting alternate variations in the concentrations of (a) CA, (b) GI, (c) St and (d) PVA.

exhibited the highest peak intensity in the mentioned spectral region. This is in comparison with the alternate formulation that constitutes a similar constitution of medium CA content and either a similar medium or higher Gl content. The mentioned alternative sample possesses a lower constitution of ester bonds due to the participation of CA in the glycerol citrate reaction but not esterification.

Further, in Figure 5.1 (c) and (d), it can be observed in the spectral region of $3272 - 3310 \text{ cm}^{-1}$ higher peak intensities exist due to the lowest PVA and St concentrations. The reason for the same has been discussed previously in this section i.e., with increasing CA and Gl content, the constitution of OH group does get enhanced. Hence, the formulation with the lowest PVA or St content facilitates higher free CA content (and hence higher free OH and COOH groups) in comparison with the formulations being achieved with higher PVA or St content. In this regard, it shall be noted that all three mentioned formulations constitute a constant GA or Gl content (27.5 wt%). Also, the peak intensity being achieved with the formulation with lowest PVA content was significantly higher than the peak being obtained for the formulation with the lowest St. The $1713 - 1716 \text{ cm}^{-1}$ spectral region affirm corroborates with both ester bonds and free COOH groups. Therefore, formulations with the highest PVA or St content exhibited the highest peak intensity due to the presence of a higher constitution of ester bonds. Firstly, condensation occurred due to the reaction of alcohol and carboxylic acid and thereby produce an ester. This effect translated into a faster disappearance of the anhydride C=O groups. Subsequently, the linked COOH groups to the polymer can be analyzed to foster once again the formation of anhydride and thereby enhance the coalescence peak intensity (Uliniuc et al., 2013). The sample with the highest PVA content was found to have lower peak intensity in comparison with the

sample being prepared with the highest St content. This might be due to the better esterification ability of St-CA interaction in comparison with the PVA-CA interaction.

5.3 Interdependence of gel fraction and solubility with macromolecular constitutions of percentage elongation inclusive data sets

5.3.1 Identification of best fit models

Table 5.2 summarizes the alternate model fitness parameters for GF and S as response variables. Among these, the best fit model can be identified with that possessing the highest F value and R^2 and lowest p -value. The CCD based model analysis inferred that the two function interaction model has been the best fit model for both cases and can be expressed as follows for GF and S:

$$GF = +35.32 + 4.15A + 0.43B - 8.81C + 0.27D + 2.95AB + 1.39AC - 0.65AD + 4.39BC + 0.50BD - 0.12CD \quad (10)$$

$$S = +64.68 - 4.15A - 0.43B + 8.81C - 0.27D - 2.95AB - 1.39AC + 0.65AD - 4.39BC - 0.50BD + 0.12CD \quad (11)$$

The two function interaction model expression being presented for both cases indicates bilinear or moderately non-linear responses of the entire region for associated interactions of PVA-St, PVA-CA, PVA-Gl, St-CA, St-Gl, and CA-Gl. For both cases, the best fit model possessed a high F value (67.2 for both GF and S) and a low p -value (< 0.0001 for both GF and S).

5.3.2 Response surface analysis

The three dimensional response surface graphs of GF and S with respect to any two independent variables are depicted in Figures 5.2 and 5.3, respectively. Among binary interactions, PVA-St

Table 5.2: (a) Fitness parameters of alternate models and (b) ANOVA parameters of best fit models representing gel fraction and solubility characteristics of percentage elongation response inclusive data sets of PVA-St-CA-GI composite films.

(a)

Source	Gel fraction					Solubility				
	Sum of squares	df	Mean square	F value	p-value prob > F	Sum of squares	df	Mean square	F value	p-value prob > F
Mean vs Total	37417.7	1	37417.7			1.3E+05	1	1.3E+05		
Linear vs Mean	2281.8	4	570.4	24.0	< 0.0001	2281.8	4	570.4	24.0	< 0.0001
2FI vs Linear	490.1	6	81.7	15.0	< 0.0001	490.1	6	81.67	15.0	< 0.0001
Quadratic vs 2FI	38.8	4	9.7	2.3	0.1109	38.8	4	9.7	2.3	0.1109
Cubic vs Quadratic	30.4	8	3.8	0.8	0.6345	30.4	8	3.8	0.8	0.6345
Residual	34.0	7	4.9			34.0	7	4.9		
Total	40292.8	30	1343.1			1.3E+05	30	4279.8		

(b)

Gel fraction/ Solubility					
Source	Sum of squares	df	Mean square	F value	p-value prob > F
Model	2771.9	10	277.2	51.0	< 0.0001
A-PVA	413.3	1	413.3	76.0	< 0.0001
B-St	4.4	1	4.4	0.8	0.3782
C-CA	1862.3	1	1862.3	342.8	< 0.0001
D-GI	1.8	1	1.8	0.3	0.5745
AB	139.5	1	139.5	25.7	< 0.0001
AC	31.0	1	31.0	5.7	0.0275
AD	6.8	1	6.8	1.3	0.2777
BC	308.7	1	308.7	56.8	< 0.0001
BD	4.0	1	4.0	0.7	0.4045
CD	0.2	1	0.2	0.04	0.836
Residual	103.2	19	5.4		
Lack of fit	78.4	14	5.6	1.1	0.4847
Pure error	24.8	5	5.0		
Cor total	2875.1	29			



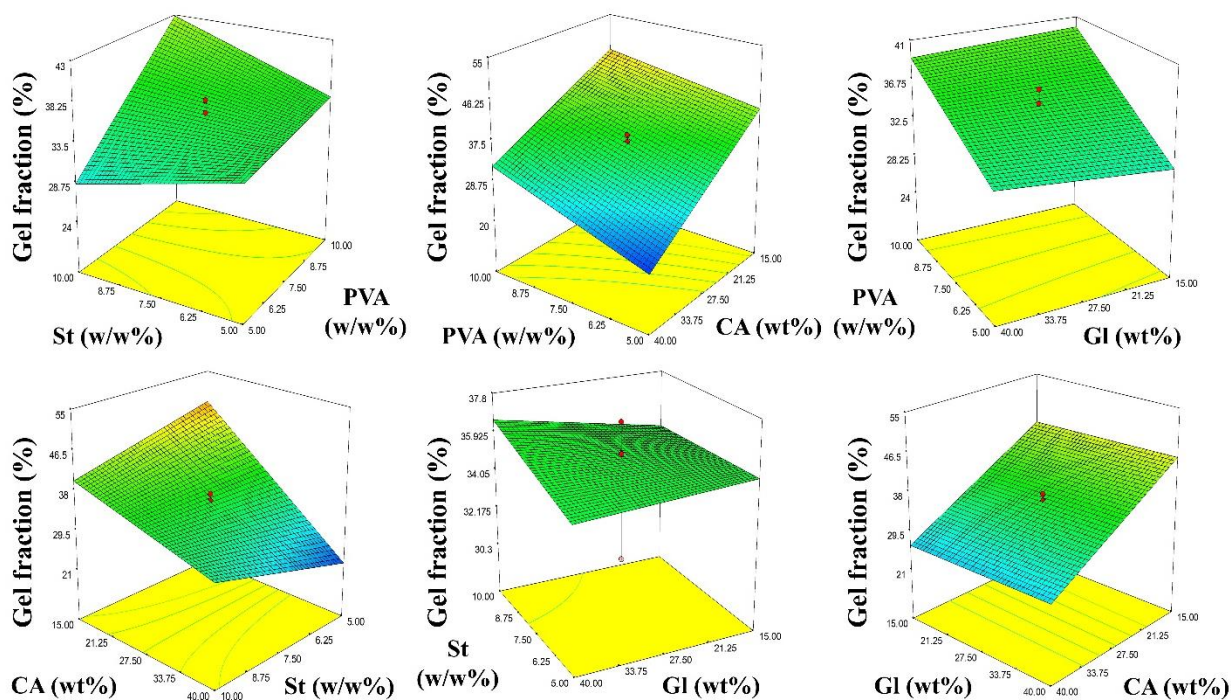


Figure 5.2: Response surface plots of gel fraction characteristics of %E response inclusive investigations of PVA-St-CA-Gl composite films.

and St-CA interactions had a predominant effect. The GF enhanced significantly with an enhancement in the PVA concentration. On the other hand, CA had a significantly negative influence upon the GF. Excluding PVA-Gl and CA-Gl interactions, the binary interactions of any two of the four independent variables affirmed positive influence upon the GF. Among these, the St-CA interaction followed with PVA-St interaction exhibited the most prominent influence upon the response characteristics. The depicted response graphs affirm the following key observations and inferences with respect to binary interactions:

- a) PVA-St interaction: The GF increased with an increase in the PVA content. Similar trends also exist for enhanced St content and higher PVA concentration case. However, for the low PVA concentration case, the GF was reduced with an increase in the St content. The associated reason for the GF increase with an increase in the PVA content is

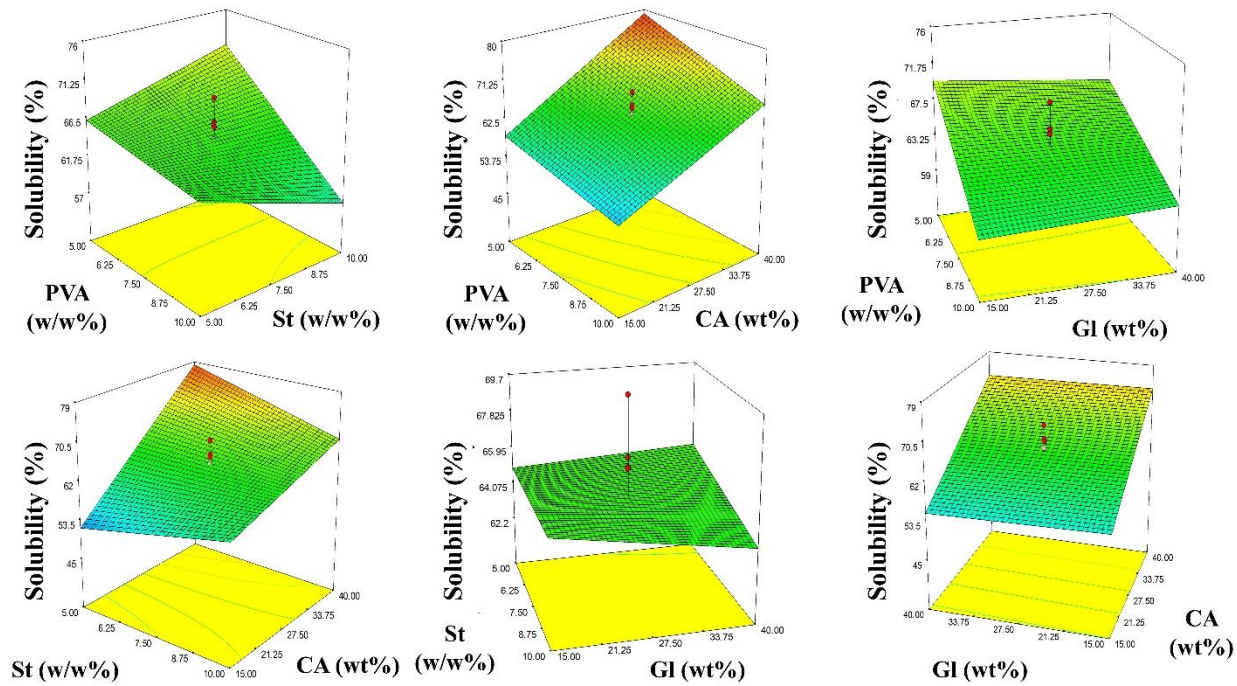


Figure 5.3: Response surface plots of solubility characteristics of %E response inclusive investigations of PVA-St-CA-Gl composite films.

due to the higher degree of crosslinking being facilitated by the PVA in the composite matrix (Ahmed et al., 2018).

- b) PVA-CA interaction: The PVA content influence was similar to that being discussed for the PVA-St interaction case. However, with CA increment, the GF reduced significantly. Conventionally, an enhancement in crosslinking agent concentration would contribute towards enhanced GF value. However, while addressing the first objective of the Ph.D. thesis, it has been mentioned in chapter 3 that the higher CA concentration had a contrary influence due to both plasticization and crosslinking effects. Once again, for the St-CA interaction case, the CA content influence on the response was similar. For higher CA concentration cases, the St increment enabled higher GF. This is due to the greater number of ester bonds. However, for the lower CA concentration case, the St increment

enabled polymer increment in the film matrix. In summary, lower crosslinking and poor mechanical property characteristics of St contributed towards lower GF values.

- c) PVA-Gl and St-Gl interactions: The influence of Gl on GF was not very significant (Basiak et al., 2018). However, several cases indicated that the GF decreased with an increment in the Gl content. This is due to its higher plasticization tendencies. The influence of PVA or St was similar to that being discussed for the PVA-CA and St-CA binary interaction cases. With respect to the CA-Gl interaction case, it can be analyzed that Gl did not influence the response variable. On the other hand, CA reduced the GF.

For all binary interaction cases namely PVA-St, PVA-CA, PVA-Gl, St-CA, St-Gl and CA-Gl, the S trends were exactly opposite to those being observed for the GF. This is because the S and GF are inversely proportional to one another. Hence, greater crosslinking facilities greater and enhancement in the GF and reduction in the solubility and subsequently plasticization accelerate the vice-versa.

5.4 Interdependence of percentage elongation with macromolecular composite film constitutions

5.4.1 Best model fitness

Based on CCD design and model fitness studies, the %E response variable has been found to be the best fit using a two function interaction model with respect to the independent variables. Accordingly, the best fit model for %E can be expressed as:

$$\begin{aligned} \%E = & +82.79 + 27.2A - 17.12B + 29.73C + 3.45D - 34.61AB - 6.16AC \\ & - 21.44AD - 1.19BC + 18.03BD + 14.22CD \end{aligned} \quad (12)$$

The best fit model parameters affirm high significance (p -value < 0.0001 , moderate F value 10.6 and R^2 value of 0.8478) (Table 5.3) and hence a very good degree of fitness for the two function interaction model. Among all measured data sets, the data set numbered 14 affirmed the highest %E characteristics. The data set referred to 5 w/w% PVA, 10 w/w% St, 40 wt% CA and 40 wt% Gl (Table 5.4).

Table 5.3: (a) Fitness parameters of alternate models and (b) ANOVA parameters of best fit models representing percentage elongation characteristics of PVA-St-CA-Gl composite films.

(a)					
Percentage elongation					
Source	Sum of squares	df	Mean square	F value	p -value prob $> F$
Mean vs Total	2.1E+05	1	2.1E+05		
Linear vs Mean	46290.8	4.0	11572.7	5.8	0.002
2FI vs Linear	35588.4	6.0	5931.4	7.7	0.0003
Quadratic vs 2FI	1802.1	4.0	450.5	0.5	0.7198
Cubic vs Quadratic	4078.3	8.0	509.8	0.4	0.8859
Residual	8821.9	7.0	1260.3		
Total	3.0E+05	30	10073.93		

(b)					
Percentage elongation					
Source	Sum of squares	df	Mean square	F value	p -value prob $> F$
Model	81879.2	10.0	8187.9	10.6	< 0.0001
A-PVA	17755.1	1.0	17755.1	23.0	0.0001
B-St	7034.7	1.0	7034.7	9.1	0.0071
C-CA	21215.5	1.0	21215.5	27.4	< 0.0001
D-Gl	285.5	1.0	285.5	0.4	0.5508
AB	19169.2	1.0	19169.2	24.8	< 0.0001
AC	608.1	1.0	608.1	0.8	0.3864
AD	7351.8	1.0	7351.8	9.5	0.0061

Source	Percentage elongation				
	Sum of squares	df	Mean square	F value	p-value prob > F
BC	22.7	1.0	22.7	0.0	0.8658
BD	5199.4	1.0	5199.4	6.7	0.0179
CD	3237.2	1.0	3237.2	4.2	0.0549
Residual	14702.3	19.0	773.8		
Lack of fit	12762.4	14.0	911.6	2.4	0.1767
Pure error	1939.9	5.0	388.0		
Cor total	96581.5	29.0			

5.4.2 Response surface analysis

Figure 5.4 depicts the contour plots and corresponding response surface plots of %E with respect to the variations in concentrations of all four constituents (PVA, St, CA and Gl). For all cases, the %E varied from 4.74 – 204.49 % (Table 5.4) for a change in PVA, St, CA and Gl concentration as 2.5 – 12.5 w/w%, 2.5 – 12.5 w/w%, 2.5 – 52.5 wt% and 2.5 – 52.5 wt%. In this regard, it can be inferred that the results being reported to fulfill the 1st objective being set in the Ph.D. thesis to affirm that the PVA-St-CA-Gl composite film prepared at 50 °C possessed lower %E values (in the range of 10.43 – 49.69 %). From the response surface plots, it can be analyzed that the %E enhanced with PVA, CA and Gl increment but reduced with St increment. The binary interactions illustrated that the St-CA, St-Gl and CA-Gl interactions contributed positively towards %E and PVA-St, PVA-CA and PVA-Gl interactions resulted in a detrimental (negative) influence upon the %E. Among all binary interactions, the PVA-St interaction had a prominent effect on the %E i.e., film strength. The response surface plots being prepared for the %E case can be analyzed as follows:

- a) PVA-St interactions: For lower range St concentration, PVA enhancement increased %E. However, for higher St concentration, the increase has been marginal. This is due to the fact that the addition of St to PVA detracts the physical properties of the blend as PVA

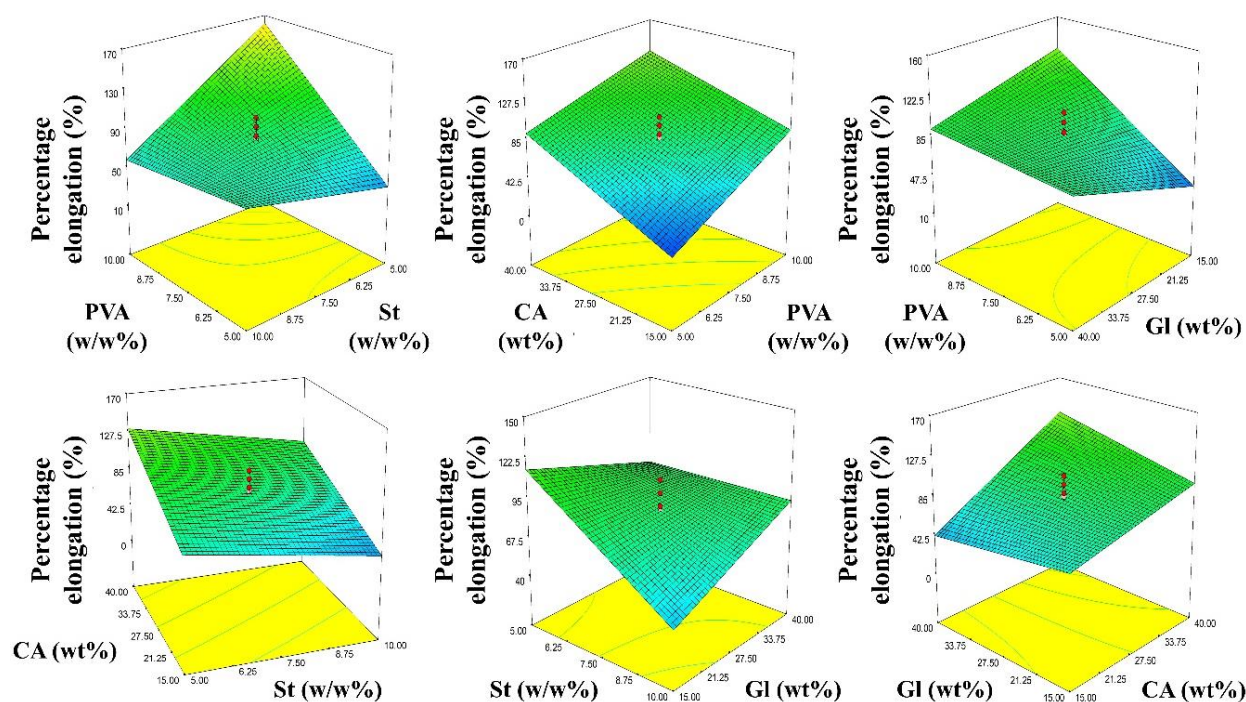


Figure 5.4: Response surface plots of percentage elongation characteristics of PVA-St-CA-Gl composite films.

and St has poor compatibility with one another (Raj and Somashekar, 2004). However, for the lower PVA case, the St addition indicated a marginal increment in the %E. Hence, an increment in both polymer (St or PVA) concentrations in the composite matrix detracts its physical stability. Therefore, either an enhancement in the concentration of PVA or St is required to achieve improved physical stability and mechanical property of the film.

- b) PVA-CA and St-CA interactions: The %E enhanced with increasing content of both PVA and CA. However, for the St-CA interaction case, an increase in St reduced %E. For the CA increment, the trends for %E were similar as discussed for the PVA-CA interaction case. Thus, PVA and CA favored %E or flexibility in the composite due to the

- plasticization property of the CA. However, similar interaction did not exist for the St-CA case. This is due to the detrimental mechanical characteristics of St.
- c) PVA-Gl interaction case: An increment in PVA facilitated better %E. However, at lower PVA concentration, with increasing Gl concentration, the %E enhanced. On the contrary, at higher PVA concentration, the %E reduced. Once again, an increment in St reduced %E for the St-Gl interaction case. On the other hand, with increasing Gl concentration, the trends were fully reverse for Gl and both cases of lower and higher St concentrations in comparison with the trends being obtained for the PVA-Gl interaction case. Such a trend is very likely due to the phase separation between Gl and St on highly plasticized St films. For a lower range of the St concentration, the excess constitution of Gl favored its effective interaction with itself and hindered macromolecular mobility. On the contrary, for the higher St concentration case, the higher constitution of St molecules (amylose/ amylopectin) prevented the anti-plasticization effect of the Gl (Sanyang et al., 2015).
- d) CA-Gl interaction: The CA concentration positively influenced %E. However, for the lower range CA concentration, an enhancement in the Gl only varied %E marginally. On the contrary, for the higher range CA concentration case, the %E enhanced with increasing Gl content. This is due to the plasticization effect of both CA and Gl.

5.5 Coherence of alternate characterization parameters associated to percentage elongation inclusive data of PVA-St-CA-Gl composite films

The available prior art affirms the coherence of few characterization parameters and hence similar associated trends. An increment in GF enhanced TS. This is due to either higher solid/gel content in the matrix or a higher degree of crosslinking (Ahmed et al., 2018, Fahmy et al., 2015).

The literature reported in-vitro degradation characteristics have been expressed in terms of WL. In other words, the characteristics are similar to the solubility characteristics of the composite films. The primary lacunae of the available prior art is with respect to each research group targeting and addressing alternate and different sets of characterization parameters and eventually converged upon the optimality of the film constitution. Therefore, a unified approach is the need of the hour to further the existing knowledge base associated with the complex interactions of the quaternary formulations and potentially influence or alter the evaluated characterization parameters.

The ATR-FT-IR analysis being conducted in the Ph.D. thesis work indicated that with three –COOH groups, the CA possessed better intermolecular bonding with the –OH groups of PVA and St in comparison with the Gl that only possessed three –OH groups. The SI and solubility trends corroborate this hypothesis. In chapter 4, a comparative assessment of PVA-CA and PVA-Gl or St-GA and St-Gl interactions indicated that compared to the Gl, the CA facilitates better crosslinking and this reflects in the lower combinations of SI and WL or in-vitro degradation in the lower CA concentration range. However, for the enhanced CA concentration range, the prevalent free CA content in the polymer matrix reaches a higher constitution. This is being confirmed with the peak intensities observed in the spectral region of $1713 - 1716 \text{ cm}^{-1}$. Such free CA –COOH groups have a stronger affinity towards water molecules and thereby enhance CA's ability to hydrolytically degrade and mitigate its ability to reduce SI. Again for the CA-Gl interaction case, higher CA and Gl combinations reduce WL. This is due to the formation of glycerol citrates being driven by the improvement of the reaction between these two constituents. Henceforth, the effect translates into a reduction in the number of hydrogen bonds prevalent in the polymer matrix. This has been confirmed in the ATR-FT-IR analysis.

Finally, ATR-FT-IR analysis affirmed that the CA and St esterification ability has been high in comparison with the PVA. For both SI and WL cases, a similar phenomenon can be observed for both PVA-CA and St-CA interactions. For any range of St-CA concentration range, lower SI and WL have been obtained. Such observation affirms a higher degree of crosslinking in comparison to the prevalent effect in the entire PVA and CA concentration range.

A useful analysis of the response surfaces indicates that the achieved trends of the binary interactions of all constituents (PVA, St, CA and Gl) are almost similar for the solubility and in-vitro degradation responses. In the lower PVA concentration and higher St concentration range, both solubility and WL or in-vitro degradation have been the highest and vice-versa. In the lower additive (Gl or CA) or higher PVA concentration range, both properties did reach higher values for lower CA content but not Gl. Such an effect is due to the better crosslinking ability of CA in conjunction with the Gl. Once again, for the lower PVA and CA concentration range, both solubility and WL have been higher than those being prevalent for the lower St and CA concentration range. This affirmed that CA reacts more easily with St in comparison with the PVA (Shi et al., 2008). Henceforth, the crosslinked St with a better networking structure fostered lower solubility and in-vitro degradation characteristics.

Also, for the GF and TS, almost similar trends have been observed for the binary interactions. Both GF and TS were reduced for lower PVA and higher CA concentration range. The reduction in PVA content reduced the crosslinking extent and facilitated the abundant existence of free –COOH groups. Further, in a relevant prior art, it has been indicated that for the reduced GF and TS case, the crosslinking extent or associated solid content reduces to thereby facilitate an enhanced %E trends (Fahmy et al., 2015). However, among relevant binary interactions, such

trends have not been observed for all scenarios of GF or TS variations. Further, such trends have not been analyzed to be applicable for the CA-GI interaction case.

In summary, all measured characterization parameters such as SI, solubility, in-vitro degradation, GF, TS, and %E can be concluded to be very important characteristics to infer upon the optimality of PVA composite films. Among these, while swelling enabled a useful analysis towards the fluid holding capacity, a critical understanding of the healing can be better learned from solubility based WL or in-vitro degradation. Similarly, TS and %E trends can be analyzed to infer upon the extent of crosslinking or solid content and shape stability characteristics of the composite film system. Henceforth, SI, WL, TS and %E can be concluded to be the most critical response variables to facilitate the identification of optimal PVA-St-CA-GI wound dressing film formulations.

5.6 Optimality of PVA-St-CA-GI film constitution

5.6.1 Optimality based on experimental investigations

The experimental data set being summarized in Table 5.4 affirmed the best values of only one response among all responses namely SI or in-vitro degradation or TS or %E. Thus, the best data set cannot indicate the highest (optimal) combinations of all four response characteristics. Further, despite indicating the best characteristics of one of the responses, the identified data sets may not indicate acceptable values for other characterization parameters. For example, run 15 indicates the best SI and acceptable TS values but a poor in-vitro degradation value. Based on extensive literature search, the acceptable combinatorial characteristics of wound dressing films have been concluded as 260 % SI for a super absorbent (Ahmed et al., 2018), good in-vitro degradation characteristics of 48 % for 27 days incubation (Fahmy et al., 2015), 3.5 MPa

Table 5.4: A summary of experimental data associated to percentage elongation inclusive trails of CA based PVA composite films.

S. no.	A: PVA concentration (w/w%)	B: St concentration (w/w%)	C: CA concentration (wt%)	D: Gl concentration (wt%)	Response 1: Swelling index (%) ¹	Response 2: In-vitro degradation (%) ¹	Response 3: Tensile strength (MPa) ²	Response 4: Percentage elongation (%) ¹
1	7.5	7.5	27.5	27.5	261.48	53.04	5.12	95.14
2	7.5	7.5	27.5	27.5	218.74	49.89	4.61	54.1
3	10	5	15	40	184.63	34.65	8.5	59.26
4	7.5	7.5	27.5	2.5	371.11	44.41	4.99	76.65
5	7.5	7.5	2.5	27.5	398.45	31.09	9.35	4.74
6	5	5	15	40	338.94	38.61	6.55	7.37
7	7.5	7.5	27.5	27.5	297.88	53.14	5.31	82.13
8	5	10	40	40	114.15	71.72	2.14	176.55
9	5	10	15	15	338.37	53.27	7.65	9.13
10	7.5	12.5	27.5	27.5	170.14	58.07	3	92.22
11	10	10	15	15	258.95	34.19	6.53	32.52
12	10	10	40	15	237.64	52.3	1.77	81.57
13	10	10	15	40	167.89	43.7	5.01	39.49
14	10	5	15	15	317.58	29.34	9.93	204.49
15	5	5	15	15	473.93	32.43	6.41	14.15
16	2.5	7.5	27.5	27.5	229.32	65.79	3.38	18.35
17	5	10	15	40	218.06	60.14	8.01	61.83
18	10	5	40	15	248.69	45.15	4.77	167.08
19	12.5	7.5	27.5	27.5	180.39	39.28	5.73	150.76
20	7.5	7.5	52.5	27.5	201.49	59.69	0.54	162.45

¹ All samples were examined thrice and the overall SD value were $\leq \pm 5$ ² All samples were examined thrice and the overall SD value were $\leq \pm 1$

S. no.	A: PVA concentration (w/w%)	B: St concentration (w/w%)	C: CA concentration (wt%)	D: Gl concentration (wt%)	Response 1: Swelling index (%) ¹	Response 2: In-vitro degradation (%) ¹	Response 3: Tensile strength (MPa) ²	Response 4: Percentage elongation (%) ¹
21	7.5	7.5	27.5	52.5	149.01	48.11	3.81	96.6
22	7.5	2.5	27.5	27.5	240.4	37.13	7.85	145.83
23	7.5	7.5	27.5	27.5	260.09	56.33	4.04	104.4
24	5	10	40	15	188.59	68.74	1.1	31.7
25	5	5	40	40	193.34	55.55	2.17	67.39
26	7.5	7.5	27.5	27.5	260.52	55.9	4.51	59.85
27	5	5	40	15	279.39	53.93	2.78	65.23
28	10	5	40	40	155.08	45.6	4.37	194.18
29	7.5	7.5	27.5	27.5	265.99	56.38	3.47	85.88
30	10	10	40	40	168.93	51.55	1.35	42.7



standard strength (Basiak et al., 2018) and 12.5 % elongation to provide adequate and promising mechanical stability (Devi et al., 2012). Among all data being summarized in Table 5.4, the best data set corresponds to the composition of 7.5 w/w% PVA, 7.5 w/w% St, 27.5 wt% CA and 27.5 wt% Gl which indicates the central point. Corresponding response characteristics were 297.88 % SI, 53.14 % WL, 5.31 MPa TS and 82.13 % elongation. Also, for the case %E being not considered as an additional response, data sets indicated by all central points and run no. 9 qualify in terms of the above mentioned values of the wound dressing film characteristics. However, among these, run 9 corresponds to the best acceptable values of 338.37 % SI, 53.27 % WL and 7.65 MPa TS for the wound dressing film being prepared with 5 w/w% PVA, 10 w/w% St, 15 wt% CA and 15 wt% Gl concentration. In conclusion, the consideration of %E as an additional response variable affirms only one composition to qualify among the experimental data set to achieve optimally performing PVA composite films for wound dressing applications.

5.6.2 Optimality based on numerical methodology

From design expert software analysis, the numerical optimization based composition has been 5.46 w/w% PVA, 8.09 w/w% St, 22.77 wt% CA and 22.69 wt% Gl for the case that did not consider %E as an additional response variable. For the film, the software indicated the optimal response characteristics as 305.73 % SI, 56.41 % WL and 5.39 MPa TS. However, for the case that considered %E as an additional response variable, the optimal composition has been obtained as 7.45 w/w% PVA, 6.96 w/w% St, 28.57 wt% CA and 18.38 wt% Gl. Corresponding optimal response characteristics have been 300.48 % SI, 51.4 % WL, 4.98 MPa TS and 87.54 % elongation. For the second case, the model complexity was enhanced significantly. Thus, numerical optimization affirmed that except for the Gl constitution, the constitution of other ingredients remained fairly similar for both cases of numerical and experimental data based

optimization. Also, in comparison with the experimentally obtained values, the RSM optimized model that considered %E as an additional response indicated higher values and better combinations of SI and %E responses and marginally variant values for in-vitro degradation and TS responses.

5.6.3. Comparative analysis with most relevant prior art

A comparative assessment of the experimentally obtained data, design expert software based optimal formulation and best prior art data have been summarized in Table 5.5. It can be observed that compared to the findings reported in chapter 3, the film properties increased significantly (260.5 to 300.48 % for SI, 2.54 to 4.98 MPa for TS and 38.55 to 87.54 % for %E) for a lower in-vitro degradation value range (59.77 to 51.4 %) but appropriate characteristics (promising value being inferred as 48 % WL in 27 days for wound dressing characteristics (Fahmy et al., 2015)). Other prior art has not been considered for the comparative assessment with the fact that the degradation WL response was not addressed in the other relevant prior art related to the optimal fabrication of PVA-St-CA-Gl composite films. Further, it is interesting to note that the films with very high tensile strength have been achieved in comparison with the best film characteristics being reported in chapter 3.

According to conventional standards, a minimum TS value of 3.5 MPa is mandatory for packaging films. However, for wound dressing films, the TS value range could be lower than this value to simultaneously indicate a higher stretchability index or %E value. In terms of RGR characteristics, according to Shi et al. (2008), 20 wt% CA concentration has been effective to achieve more than 99 % cell growth (Shi et al., 2008). With 28.6 % CA in the optimized film constitution, the CA content is comparably lower than that being reported for the best fabricated film in chapter 3 (35.58 wt% of CA). In summary, compared to those findings, for every

Table 5.5: A summary of optimal data sets associated to percentage elongation inclusive data trails and most relevant prior art of CA based PVA composite films.

S. No.	Work	Drying Temperature (°C)	Drying Time (h)	Optimized aqueous precursor constitution (w/w%)				Swelling index (%)	In-vitro degradation (%)	Tensile strength (MPa)	Percentage elongation (%)
				PVA	St	CA	GI				
1.a	EXP-%E	50	12	4.79	9.59	2.16	2.16	338.37	53.27	7.65	9.13
1.b	RSM-%E	50	12	5.16	7.64	2.91	2.9	305.73	56.41	5.39	-
1.c	EXP+%E	50	12	6.94	6.94	3.82	3.82	297.88	53.14	5.31	82.13
1.d	RSM+%E	50	12	6.99	6.53	3.87	2.49	300.48	51.4	4.98	87.54
2.	Objective 1	50	12	7.08	7.08	5.04	5.32	260.5	59.77	2.54	38.55
3.	Wu et al. (2017)	-	4.5	7.27	7.27	2.59	5.46	5431.0	-	45.54	-
4.	Shi et al. (2008)	140 (Ventilated)	1	3.0	1.0	0.8	0.8	119.6	-	44.0	178.9

constituent, the optimized film constitution indicated lower ingredient content for all other constituents but not the PVA. Thereby, such remarkable reduction in the expensive ingredients of the composite film translate into a reduced materials cost in comparison with the optimal film constitution reported in the chapter 3 of the thesis. Thus, better response characteristics have been achieved through the RSM design based numerical optimization along with a reduction in the materials cost. In summary, compared to the best findings reported in the previous chapter, better and inexpensive PVA-St-CA-Gl composite films have been obtained as optimal films in this chapter.

5.7 Qualitative assessment of optimally fabricated CA based PVA composite films

5.7.1 Antibacterial effectiveness

The optimally formulated PVA-St-CA-Gl films and a control sample (kanamycin, 0.2 µg/mL) were subjected to antibacterial activity investigations against *E. coli* and *L. monocytogenes*. In due course of such investigations, it was observed that all PVA-St-CA-Gl films exhibited negative bacterial growth around the film surface area and fostered the generation of a clear inhibition zone against both investigated microbes. The actual inhibition area around the film was tabulated in Table 5.6.

Among all samples, the PVA-St-CA-Gl film formulation being obtained from the design expert based analysis (deduced with the methodology that does not consider %E as an additional response variable) with the composition of 5.46 w/w% PVA, 8.09 w/w% St, 22.77 wt% CA and 22.69 wt% Gl exhibited highly promising results in terms of antibacterial activity in comparison

with those being obtained by considering %E as the additional response variable (optimal composition being obtained as 7.45 w/w% PVA, 6.96 w/w% St, 28.57 wt% CA and 18.38 wt% Gl) (Figure 5.5).

Table 5.6: Actual inhibition zone area (cm²) data of alternate optimal formulation based PVA-St-CA-Gl composite films with respect to *E. coli* and *L. monocytogenes*.

Sample name	Actual area of inhibition (cm ²)	
	<i>Escherichia coli</i>	<i>Listeria monocytogenes</i>
EXP-%E	0.9	0.2
EXP+%E	0.3	0.1
RSM-%E	4.2	0.8
RSM+%E	1.6	0.5

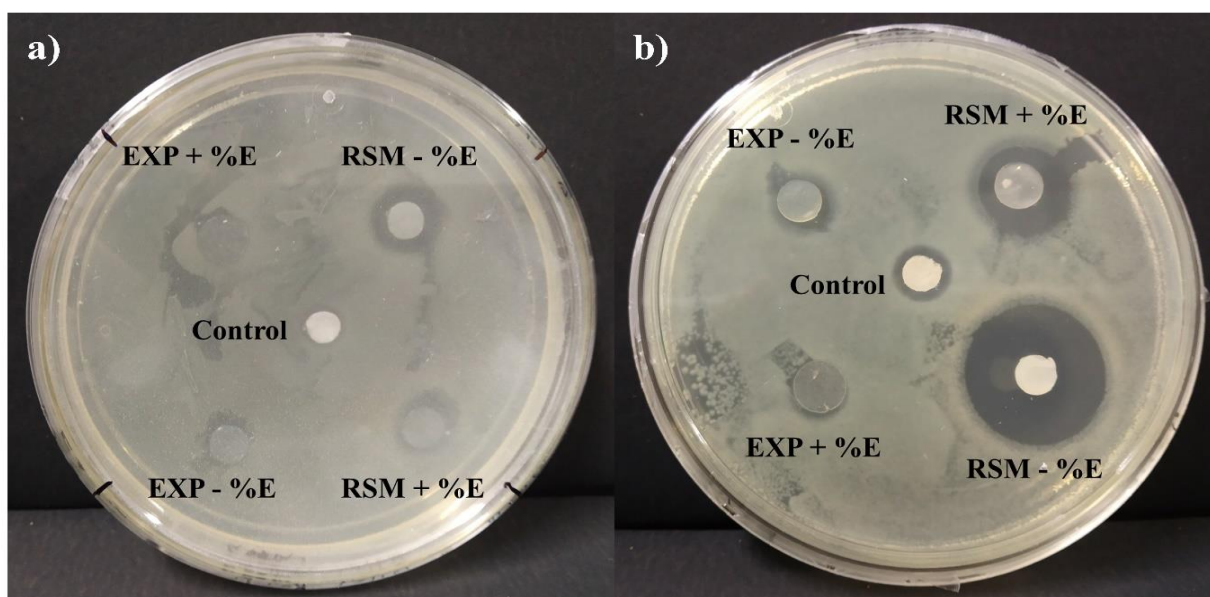


Figure 5.5: Photographs depicting antibacterial efficacy of optimally formulated PVA-St-CA-Gl composite films with respect to (a) *E. coli* and (b) *L. monocytogenes*.

Among these two compositions, it can be observed that the CA content in the latter case is higher. However, the total polymer content for the case is much higher (13.53 w/w%) than that being prevalent (12.8 w/w%) for the RSM-%E case. Hence, this indicates a reduction in the free CA content of the composite matrix. Thereby, this effect influences the leaching extent and acidic environment being facilitated to break the synthesis associated with the protein and cell membrane of the microbes (Wu et al., 2017). Additionally, it has been as well observed that the experimentally optimized formulation for both cases (EXP+%E) and (EXP-%E) indicated comparatively lesser effectiveness towards antibacterial effects with respect to the RSM optimized formulation. This is due to the fact that the RSM optimized formulation has a higher CA in comparison with the CA increment prevalent in the experimental data sets. Hence, along with most responses, the CA had a positive influence on the mitigation of microbial propagation. However, it is interesting to observe that, compared to *E. coli*, the *L. monocytogenes* exhibited far prominent positive antimicrobial activity.

5.7.2 Evaluation of in-vitro biocompatibility

5.7.2.1 Relative growth rate

For the medical application of wound dressing films, cytotoxicity assessment through MTT assay is being considered as an initial step to evaluate the wound dressing material potential. The in-vitro biocompatibility of the optimized PVA-St-CA-GI films was evaluated for their cell growth potential by evaluating their RGR using HEK cells. After being subjected to extraction or soaking of 24 or 48 h, the concentration of the quaternary soluble blend of the PVA-St-CA-GI composite films in the media was determined. Table 5.7 summarizes the obtained results with the due consideration that the extract dilution is to be regarded as the treatment.

According to the ISO guidelines (GB/T16175-1996 standard), <75 % RGR characteristics of the test material was achieved with respect to the control sample. Thereby, the bio-compatibility potential of the film could be analyzed to be promising (Shi et al., 2008). On the contrary, no toxic effect was observed on the cell growth for applying the optimized film sample extracts as treatment. Comparing all tested films, the PVA-St-CA-GI film formulation indicated by the design expert with %E as an additional response (7.45 w/w% PVA, 6.96 w/w% St, 28.57 wt% CA and 18.38 wt% GI) exhibited the highest RGR of 145.48 % (significantly higher than control; level $p \leq 0.05$) after using 48 h of extracted sample as treatment (Figure 5.6). Also, an interesting pattern can be analyzed with the fact that except for (EXP-%E) formulation, the cell growth rate has been relatively higher for 48 h of extraction time in comparison to those being evaluated with 24 h extraction time.

In summary, the achieved results demonstrate that all optimized formulations have better RGR than that of the control samples. Further, apart from being only biocompatible, optimized PVA films also have a proliferative effect on cell growth. After co-culturing, the RGR for the

Table 5.7: Concentrations of the quaternary soluble blend in the FBS supplemented DMEM media being obtained after extraction of the PVA-St-CA-GI composite films.

Sample name ¹	Initial weight, W ₀	Weight Loss, WL (%)		Extract concentration g/mL of media ²	
		24 h	48 h	24 h	48 h
EXP-%E	0.0153	46.0	52.4	7.0×10^{-4}	1.6×10^{-3}
RSM-%E	0.0139	71.2	77.6	9.9×10^{-4}	2.2×10^{-3}
EXP+%E	0.0122	44.2	49.5	5.4×10^{-4}	1.2×10^{-3}
RSM+%E	0.0115	60.4	64.8	7.0×10^{-4}	1.5×10^{-3}

¹ All the samples were tested based on control (Control: 0 μ L of extract with 100 μ L of media)

² 3 dilutions of each extract were chosen as treatment. Such as; 5, 10 and 100 % volume proportions, in 100 μ L of total treatment (Example: For case 1, 5 μ L of extract with 95 μ L of media and likewise)

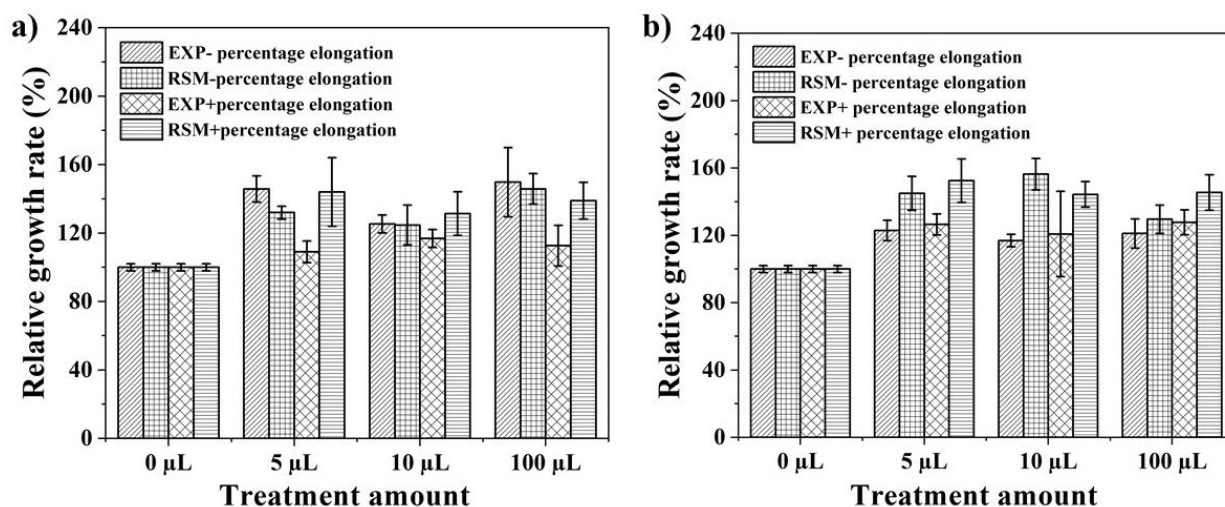


Figure 5.6: In-vitro biocompatibility characteristics of optimally fabricated PVA-St-CA-Gl composite films in terms of relative growth rate of the HEK cells with respect to (a) 24 h and (b) 48 h extraction period.

optimized film increased significantly for the 48 h extracted sample (145.48 %) in comparison with 24 h extracted (138.85 %) and control sample (100 %). This is due to the proliferative effect on the cell growth being driven by the enhanced leaching based on higher WL of the film. The thesis findings associated with the first objective affirm upon such WL trends and thereby validate the stated hypothesis.

5.7.2.2 Visual measurements of relative growth rate

Annexin V-FITC and PI dual staining using confocal microscopy facilitated qualitative analysis was conducted to obtain images of the living and dead cells. Among these precursors, the Annexin V-FITC selectively binds to the flipped phosphatidylserine exposed to the outer membrane surface of the early apoptotic cells. Thereby, green fluorescence can be achieved for such cells upon excitation with a laser beam at a wavelength of 488 nm. On the other hand, the PI precursor emits red fluorescence due to associated intercalation with the DNA of the

membrane compromised dead cells. Hence, early apoptotic cells and dead cells emit green and red fluorescence respectively and the live cells possess no stain. Further, Paclitaxel (anticancer drug) has been used as a cytotoxic agent in the conducted experiments. Eventually, the HEK cells being treated with the drug exhibit abundance of green and red cells and henceforth confirm the existence of early apoptotic and dead cells respectively. Thereby, the RGR % data being obtained from the MTT assay can be corroborated. Figure 5.7 (a-f) illustrate the confocal images of cell growth being obtained for various investigated cases namely untreated control (a), cytotoxic agent (b), RSM+%E (c), RSM-%E (d), EXP+%E (e) and EXP-%E (f) treated cases.

The cell proliferation indicated by the MTT assay affirmed that the HEK cells treated with samples prepared with optimized film composition (7.45 w/w% PVA, 6.96 w/w% St, 28.57 wt% CA and 18.38 wt% Gl) had higher cell density in comparison with the control group for all cases namely RSM-%E and EXP+%E and EXP-%E. On the other hand, for the cases (c-f) illustrated in Figure. 5.7, higher cell density is being indicated by the images in comparison with the image analyzed for the untreated total case. Compared to the EXP+%E and EXP-%E cases, the best data was obtained for the RSM+%E and RSM-%E cases. Further, the cell density for the untreated and EXP-%E cases also appear similar. Further subjective analysis of the images to achieve the extent of cellular density could not be carried out. This is due to limitations in the Image analysis software in interpreting the intensities of grey and dark grey portions of the images.

In summary, the above mentioned findings are in good agreement with those being obtained from the RGR characteristics. Further, it is apparent that images represented in Figure 5.7 (b-f) affirm higher proportions of red coloured cells in comparison with those being apparent in Fig. 7 (a). These observations affirm that the film does have a role in the alteration of apoptosis and

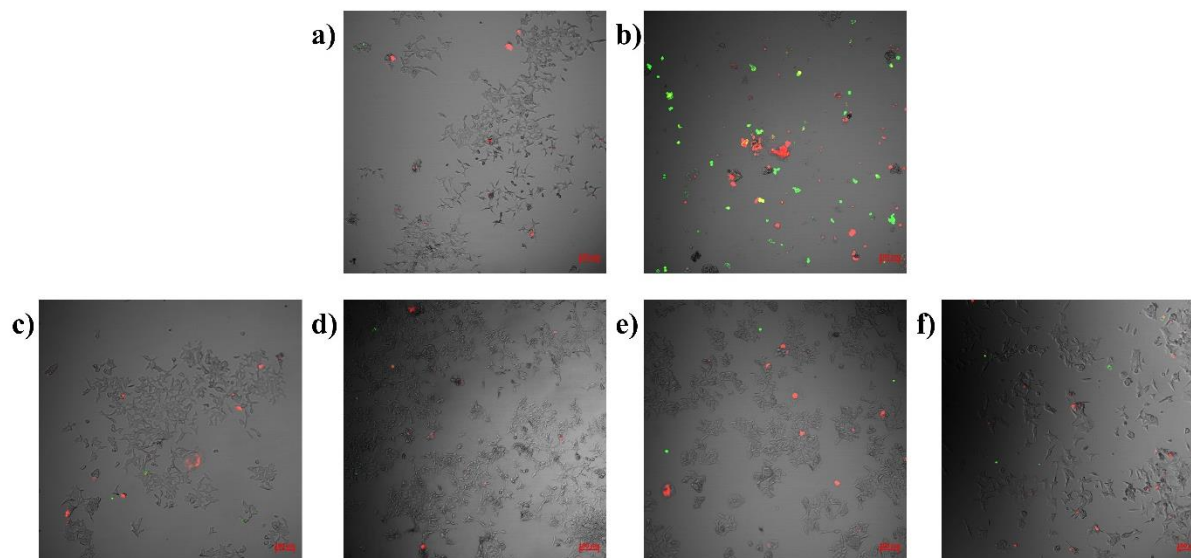


Figure 5.7: Confocal microscopy images depicting relative growth rate characteristics of (a) untreated control, (b) cytotoxic agent, (c) RSM+%E, (d) RSM-%E, (e) EXP+%E and (f) EXP-%E treatment cases.

living cells becoming dead cells. However, such a role is marginal, as the living cell density increased for the optimized film cases in comparison with the control case. Compared to all cases, the image obtained for the cytotoxic agent treated cells indicated significant red and green portions and hence a higher degree of apoptosis and cell death. This is due to the greater role of the anticancer drug to influence the apoptosis and cell death mechanisms, as cells about to undergo cancerous effects are very likely to have been strongly influenced. Further, the image (Figure 5.7 (b)) indicates that cell growth is poor and hence converges with the hypothesis that the cancer drug had a potential negative influence upon cell growth.

The most important inference being drawn from both RGR assay and Annexin V FTIC and PI studies are with respect to the wound dressing film's non-significant cytotoxicity and significant bio-compatibility. Further, the observations do indicate upon the difficulty associated with the formulation of a hypothesis or rule of thumb to indicate strong coherence of other properties with

cytotoxicity results. For example, the RSM+E% case can be concluded as the best based on other characterization parameter values. However, except for the EXP-E% case, the cytotoxicity performance for all cases has been similar. Henceforth, significant difficulty exists to comprehend the role of chemical interactions during cytotoxicity analysis for cases that refer to near optimal combinations of ingredients in the wound dressing compatible PVA composite films.

5.8 Visual appearance of optimally fabricated films

Photographs depicting the transparency and colour of alternate PVA-St-GA-Cl composite films have been depicted in Figure 5.8. As shown, all composite films have similar colour characteristics and are highly transparent. For certain cases, the film texture has been contrasting and different. For most cases, films being prepared with greater PVA content possessed greater transparency and smoother texture. On the other hand, films consisting of greater St and PVA content possessed marginally rougher surfaces and hence partial reduction in the film transparency (Figure 5.9). This is possibly due to the reduced mobility of the composite solution

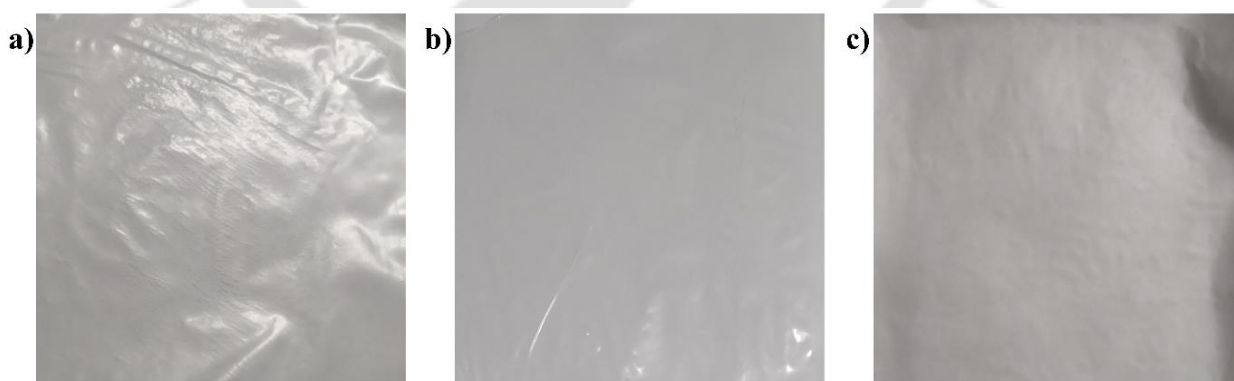


Figure 5.8: Photographs depicting colour characteristics of optimally fabricated PVA-St-Gl-CA composite films (a) RSM+%E, (b) RSM-%E and (c) Run 12.



Figure 5.9: Photographs depicting transparency characteristics of optimally fabricated PVA-St-CA-Gl composite films (a) RSM+%E, (b) RSM-%E and (c) Run 12.

with higher St and PVA content. The %E and TS property trends can be analyzed to be in good agreement with the hypothesis of the composite solution reduced mobility.

5.9 Summary

A summary of the critical findings associated with the optimality of PVA composite films can be presented as follows:

- The due consideration of %E as an additional response variable significantly altered and influenced the conclusions being deduced with the RSM based optimization. Thereby, the greater utility of RSM based optimization is evident for the case.
- For each response variable, except for the SI, all binary interactions involving Gl (*D*) had a marginal effect and *F* value. Among all responses, Gl had a minimal influence upon all other evaluated characteristics. The binary interactions involving Gl also indicated a similar trend as the Gl only influenced all responses to vary marginally.
- The (RSM+%E) case indicated similar characteristics as those being obtained for the (EXP+%E) case. Hence, RSM based optimization is not necessary for the case as only

experimentation is required. Such an effort reduces the significant time being required for the analysis of the obtained data in terms of the associated sensitivity characteristics. On the other hand, this was not the case for the (EXP-%E) and (RSM-%E) data sets, as significant disparities exist in these two data sets. Hence, RSM based optimization is required for the data sets being that did not consider %E as an additional response variable.

- The best composition achieved through RSM optimization (7.45 w/w% PVA, 6.96 w/w% St, 28.57 wt% CA and 18.38 wt% Gl and approach RSM+%E) exhibited very high SI (300.48 %), in-vitro degradation (51.4 %), acceptable TS (4.98 MPa), good %E (87.54 %), positive antibacterial effectiveness against both *L. monocytogenes* and *E. coli* and promoted cell growth (145.48 %) using HEK cells.

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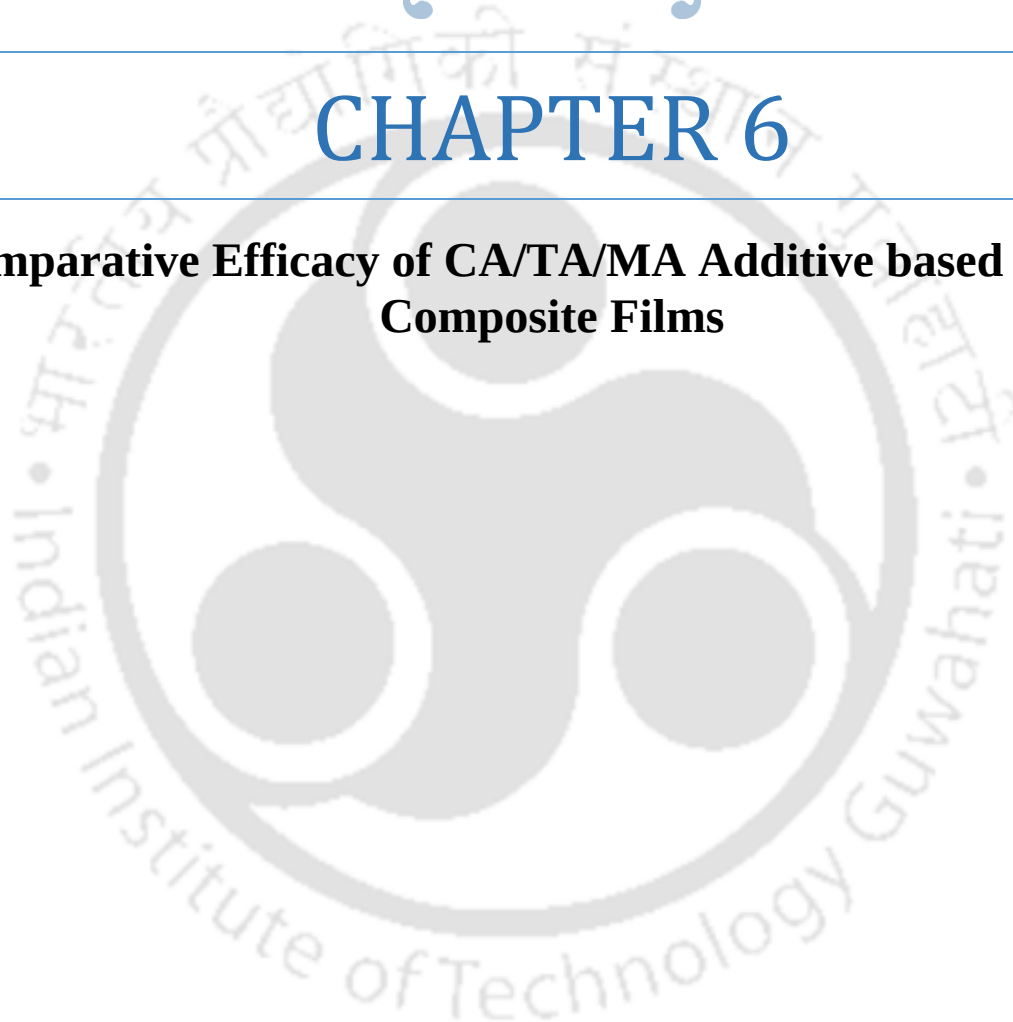
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CHAPTER 6

Comparative Efficacy of CA/TA/MA Additive based PVA-St Composite Films





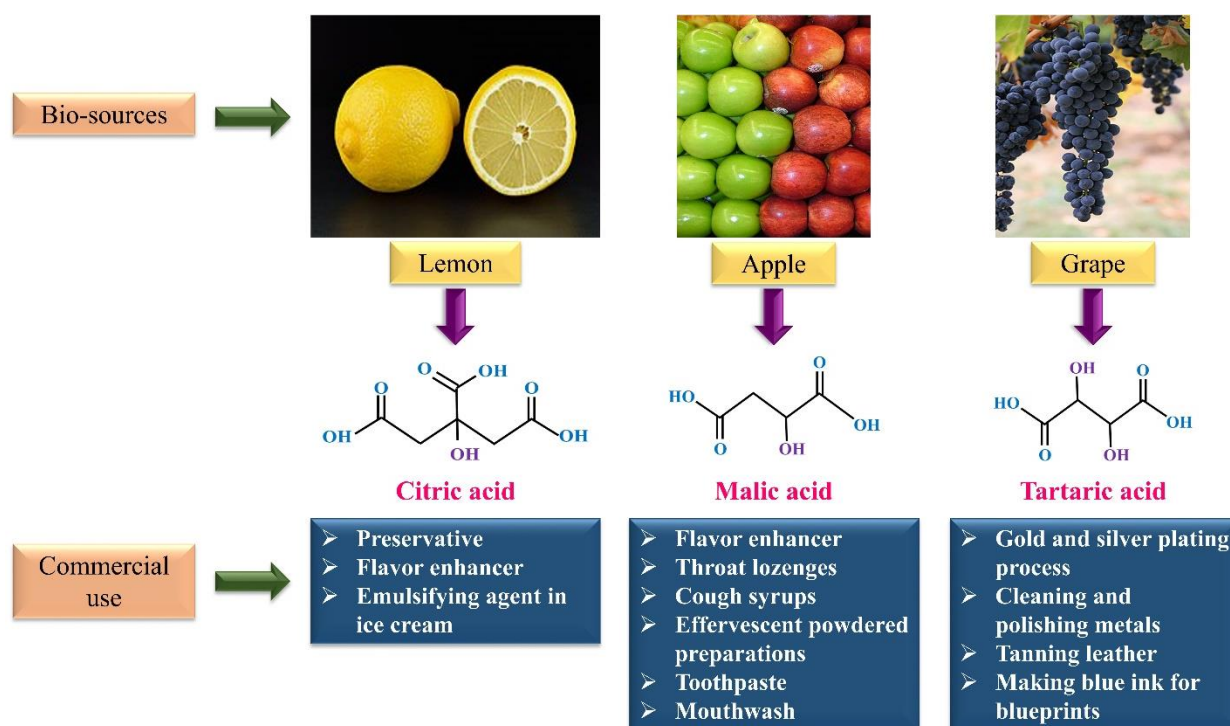
Comparative Efficacy of CA/TA/MA Additive based PVA-St Composite Films

In a comparative framework, the chapter addresses the influence of alternate organic acids namely CA, TA and MA on the optimal performance characteristics of PVA-St-Gl composite films being fabricated for wound dressing applications. For all cases, the optimal film constitution has been based on that being evaluated for the PVA-St-CA-Gl composite films. After summarizing relevant introductory information in section 6.1, section 6.2 of the chapter devotes towards the SI characteristics of CA/MA/TA based PVA-St composite films. Thereafter, sections 6.3 and 6.4 respectively address the solubility/GF and in-vitro degradation characteristics. Thereafter, section 6.5 delineates upon the mechanical characteristics of the composite films. Following this, the antibacterial and in-vitro biocompatibility characteristics of the composite films have been comparatively analyzed in sections 6.6 and 6.7 respectively. Finally, section 6.8 summarizes the key research findings of the comparative analysis and efficacy studies.

Overview

The efficacy of alternate organic acids CA, TA and MA has been targeted in this chapter in terms of the physical and biological characteristics of the PVA-St composite films being fabricated for wound dressing applications. Among these three organic acids, the TA is the most costly organic acid followed with MA and CA. The physical properties of the three alternate organic acid based PVA composite films affirmed best performance of MA (SI 739.29 %, TS 4.88 MPa and %E 103.68 %), which is comparable to that of the CA based PVA composite film. Further, the MA

based films exhibited highest RGR of 215.59 % after 24 h incubation period. Henceforth, MA has been identified to be the most relevant organic acid for further optimization of film constitution, cost, physical and biological characteristics.



6.1 Introduction

The comparative efficacy of three alternate organic acids namely CA, MA and TA towards the optimal combinatorial functionalities of PVA-St composite wound dressing films has been targeted in this chapter. The optimized formulations being obtained for the PVA-St-CA-GI composite films in chapters 4 and 5 were considered as the constitution of the fabricated films. Thus, only organic acid was replaced in the fabricated films i.e., maintaining similar film constitution, CA was being replaced with either TA or MA to obtain defect free composite films for subsequent characterizations and analysis. For the alternate films, the characterizations being targeted include SI, solubility, GF, in-vitro degradation, TS and %E. These affirmed upon the

comparative efficacy of the films towards physical characteristics. Further, the efficacies of the films have also been targeted through the antibacterial effectiveness and in-vitro biocompatibility characterizations.

6.2 Swelling index measurements

The PVA-St composite film SI characteristics with respect to CA, TA and MA concentration have been depicted in Figure 6.1. For all cases, the optimal formulations refer to those being obtained from the 4th and 5th chapters. These have been denoted as EXP-%E (5 w/w% PVA, 10 w/w% St, 15 wt% CA/TA/MA and 15 wt% Gl), EXP+%E (7.5 w/w% PVA, 7.5 w/w% St, 27.5 wt% CA/TA/MA and 27.5 wt% Gl), RSM-%E (5.46 w/w% PVA, 8.09 w/w% St, 22.77 wt% CA/TA/MA and 22.69 wt% Gl) and RSM+%E (7.45 w/w% PVA, 6.96 w/w% St, 28.57 wt% CA/TA/MA and 18.38 wt% Gl). Thereby, the SI values increased from 292.63 ± 4.97 to 338.37 ± 4.95 , 322.68 ± 2.54 to 355.45 ± 4.34 and 347.56 ± 4.49 to 739.29 ± 5.24 %, respectively for variations in CA, MA and TA. Thus, among all formulations, MA based films possessed highest SI characteristics (level $p \leq 0.001$, for EXP+%E, RSM-%E and RSM+%E formulations, in comparison with both CA and TA) followed by TA based films. In this regard, it shall be noted that while MA possessed one –OH and two –COOH groups, the TA possessed two –OH and two –COOH groups. However, the CA possessed one –OH and three –COOH groups (Yun et al., 2006). Hence, it can be analyzed that since CA possessed the highest number of –COOH groups among the chosen organic acids, the CA facilitated higher and efficient crosslinking with the –OH groups of PVA-St blend (Shi et al., 2008). Apart from CA, among the other two organic acids (TA and MA), the MA possessed the lowest number of relevant functional groups for participation in the esterification reaction. Typically, wound dressing materials shall possess

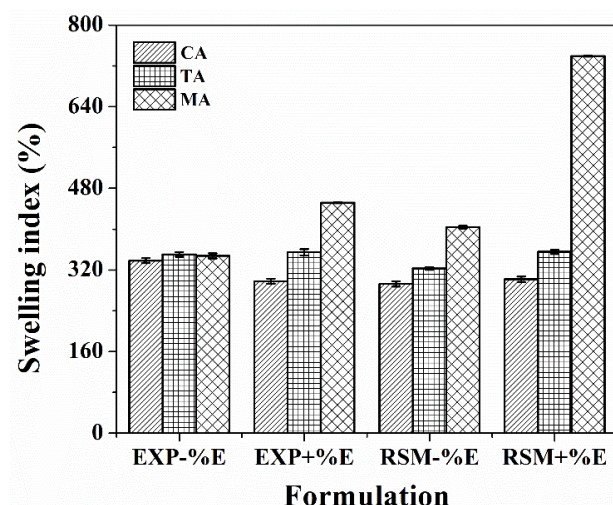


Figure 6.1: Influence of CA, TA and MA on the swelling index characteristics of optimally fabricated PVA-St composite films.

significantly higher swelling capacity to facilitate the effective absorption of wound exudates. Since films fabricated with all three alternate organic acids based formulations procured a SI value greater than 260 %, they can be concluded to be super absorbents and compatible for wound dressing applications from SI analysis perspective (Ahmed et al., 2018). However, among all of them, the MA depicted lowest crosslinking ability and highest SI characteristics. Hence, MA needs to be explored further with respect to the optimality of the film constitution for wound dressing applications. Further, it can be observed that, for the case of EXP-%E formulation based film that possessed lowest additive concentration, the SI variation has been negligible (Only TA possessed higher SI with a significance level of $p \leq 0.05$, in comparison to CA). Thus, it is anticipated that any of the alternate organic acids namely MA or CA or TA would achieve a similar crosslinking extent (and swelling characteristics) up to a concentration of 15 wt% of the organic acid additive.

6.3 Solubility and gel fraction measurements

Figure 6.2 (a) and (b) respectively depicted the PVA-St composite film solubility and GF trends with respect to variations in organic acid (CA, TA and MA) concentrations for four different case films being designated as EXP-%E, EXP+%E, RSM-%E and RSM+%E. It can be analysed, for the optimized PVA-St composite films and corresponding constituent concentration, the solubility characteristics varied as 66.01 ± 4.47 to 75.88 ± 4.34 , 51.81 ± 2.25 to 74.88 ± 0.41 and 61.59 ± 1.21 to 82.72 ± 2.28 %, respectively, for CA, TA and MA. The corresponding variation in GF trends have been as 24.12 to 66.11, 25.12 to 48.19 and 17.28 to 38.41 % for CA, TA and MA based films respectively. To portray upon the enhancement or detrimental trends of solubility or GF with respect to the organic acid concentrations, the obtained trends have been inconclusive. This was possibly due to the intermolecular reaction between the polymer (PVA and St) and the additives (Gl and CA/TA/MA organic acid). However, among all organic acids, the MA based PVA-St composite film exhibited versatile solubility range data and indicates likely promising performance upon further exploration. For the case of EXP-%E alternate formulations (5 w/w% PVA, 10 w/w% St, 15 wt% CA/TA/MA and 15 wt% Gl), The TA based films possessed highest GF or lower solubility in comparison to those being obtained with CA (significance level of $p \leq 0.05$) and MA (significance level of $p \leq 0.01$) based films. In this regard, it can be recalled from section 6.2 that for lower organic acid concentration (15 wt%), the extent of esterification reaction remained similar for all organic acids. Hence, the GF characteristics can be anticipated to be of similar trends for all organic acid cases and similar lower additive concentrations. However, the trends did not corroborate with the sole hypothesis of crosslinking extent. On the contrary, the pertinent trends indicated highest, higher and lower solubility characteristics of MA, CA and TA based films, respectively. This was possibly due to the

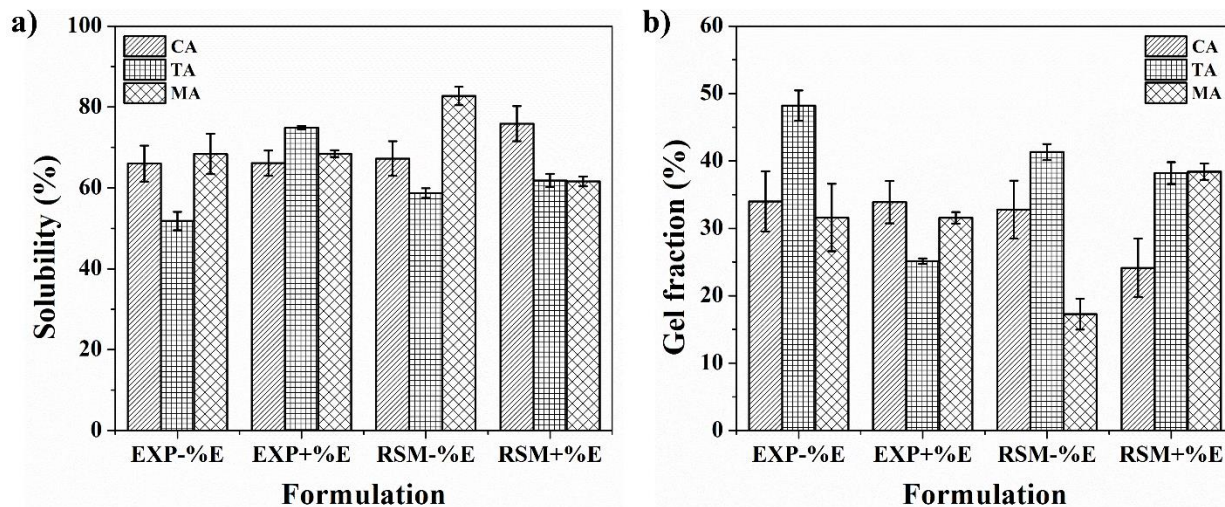


Figure 6.2: Influence of CA, TA and MA on the (a) solubility and (b) gel fraction characteristics of optimally fabricated PVA-St composite films.

effective bonding of TA with St but not the PVA to thereby indicate lowest solubility of the TA based PVA-St composite film. Similar trends existed for the RSM-%E (5.46 w/w% PVA, 8.09 w/w% St, 22.77 wt% CA/TA/MA and 22.69 wt% Gl) formulations, where St concentration was again higher than PVA concentration in the film matrix (significant low solubility with p -value ≤ 0.05 in comparison to CA, with p -value ≤ 0.001 in comparison to MA). For the RSM+%E (7.45 w/w% PVA, 6.96 w/w% St, 28.57 wt% CA/TA/MA and 18.38 wt% Gl) case, the CA based film possessed highest solubility or lowest GF (with a significance level of $p \leq 0.01$, in comparison to both TA and MA). This was possibly due to its comparatively improvised plasticization characteristics that are being facilitated with the larger number of free $-\text{COOH}$ groups in the matrix or blend at higher CA concentrations (Shi et al., 2008).

6.4 In-vitro degradation measurements

Figure 6.3 illustrated the in-vitro degradation data trends (being measured as WL obtained after 27 days of incubation of PVA-St composite films in PBS solution (7.4 pH at 37 °C)) with time for alternate organic acid based PVA-St composite films. For the alternate formulations based on the EXP-%E data set (5 w/w% PVA, 10 w/w% St, 15 wt% CA/TA/MA and 15 wt% Gl), the WL during 27 days of incubation varied from 25.56 ± 0.57 to 53.27 ± 2.77 , 31.05 ± 4.62 to 76.75 ± 4.7 and 32.54 ± 3.37 to 61.73 ± 0.69 % for CA, TA and MA respectively. For the EXP+%E formulation based PVA-St composite films (7.5 w/w% PVA, 7.5 w/w% St, 27.5 wt% CA/TA/MA and 27.5 wt% Gl), the corresponding values altered in the range of 40.62 ± 2.05 to 56.38 ± 2.8 , 51.65 ± 4.25 to 65.9 ± 4.8 and 37.57 ± 1.41 to 57.44 ± 0.9 %, respectively. On the other hand, for the alternate organic acid based PVA-St composite films being fabricated with RSM-%E formulation (5.46 w/w% PVA, 8.09 w/w% St, 22.77 wt% CA/TA/MA and 22.69 wt% Gl), the in-vitro degradation characteristics varied as 54.51 ± 2.3 to 76.78 ± 3.93 , 37.42 ± 2.3 to 73.96 ± 3.69 and 43.57 ± 1.1 to 73.99 ± 3.27 % for CA, TA and MA, respectively. Similarly, for the composite films being prepared with the RSM+%E formulation (7.45 w/w% PVA, 6.96 w/w% St, 28.57 wt% CA/TA/MA and 18.38 wt% Gl), the variable trends altered correspondingly in the range of 51.93 ± 2.71 to 64.79 ± 3.65 , 35.32 ± 0.8 to 93.47 ± 3.77 and 27.96 ± 1.81 to 66.56 ± 3.51 %, respectively.

For most cases, the time dependent characteristics affirmed firstly significant, then gradual increment and finally steady degradation characteristics during the 1st day, 3 to 7 days and 27 days time duration, respectively. Except for the films being fabricated with the EXP+%E formulation, for all other cases, the TA based PVA composite films confirmed poor overall degradation characteristics up to 14 days of time duration. However, after 14 days, a sharp

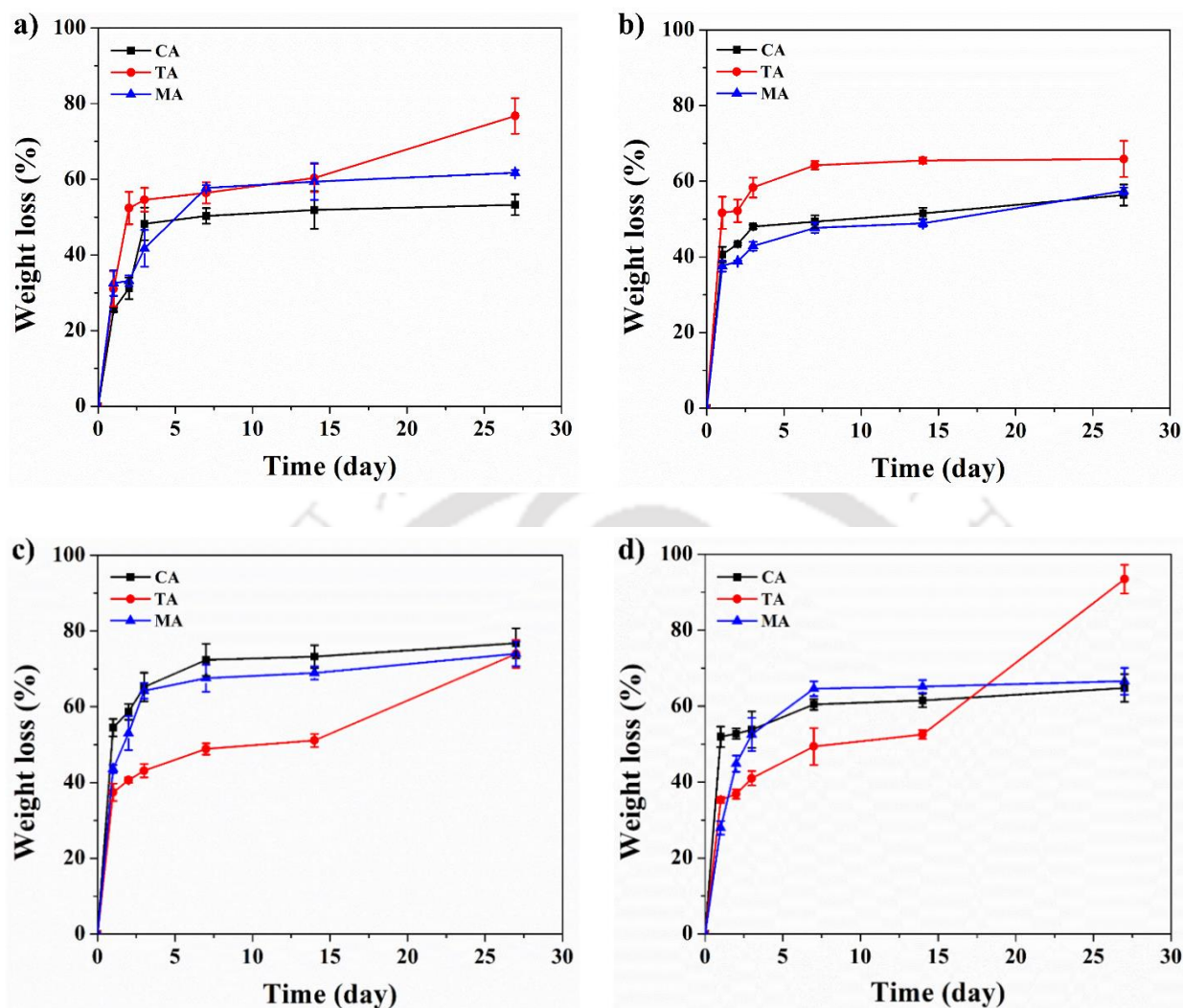


Figure 6.3: Influence of CA, TA and MA on the in-vitro degradation characteristics of alternate optimally fabricated PVA-St composite films (a) EXP-%E (b) EXP+%E (c) RSM-%E and (d) RSM+%E constitutions.

increment (with a significance level of $p \leq 0.001$, for RSM+%E, RSM-%E formulation and $p \leq 0.01$, for EXP-%E formulation) can be observed for all cases that considered TA as the organic acid additive in the optimized PVA composite film formulation. The hydrogel composite films crosslinked with TA appear to be relatively stable in comparison with the CA and MA based hydrogel composite films. This is possibly due to the presence of two $-OH$ groups in TA and

henceforth improved participation in the crosslinking reaction of the –OH groups in comparison with the CA and MA that possess only one –OH group. Thus, TA is very likely to induce prominent crosslinking but not plasticization effect. Henceforth, despite being subjected to longer exposure to water, the TA crosslinked hydrogel film exhibited lesser weight loss in comparison with those being prepared with the MA and CA.

Also, for the films being fabricated with EXP+%E formulation, it has been delineated in section 6.3 that the films being prepared with TA possessed higher solubility than the CA and MA based films. Thus, it is evident that the same formulation shall have highest degradation characteristics with respect to time in comparison to those being fabricated with CA and MA. Except for the films being fabricated with EXP-%E optimized formulation, the CA and MA based films affirmed similar degradation characteristics on the 27th day. However, the degradation rate for each day was comparatively prominent for the MA based film. During the 1st day, the MA based film indicated lower degradation content or WL in comparison with the CA based film (with a significance level of $p \leq 0.001$ and ≤ 0.01 for RSM+%E and RSM-%E formulation, respectively). However, the degradation or WL enhanced gradually to compete with the WL value indicated by the CA based film after 27 days' time frame. Ideally, a wound dressing film shall facilitate a regular and gradual increment in release of the drugs or antibiotic components from the hydrogel film matrix in comparison with the enhanced release during the initial time frame and subsequent reduction in the release of the desired materials. The latter case refers to the reduced shelf life of the hydrogel film. The CA based films exhibited the latter case and the MA based films exhibited in-vitro degradation characteristics as being desired in the ideal scenarios. Thus, MA can be very beneficial to facilitate the release characteristics of desired drugs in the composite films in comparison with the CA in the PVA composite films for wound dressing applications.

Also, for the films being fabricated with the EXP-%E formulations, irrespective of similar crosslinking extent being facilitated at the lower additive concentrations (being discussed in the SI characteristics section or section 6.2), the CA based films can be analyzed to facilitate lower WL content in comparison with the MA and TA based films (For TA and MA based film, higher WL with p -value ≤ 0.001 and ≤ 0.05 , respectively). This is being supplemented with the hypothesis that the CA facilitates stronger bonds with the PVA-St blended matrix in comparison with the MA and TA and this is due to its comparatively higher constitution of carboxylic groups than the MA and TA.

6.5 Mechanical properties measurements

Figure 6.4 (a) and (b) respectively illustrate the TS and %E variation for alternate PVA composite films with variations in the CA, TA and MA concentrations. For the all cases of optimized PVA-St composite films and corresponding constituent concentration variations, the TS and %E range were about 3.36 ± 0.12 to 7.65 ± 5.2 , 2.98 ± 0.12 to 7.75 ± 1.39 , 1.91 ± 0.23 to 5.83 ± 0.09 MPa and 9.13 to 147.86 ± 7 , 16.76 ± 1.46 to 49.58 ± 4.18 , 18.4 ± 0.84 to 103.68 ± 6.87 %, for CA, TA and MA respectively. For the case of alternate films being fabricated with the optimized EXP-%E formulation (5 w/w% PVA, 10 w/w% St, 15 wt% CA/TA/MA and 15 wt% Gl), the TS trends indicate similar values for the films being prepared with CA and TA but not MA. Fig. 6.3 indicates that compared to TA, both MA and CA based hydrogel films possess better in-vitro degradation trends. Fig. 6.4b indicates that compared to TA, both MA and CA based hydrogel films possess better %E trends. Fig. 6.4a indicates that compared to MA, both CA and TA based hydrogel films possess higher tensile strength trends for most of the cases. Thus, Fig. 6.4a and b have opposite trends. This is due to the variations in the number of –OH

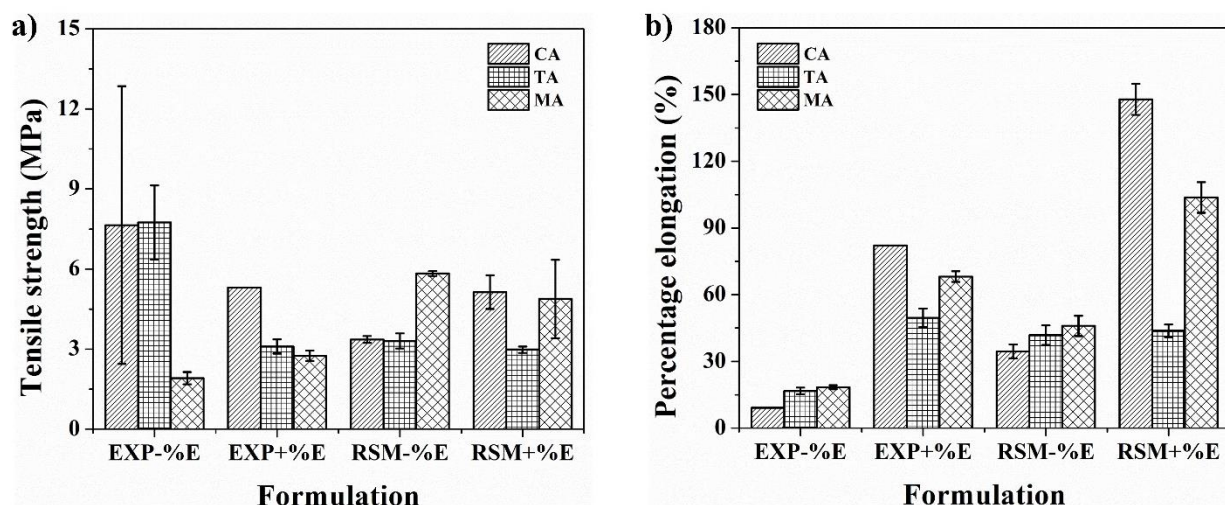


Figure 6.4: Influence of CA, TA and MA on the (a) tensile strength and (b) percentage elongation characteristics of alternate optimally fabricated PVA-St composite films.

and $-\text{COOH}$ groups in MA, CA and TA and thereby affirming that MA does not allow significant crosslinking in comparison with the CA and TA. In other words, MA does have significant plasticization but not crosslinking effect and this resulted in the trends associated to tensile strength and elongation %.

MA possessed significant weaker mechanical strength in comparison with CA and TA for the EXP-%E formulation. However, the %E characteristics affirmed lower values for the CA based EXP-%E film formulation in comparison with the TA and MA based films. These trends were similar to those being analyzed for the in-vitro degradation characteristics of the same films (elaborated in section 6.4). For both mechanical properties (TS and %E), the TA based films indicated weaker characteristics in comparison to those being prepared with the CA and MA (for RSM+%E film formulation, higher %E with $p\text{-value} \leq 0.001$) (higher TS with $p\text{-value} \leq 0.001$ for MA based RSM-%E formulation, ≤ 0.01 for CA-based EXP+%E formulation).

For films being prepared using alternate organic acid and for both EXP+%E (7.5 w/w% PVA, 7.5 w/w% St, 27.5 wt% CA/TA/MA and 27.5 wt% Gl) and RSM+%E (7.45 w/w% PVA, 6.96 w/w% St, 28.57 wt% CA/TA/MA and 18.38 wt% Gl) optimized formulations, it can be inferred that the CA based films possessed comparatively higher TS (with a significance level of $p \leq 0.01$ for EXP+%E formulation) and %E values (with a significance level of $p \leq 0.001$ for RSM+%E formulation). This is due to the reason that higher concentration facilitated better crosslinking characteristics of CA (Shi et al., 2008) in comparison with the MA and TA and henceforth higher TS values. Further, higher CA content in the polymer matrix enabled the realization of greater %E due to the higher content of free $-\text{COOH}$ groups (Shi et al., 2008). This has not been the case for the MA and TA based PVA composite films. Overall, MA can be considered as the second best option among the three organic acids in terms of the improvisation of the mechanical characteristics of the PVA-St composite films. Further, for the alternate films being prepared with EXP-%E and RSM-%E optimized formulations (5.46 w/w% PVA, 8.09 w/w% St, 22.77 wt% CA/TA/MA and 22.69 wt% Gl), the MA based films exhibited better mechanical characteristics than the CA based films (higher TS with a significance level of $p \leq 0.001$ for RSM-%E formulation). Also, these formulations can be analyzed to possess a higher constitution of the St. Henceforth, the interaction of St and MA could likely propel the pertinent characteristics. However, the same needs to be explored further in the near future investigations.

6.6 Antibacterial effectiveness of PVA-St blend films

The influence of TA and MA on the antibacterial activity of optimized formulations of PVA-St-organic acid-Gl films was investigated using *E. coli* and *L. monocytogenes*. An antibiotic, kanamycin (10 $\mu\text{g/mL}$) was deployed to serve as a positive control. Figures 6.5 and 6.6

respectively depict the photographs affirming antibacterial effectiveness of TA (a) and MA (b) based films for Gram-negative (Figure 6.5) and positive bacteria (Figure 6.6) cases respectively. For most cases, a clear inhibition zone can be observed around the film surface area. Both TA and MA based PVA-St composite films exhibited antibacterial activity towards *E. coli* and *L. monocytogenes*. However, due to the TA being strong organic acid, the TA based films affirmed more prominent effect in comparison with the MA based films. Organic acids with lower pKa values (pKa in the order of $TA < CA < MA$) indicated enhanced and efficient antibacterial activity (Ceciliani, 2017). On the contrary, the pKa level of the organic acids facilitated an acidic environment that fosters breakage of the synthesis route associated with the protein and cell membranes in the bacterial cell (Wu et al., 2017). Further, it shall be noted from the findings reported in chapter 5 that the CA based PVA composite films for all four alternate cases (EXP-%E, EXP+%E, RSM-%E and RSM+%E) exhibited even better and improvised antimicrobial activity against *L. monocytogenes* only. For the *E. coli* case, better improvisation was not evident. However, the TA and MA based PVA composite films with similar film constitutions exhibited prominent antibacterial activity for both *E. coli* and *L. monocytogenes*.

Among MA based PVA-St composite films, the EXP+%E (7.5 w/w% PVA, 7.5 w/w% St, 27.5 wt% MA and 27.5 wt% Gl) and EXP-%E (5 w/w% PVA, 10 w/w% St, 15 wt% MA and 15 wt% Gl) cases exhibited lesser antibacterial effectiveness in comparison with those being obtained for RSM+%E (7.45 w/w% PVA, 6.96 w/w% St, 28.57 wt% MA and 18.38 wt% Gl) and RSM-%E (5.46 w/w% PVA, 8.09 w/w% St, 22.77 wt% MA and 22.69 wt% Gl) cases. This is since, in comparison with the RSM+%E case, both EXP+%E and EXP-%E based PVA-MA composite films possessed lower organic acid content in the film matrix. Further, for the RSM-%E case, the PVA constitution in the film matrix has been much lower. Thereby, the matrix facilitated

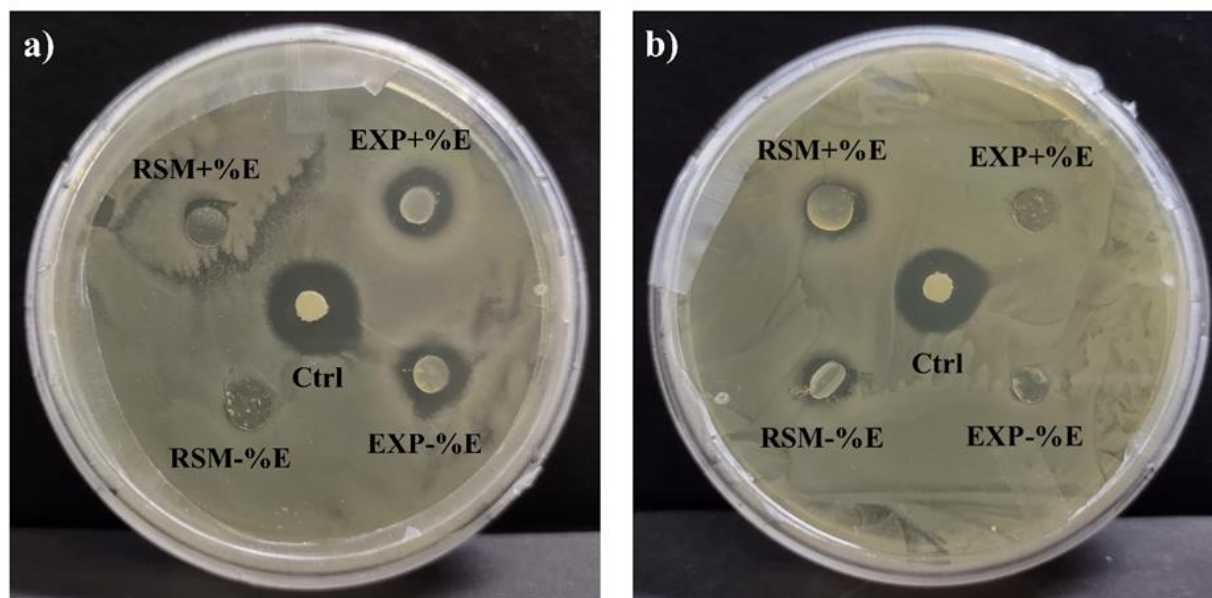


Figure 6.5: Photographs depicting antibacterial activity of optimally fabricated PVA-St composite films with respect to *E. coli* and (a) TA and (b) MA additives.

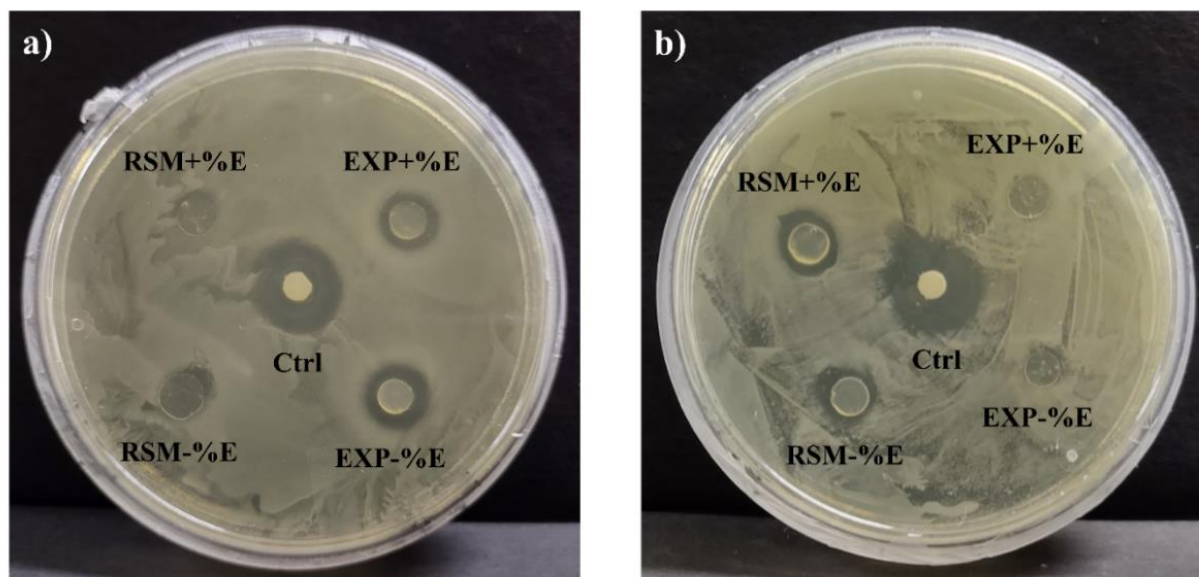


Figure 6.6: Photographs depicting antibacterial activity of optimally fabricated PVA-St composite films with respect to *L. monocytogenes* and (a) TA and (b) MA additives.

enhanced leaching of the free organic acids being prevalent in the film constitution and hence improvised acidic environment. A similar trend can be analyzed for the CA based PVA composite films. However, this has not been the case for the TA based composite films. On the other hand, it shall be noted that for all PVA composite films being prepared with EXP based film constitution and especially for the EXP+%E case, the TA based PVA composite films exhibited higher degradation characteristics (discussed in section 6.4). Hence, it is apparent that for the case that the TA content leached from the films is significantly higher in comparison with the films being prepared with any other formulation. Thereby, the same can be attributed to the possible reason for higher antibacterial activity.

6.7 In-vitro biocompatibility measurements

The in-vitro biocompatibility of the alternate organic acid (CA/MA/TA) based PVA-St composite films were investigated in terms of their cell growth potential based on RGR % after 48 h of treatment using HEK cells. Figure 6.7 and 6.8 respectively depict the RGR % of the extract (treatment solution) after 24 h and 48 h of incubation with alternate organic acid PVA-St composite film formulations. Four alternate formulations of the organic acid based PVA-St films (based on film constitution corresponding to EXP-%E, EXP+%E, RSM-%E and RSM+%E cases) were soaked in the FBS supplemented DMEM media for 24 h and 48 h. Thereafter, the films were collected from the media and the same media was diluted for three different levels of dilution namely 5, 10 and 100 μ L (5, 10 and 100 % volume proportions, respectively) for subsequent analysis.

According to the ISO guidelines (GB/T16175-1996 standard), in comparison with the control sample, the specimen sample exhibiting <75 % RGR shall be concluded to be having toxic

potential. In other words, such materials are not promising solutions to serve wound dressing applications. Thereafter, the experimental investigations affirmed that the TA based PVA composite film exhibited such toxic potential after 48 h of extraction period. For EXP-%E and EXP+%E formulation, the RGR of the HEK cells treated with 100 μ L treatment or extract sample has reduced significantly ($p \leq 0.01$ and ≤ 0.001 , respectively) in comparison to the control sample. These two formulations altering with TA were previously observed to obtain higher

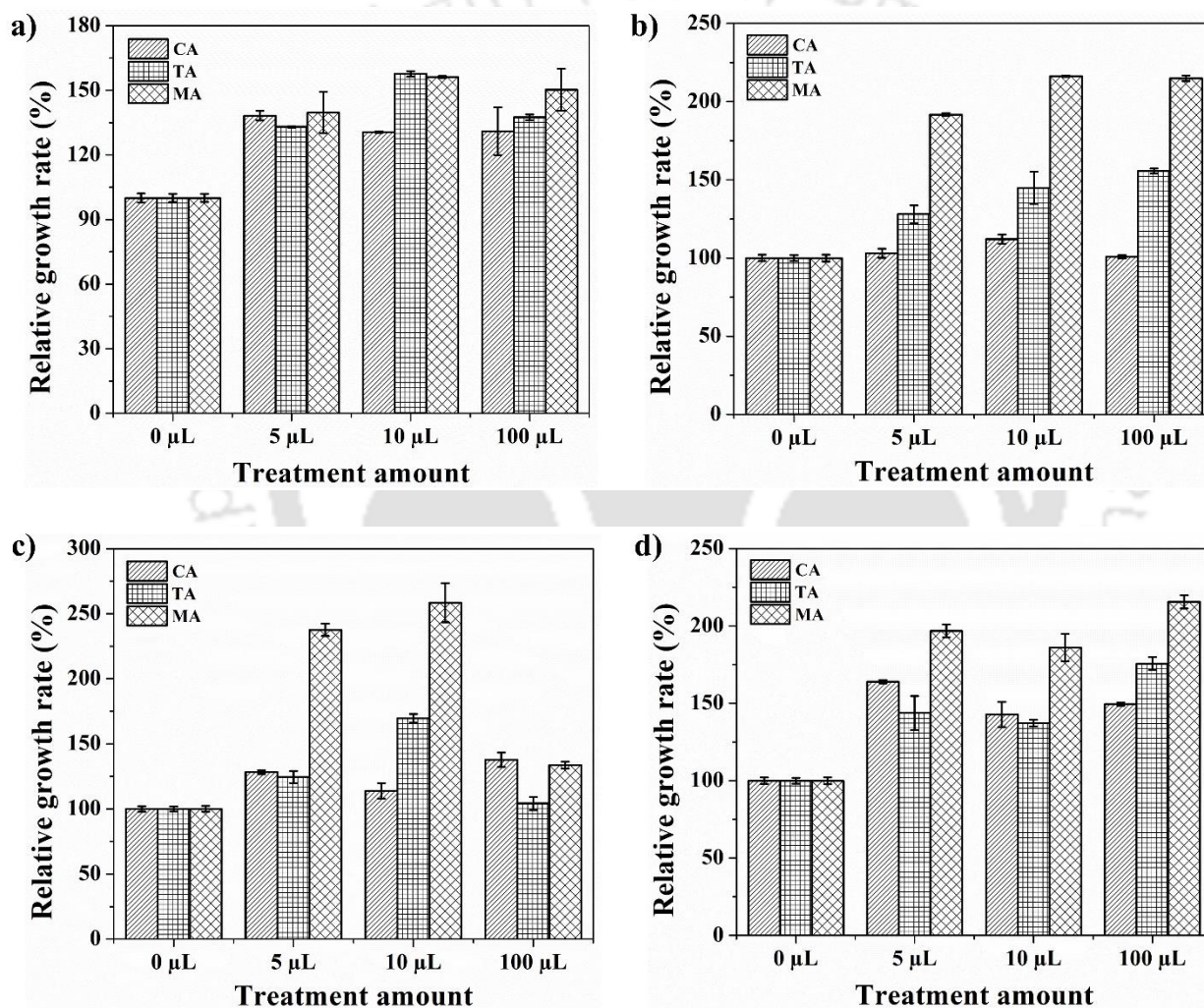


Figure 6.7: Influence of CA, TA and MA on the relative growth rate characteristics of 24 h extracted sample with HEK cells and alternate optimally fabricated PVA-St composite films (a) EXP-%E (b) EXP+%E (c) RSM-%E and (d) RSM+%E cases.

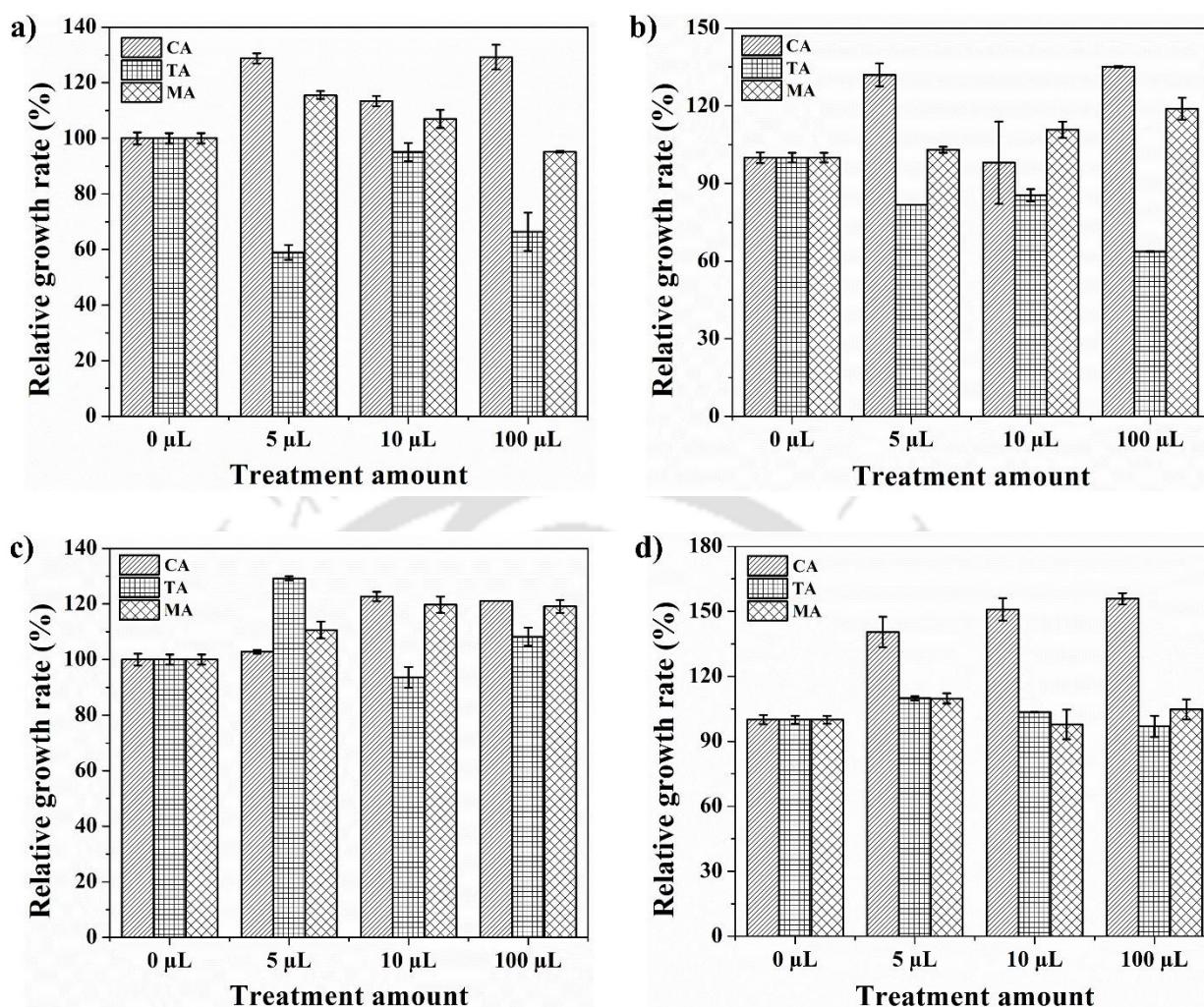


Figure 6.8: Influence of CA, TA and MA on the relative growth rate characteristics of 48 h extracted sample with HEK cells and alternate optimally fabricated PVA-St composite films (a) EXP-%E (b) EXP+%E (c) RSM-%E and (d) RSM+%E cases.

degradation characteristics in section 6.4. Subsequently, the weight loss driven leaching of TA from both of the formulation facilitated higher acidity and toxicity. Hence, TA based EXP-%E and EXP+%E formulation were observed to achieve higher antibacterial effectiveness (in section 6.6) but poor in-vitro biocompatibility in comparison with CA and MA. For 24 h extract treatment case, compared to the TA and CA based films, the MA based PVA composite films

exhibited the highest amount of RGR (with a significance level of $p \leq 0.01$ and ≤ 0.05 respectively, for both CA or TA based EXP+%E, RSM+%E formulation; For RSM+%E formulation p -value ≤ 0.01 respective to CA and ≤ 0.05 respective to TA) for all film constitutions and treatment dilution cases (5, 10 and 100 μ L). For EXP-%E, EXP+%E and RSM+%E formulation, the RGR of the HEK cells treated with 100 μ L treatment or extract sample has elevated significantly ($p \leq 0.05$ for EXP-%E and ≤ 0.001 For EXP-%E, EXP+%E and RSM+%E). However, for the case of 48 h of extract treatment, a significant reduction in RGR was observed for the MA based PVA composite films. Further, for the case of 48 h extract samples, the CA based PVA composite films indicated a steady proliferative effect on the cell growth along with incremental WL of the film driven with enhanced leaching after 48 h of incubation.

In this regard, it can be recalled from section 6.4 that the in-vitro degradation rate of MA based PVA composite films was significantly higher than those being prepared with the CA and for the 1st and 2nd days of the degradation related incubation period. On the contrary, after 48 h of extraction, the leached MA into the media is anticipated to detrimentally influence cell growth. This resulted in a lower prolific activity for the case, where 48 h of extraction sample used as treatment. However, in corroboration with the steady increment in the WL, the CA based PVA composite films exhibited steady prolific activity. Among all films being tested for the RGR characteristics, the MA based PVA composite films being prepared with the RSM+%E film constitution (7.45 w/w% PVA, 6.96 w/w% St, 28.57 wt% MA and 18.38 wt% Gl) indicated the highest RGR of 215.59 % (with respect to control sample data) after using 24 h extracted sample as treatment (Figure 6.7). Comparatively, the CA based films exhibited significantly lower values (149.55 %). Thereafter, for the same case, co-culturing procedures affirmed that the RGR of the MA based PVA composite films reduced significantly for the 48 h extracted sample

(104.78 % with respect to the control sample). However, the RGR of the CA based PVA composite films has been comparatively higher (155.87 %). This is due to the larger detrimental effect of MA leaching in due course of extraction, which is not the case for the CA. In summary, both MA and CA based PVA-St-GI composite films responded sensitively and positively towards cell growth and thereby affirmed their possible applications as customized bio-medical products.

6.8 Summary

Several useful inferences can be deduced from this work. These have been presented as follows.

- Among all alternate organic acids, (CA, MA and TA), apart from CA, the MA can be a fascinating choice for the fabrication of defect free PVA composite films for wound dressing applications.
- Endowed with comparatively weakest crosslinking characteristics, the MA based PVA composite film for RSM+%E formulation exhibited highest SI values (739.29 ± 5.24 %) but not similar solubility values. The MA based films possessed 61.59 ± 1.21 % solubility which is not significantly higher in comparison with the CA (75.88 ± 4.34 %) and TA (61.81 ± 1.63 %) based PVA composite film solubility characteristics.
- The MA based PVA composite film for RSM+%E formulation exhibited lower WL (27.96 ± 1.81 %) during the initial time period in comparison with the CA based film (51.93 ± 2.71 %). However, during 27 days time period, the WL of the MA based films increased substantially (66.56 ± 3.51 %) to reach values that are similar to those being evaluated for the CA based PVA composite films (64.79 ± 3.65 %). Such slow and steady

degradation characteristics are promising for the delayed drug release, a condition being considered as the most desired feature of wound dressing products.

- In addition to the SI and in-vitro degradation physical characteristics, despite being comparatively weaker crosslinker, the MA induced promising mechanical characteristics of the PVA composite films. This has been the case for the MA based films in comparison with the TA based films. While the mechanical characteristics of CA based PVA films for RSM+%E formulation were the highest (5.14 ± 0.63 MPa TS and 147.86 ± 7 % elongation), the MA based PVA films exhibited higher mechanical characteristics (4.88 ± 1.47 MPa TS and 103.68 ± 6.87 % elongation).
- Overall, it may seem that no difference exists for the RGR (%) trends of CA, TA and MA based hydrogel films. However, for a chosen sample formulation and dilution concentration, the TA based composite film has been analysed to possess less than 75 % RGR (63.71 %) in comparison with the MA and CA based films that possessed 118.89 and 135.09 % RGR respectively, for 48 h of extracted sample. Thereby, the TA based films can be concluded to possess greater toxicity in comparison with the CA and MA based films. Also, the MA based extracted treatment sample exhibited higher cell proliferation in comparison with the CA based sample. Thus, these variations are highly subjective and cannot be considered to be similar.
- Further, the quantitative characterization of the PVA-St composite films being prepared with alternate organic acids affirmed the efficacy of MA based films in terms of positive antibacterial effectiveness and cell growth for HEK cells. The best MA based PVA-St composite film exhibited the highest RGR of 215.59 ± 7.36 % after 24 h of incubation, which is significantly higher than the corresponding value obtained for the CA based

PVA composite films (149.55 ± 1.92 %). However, the extended experimentation affirmed further need to optimize MA content due to its detrimental effect of substantial leaching in due course of the prolonged treatment.

In summary, among all organic acids, the MA based PVA composite films exhibited promising characteristics and need to further fine tuned in terms of the film constitution to further improvise upon the physical and biological characteristics and thereby pave the way towards enriching applications as cost effective wound dressing materials.

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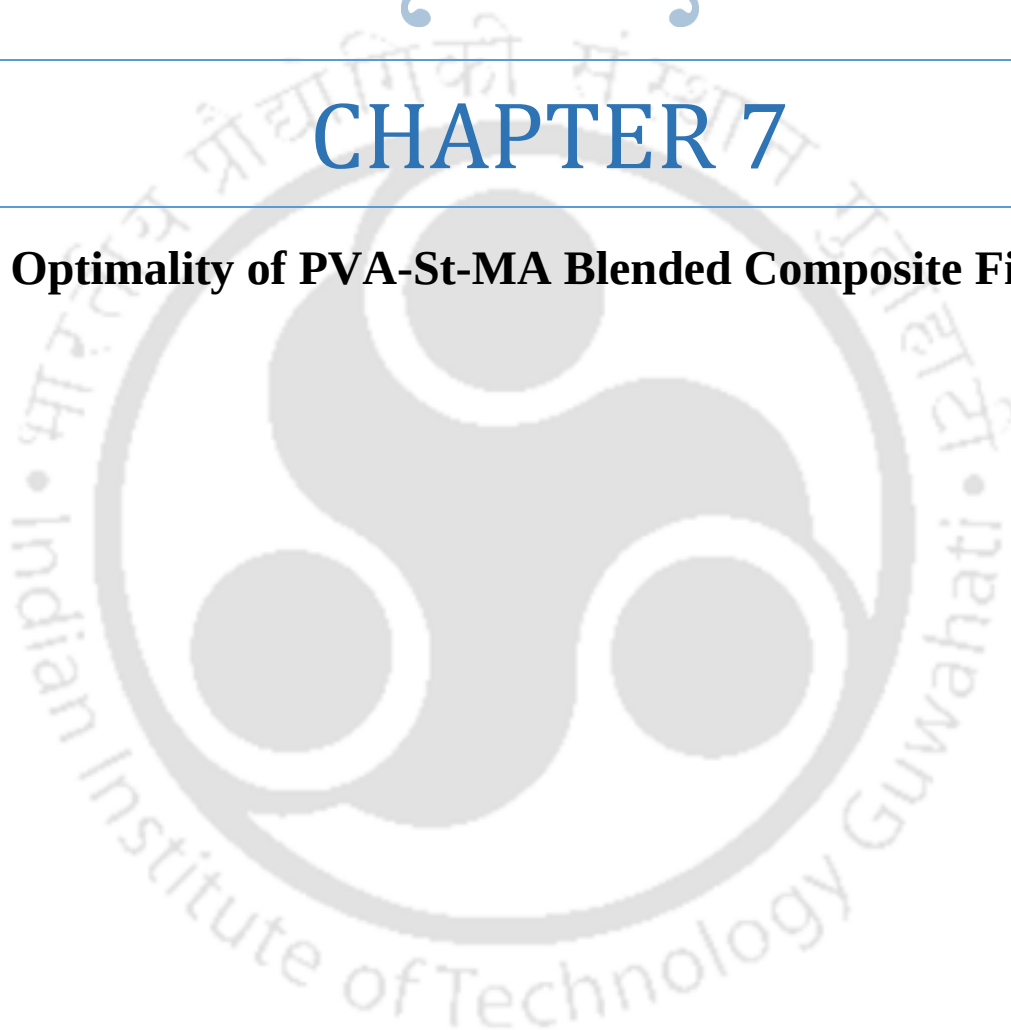
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CHAPTER 7

Optimality of PVA-St-MA Blended Composite Films





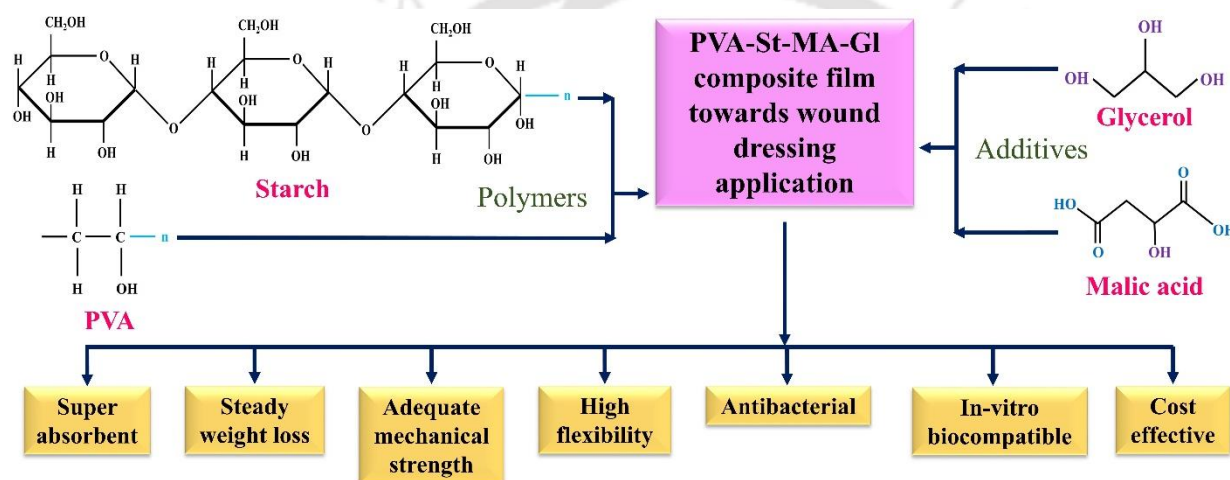
Optimality of PVA-St-MA Blended Composite Films

This chapter addresses and devotes towards the assimilation of experimental data with the numerical optimization of a ternary formulation of MA based PVA-St composite films. The findings refer to a fixed choice of GI (parameter) and variant choices of PVA, St and MA concentrations as independent variables. The PVA composite film efficacy was broadly evaluated in terms of SI, in-vitro degradation, TS and %E. The organization of the chapter's contents is as follows. Firstly, section 7.1 orients the reader with respect to the relevant background and specific novelty in the specific area of research in the field of PVA-St composite films. Thereafter, sections 7.2, 7.3, 7.4 and 7.5 respectively summarize findings related to the identification of the best fit model and the associated role of binary interaction parameters in influencing the evaluated responses namely SI, in-vitro degradation, TS and %E. Thereafter, section 7.6 briefly outlines the numerical optimization based results of the ternary PVA composite film formulation. Following this, the cost efficacy of the identified optimal formulations has been addressed in section 7.7. Eventually, section 7.8 of the thesis devotes towards a comparative assessment of the best findings with those being indicated in the literature. Finally, sections 7.9 and 7.10 devote towards the qualitative assessment related findings of the optimized film constitution and associated summary of the key findings.

Overview

The chapter summarizes the findings associated to the RSM design based experimental studies being conducted for the optimality of ternary formulations of PVA (4.5 – 10.5 w/w%), St (4.5 –

10.5 w/w%) and MA (12 – 43 wt%) and fixed choice of GI content (18.38 wt%) associated to the MA based PVA composite films for wound dressing applications. The optimality was addressed in terms of SI, 27 days based WL, TS and %E. The RSM based numerical optimization affirmed excellent combinations of water absorption (583.83 % SI), in-vitro degradation (65.47 % WL) and flexibility (99.53 %E) characteristics and acceptable TS (5.57 MPa) characteristics. These findings are novel and affirm the efficacy of MA based PVA composite films for wound dressing applications.



7.1 Introduction

This work addresses the targeted investigations to visualize upon the compatibility of MA, St and PVA to further improvise upon the composite film characteristics through a systematic variation in the constituent concentrations. The central goal of such investigations is to identify acceptable combinations of PVA composite film constitutions that serve towards wound dressing applications. The systematic variation in the constituent concentrations was achieved through a rigorous experimental approach involving the statistical design based experimental methodology. Finally, based on best fit models to represent responses as functions of constituent

concentrations, rigorous numerical optimization of the blend was targeted to achieve the optimal PVA-St-MA-Gl constitution in the solution casting based wound dressing compatible polymer hydrogel films. Thus, the methodology allowed a useful insight into the complex interaction between polymers (PVA and St) and additive (MA) using design expert software. The same was not addressed in the relevant literature being elaborated in chapter 1. Further, it shall be noted that for all cases, the glycerol content was kept fixed and only the concentrations of PVA, St and MA have been duly varied as degrees of freedom. Thereafter, using analysis of variance statistical approach, the best fit models were identified for all responses namely SI, in-vitro degradation, TS and %E. Further, contour plots were being developed for the needful analysis of the response surfaces. Thereby, useful insights have been gained with respect to the associated binary interactions of any two independent variables or degrees of freedom. Finally, numerical optimization, cost analysis, antibacterial effectiveness and cytotoxicity assay analyses have been considered to evaluate upon the efficacy of the optimized PVA-St-MA-Gl composite films towards wound dressing applications.

7.2 Swelling index

7.2.1 Identification of best fit model

Using central composite design based response surface methodology design approach in the design expert software, the model fitness studies affirmed the best fitness of quadratic expression model to represent the SI as a non-linear function of the degrees of freedom namely PVA, St and MA concentrations. The relevant expression being obtained from the response surface methodology based analysis is as follows:

$$SI = +538.85 + 49.01A - 191.54B - 2.46C + 24.34AB + 15.37AC + 33.03BC - 71.26A^2 + 23.43B^2 - 72.71C^2 \quad (13)$$

In the above expression, *A*, *B* and *C* refer to the independent variables namely PVA, St and MA concentration, respectively. The analysis of variance based statistical analysis of the above expressed best fit quadratic model affirmed upon its good fitness in terms of insignificant *p*-value (<0.0001) and higher combinations of the regression coefficient (98.09 %) and *F* values (100.01) (Table 7.2 b).

For all independent variables, associated parameters in the above expression have been positive and confirmed that the SI increased with their increment. Further, among all 20 alternate experimental data sets being suggested by the RSM (Table 7.1), the seven numbered data set affirmed the highest value of experimentally obtained SI value of 961.98 %. This corresponds to a film constitution of 7.5 w/w% PVA, 2.5 w/w% St, 27.5 wt% MA and 18.38 wt% Gl.

7.2.2 Response surface plots

The three dimensional response surface graphs of SI with respect to any two independent variables have been depicted in Figure 7.1. From experimental investigations, the obtained value range of SI varied between 961.98 – 155.75 % for the independent variable range of PVA, St and MA concentration as 2.45 – 12.55 w/w%, 2.45 – 12.55 w/w% and 1.43 – 53.57 wt% (Table 7.1). The St exhibited immense influence towards SI reduction. On the other hand, an almost negligible effect was prevalent for the MA towards the independent variable. Among single and binary interaction terms, the PVA-MA and St-MA interactions respectively indicated a significant positive influence upon the SI. Among squared terms of the independent variables, only MA affirmed a similar and strong negative effect towards the SI. The two other terms indicated a contrary effect with respect to their individual variable terms. However, the contrary

Table 7.1: CCD-RSM experimental design based data summary of PVA-St-MA blended composite films.

S. no.	A: PVA concentration (w/w%)	B: St concentration (w/w%)	C: MA concentration (wt%)	GI concentration (wt%)	Response 1: Swelling index (%)	Response 2: In-vitro degradation (%)	Response 3: Tensile strength (MPa) ¹	Response 4: Percentage elongation (%) ²
1	12.55	7.5	27.5	18.38	443.34±4.92	63.36±4.83	3.75	82.7
2	7.5	7.5	27.5	18.38	525.86±5.23	65.93±4.15	5.44	114.63
3	7.5	7.5	27.5	18.38	546.61±5.19	67.2±4.69	4.55	122.95
4	4.5	10.5	12	18.38	155.75±4.71	81±4.89	7.95	6.06
5	7.5	7.5	27.5	18.38	577.62±3.91	62.46±4.86	5.43	115.73
6	7.5	7.5	53.57	18.38	335.71±4.97	73.47±3.73	2.47	190.85
7	7.5	2.45	27.5	18.38	961.98±5.45	54.75±4.4	6.2	73.04
8	7.5	7.5	1.43	18.38	342.85±4.54	50.57±4.7	10.88	7.26
9	2.45	7.5	27.5	18.38	243.43±4.98	88.72±0.95	1.98	23.72
10	10.5	4.5	12	18.38	633.34±5.41	45.7±0.73	8.95	47.97
11	4.5	4.5	43	18.38	527.27±5.21	70.31±0.76	1.46	88.83
12	10.5	10.5	43	18.38	348.35±4.79	69.4±1.02	1.85	173.38
13	4.5	10.5	43	18.38	181.34±5.44	88.32±2.61	1.93	109.23
14	4.5	4.5	12	18.38	625.16±5.6	60.33±2.76	5.99	15.44
15	7.5	7.5	27.5	18.38	546.44±4.79	67.11±0.77	5.34	97.89
16	7.5	7.5	27.5	18.38	496.68±4.24	65.52±2.41	5.89	123.34
17	7.5	7.5	27.5	18.38	537.77±5.51	69.7±4.07	5.8	85.3
18	10.5	4.5	43	18.38	588.27±5.64	62.13±4.99	5.17	160.07
19	10.5	10.5	12	18.38	252.61±5.74	52.17±4.77	7.25	14.52
20	7.5	12.55	27.5	18.38	260.42±4.23	75.61±3.2	3.33	57.19

¹ All samples were examined thrice and the overall SD value were $\leq \pm 1$ ² All samples were examined thrice and the overall SD value were $\leq \pm 5$

Table 7.2: (a) Alternate model fitness and (b) Best fit ANOVA parameters associated to swelling index and in-vitro degradation characteristics of PVA-St-MA composite films.

(a)

Source	Swelling index					In-vitro degradation				
	Sum of squares	df	Mean square	F value	p-value prob > F	Sum of squares	df	Mean square	F value	p-value prob > F
Mean vs Total	4168564	1	4168564			88944.94	1	88944.94		
Linear vs Mean	533926.1	3	177975.4	15.95	<0.0001	2085.4	3	695.13	29.73	<0.0001
2FI vs Linear	15359.09	3	5119.7	0.41	0.75	111.65	3	37.22	1.84	0.1891
Quadratic vs 2FI	155351.6	3	51783.86	66.15	<0.0001	217.19	3	72.4	16	0.0004
Cubic vs Quadratic	3890.03	4	972.51	1.48	0.3172	7.99	4	2	0.32	0.8541
Residual	3938.86	6	656.48			37.27	6	6.21		
Total	4881030	20	244051.5			91404.43	20	4570.22		

(b)

Source	Swelling index					In-vitro degradation				
	Sum of squares	df	Mean square	F value	p-value prob > F	Sum of squares	df	Mean square	F value	p-value prob > F
Model	704636.8	9	78292.97	100.01	< 0.0001	2414.24	9	268.25	59.27	< 0.0001
A-PVA	32797.81	1	32797.81	41.89	< 0.0001	938.81	1	938.81	207.42	< 0.0001
B-St	501045.5	1	501045.5	640	< 0.0001	560.5	1	560.5	123.84	< 0.0001
C-MA	82.82	1	82.82	0.11	0.7517	586.08	1	586.08	129.49	< 0.0001
AB	4738.14	1	4738.14	6.05	0.0337	77.71	1	77.71	17.17	0.0020
AC	1890.71	1	1890.71	2.42	0.1512	33.51	1	33.51	7.4	0.0215
BC	8730.24	1	8730.24	11.15	0.0075	0.43	1	0.43	0.09	0.7647
A ²	73175.67	1	73175.67	93.47	< 0.0001	137.15	1	137.15	30.3	0.0003
B ²	7912	1	7912	10.11	0.0098	8.2	1	8.20	1.81	0.2079
C ²	76183.49	1	76183.49	97.31	< 0.0001	50.52	1	50.52	11.16	0.0075
Residual	7828.89	10	782.89			45.26	10	4.53		
Lack of fit	4260.63	5	852.13	1.19	0.4252	16.79	5	3.36	0.59	0.7118
Pure error	3568.26	5	713.6518			28.47	5	5.69		
Cor total	712465.7	19				2459.5	19			

influence of St was not that significant in comparison to the influence of the other two variables (PVA and MA). From these response graphs, key observations with respect to the binary interactions can be summarized as follows:

- a) PVA-St interaction: The SI reduced significantly with an enhancement in the St concentration. This is due to the reason that with an increase in the St polymer concentration, crosslinking was promoted and porosity was reduced. However, an enhancement in the PVA enabled moderate initial enhancement in the SI and subsequent achievement of a steady value at higher PVA concentrations.
- b) PVA-MA interaction: The impact of PVA concentration in due course of the analysis of the PVA-MA interaction was similar to that being indicated for the PVA in the PVA-St interaction case. Despite affirming a steady but moderate SI increment up to about 9 w/w% PVA concentration, the trends confirm upon a moderate reduction in the SI value for a further increment in PVA concentration (Ahmed et al., 2018). Also, for the lower PVA concentration case, with MA increment, the SI enhanced initially and thereafter reduced significantly. This may be due to the presence of two –COOH groups in MA that enabled a lower degree of crosslinking and hence higher SI. In this regard, it can be recalled from our observations in chapter 5 that the citric acid fostered a higher crosslinking effect. This is due to the existence of three –COOH groups and hence comparatively lower SI with respect to the trends being obtained with the MA (Yoon et al., 2006, Shi et al., 2008). However, for higher MA and lower PVA concentrations, the enhanced existence of –COOH groups facilitated esterification reaction and subsequent effective bonding with the PVA –OH groups. Thereby, this effect reduced the SI.
- c) St-MA interaction: The influence of MA concentration was similar to the MA influence

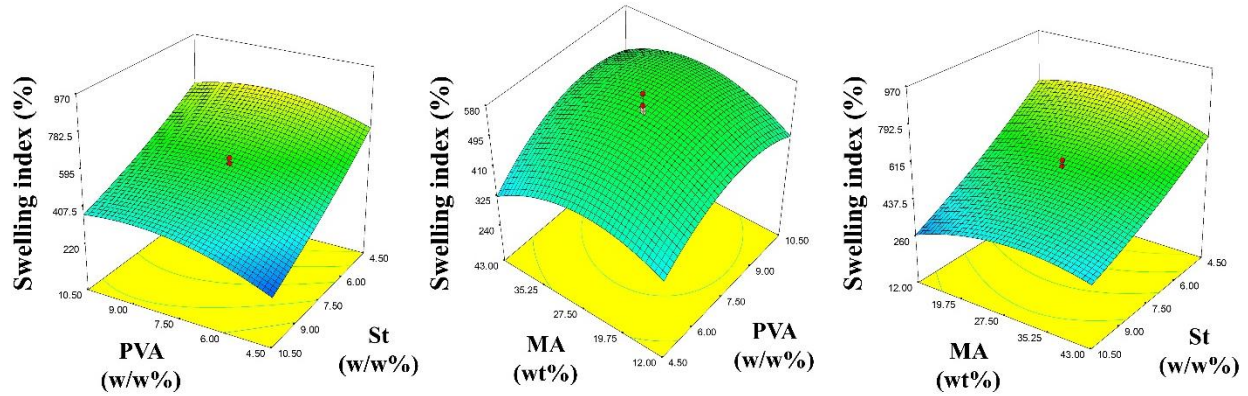


Figure 7.1: Response surface plots of swelling index characteristics of PVA-St-MA composite films.

being elaborated for the PVA-MA interaction case. However, the SI reduction was not highly significant. Despite indicating this, the influence of St concentration was similar to that being mentioned for the St in the PVA-St interaction case. Hence, it can be inferred that among PVA, MA and St, the MA influenced or interacted better with the PVA but not the St in due course of influencing the SI trends.

7.3 In-vitro degradation

7.3.1 Identification of best fit model

Among alternate algebraic models, the RSM based design expert software indicated that the quadratic expression could be the best fit model to represent the in-vitro degradation or WL response variable. Accordingly, the model expression with relevant best fit parameters is as follows:

$$WL = +66.37 - 8.29A + 6.41B + 6.55C - 3.12AB + 2.05AC - 0.23BC + 3.08A^2 - 0.75B^2 - 1.87C^2 \quad (14)$$

ANOVA of the best fit model affirmed high significance level (p -value <0.0001), high F value (59.27) (Table 7.2 b) and good R^2 value (0.9816). Thereby, the model fitness has been concluded to be relevant from the analysis of variance. Further, it shall be noted that the data set numbered 9 affirmed the highest WL and corresponds to the composition of 2.45 w/w% PVA, 7.5 w/w% St and 27.5 wt% MA and 18.38 wt% Gl (Table 7.1).

7.3.2 Response surface plots

Figure 7.2 depicts the 3D response surface plots of in-vitro degradation or WL with respect to the variations in any two binary interactions. Considering 27 days in-vitro degradation period, the WL value range was in the range of 88.72 – 45.7 % for a corresponding variation in the independent variables as 2.45 – 12.55 w/w%, 2.45 – 12.55 w/w% and 1.43 – 53.57 wt% for PVA, St and MA concentrations respectively (Table 7.1). For the wound dressing films, the most relevant literature by Fahmy et al. (2015) and Hajian et al. (2017) that indicated promising hydrolytic in-vitro degradation values refer to 50 – 60 % for 30 % AV-PVA and 28 – 58 % for 20 & 30 % HA-PVA composite membranes (Fahmy et al., 2015, Hajian et al., 2017). Compared to these values affirmed in the relevant prior art, the design expert software affirmed a better hydrolytic WL value range. In general, the WL reduced significantly with an increase in the PVA concentrations. On the contrary, St and MA had a significant positive influence upon the WL. For the WL, among binary interactions of the independent variables, the PVA-St interaction can be identified to be the most distinguished but negative interaction. All square terms exhibited a contrary influence with respect to their individual variable terms. However, their significance level was comparatively lower. A vivid summary of the associated binary interactions is as follows:

- a) PVA-St interaction: For increasing PVA concentration scenarios, the WL reduced rapidly

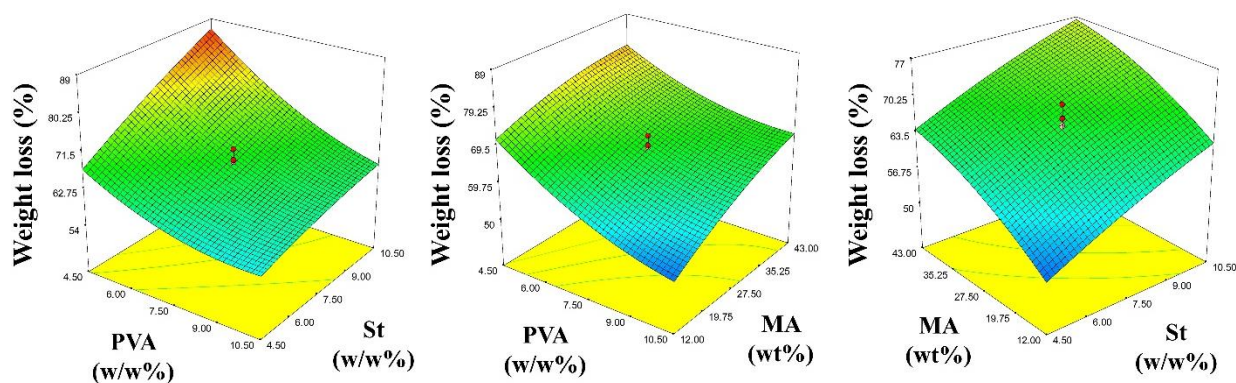


Figure 7.2: Response surface plots of in-vitro degradation characteristics of PVA-St-MA composite films.

and this is due to an enhancement in the composite matrix solid content (Ahmed et al., 2018). Further, with St being a degradable natural polymer, its influence was towards enhanced WL. Thus, the highest WL characteristics have been achieved for the combination of highest St and lowest PVA concentration in the specified independent variable range.

- b) PVA-MA interaction: For an increment in the MA, the WL trends indicated enhanced values. However, the PVA behavior for the PVA-MA interaction case was similar to that being discussed for the PVA in the PVA-St interaction case. Also, it was observed that for the case of higher PVA and lower MA concentrations, the lowest WL values were obtained. The effect has been contrary to the vice-versa influence of the independent variables. For the case of higher PVA and lower MA, most residual free -COOH groups of MA were engaged in due course of hydrogen bond formation with the PVA (Shi et al., 2008). Thereby, better intermolecular interactions were favored and thereby resulted in the discussed trend.

- c) St-MA interaction: The enhancement in the WL was similar and proportional to the corresponding enhancements in both St and MA concentrations. This is due to the reason that both St and MA are organic biodegradable materials and thereby had minimal intermolecular interaction or reaction with one another.

7.4 Tensile strength

7.4.1 Identification of best fit model

For the experimentally determined TS variable data set, using RSM based model analysis, the TS response variable has been evaluated to be the best fit with the following quadratic expression:

$$TS = +5.40 + 0.65A - 0.54B - 2.48C - 0.93AB + 0.17AC - 0.39BC - 0.83A^2 - 0.16B^2 + 0.52C^2 \quad (15)$$

The best fit regression model can be analyzed to have good fitness parameters (Table 7.3b) in terms of high significance (p -value < 0.0001) and a high F value (50.83). The ANOVA based regression expression affirmed 97.86 % fitness of the response variable in terms of statistical analysis. Table 7.1 summarizes the relevant data and affirms that the best data set corresponds to the eight numbered data set for which the highest TS of 10.88 MPa was obtained. Corresponding film constitution refers to 7.5 w/w% PVA, 7.5 w/w% St, 1.43 wt% CA and 18.38 wt% Gl.

7.4.2 Response surface plots

The 3D response surface plots of the TS variable with respect to variations of any two independent variables have been depicted in Figure 7.3. Corresponding TS variable value range refers to the range of 10.88 – 1.46 MPa for a variation in the independent variables as 2.45 – 12.55 w/w%, 2.45 – 12.55 w/w% and 1.43 – 53.57 wt% for PVA, St and MA concentrations

Table 7.3: (a) Alternate model fitness and (b) Best fit ANOVA parameters associated to tensile strength and percentage elongation characteristics of PVA-St-MA composite films.

(a)

Source	Tensile strength					Percentage elongation				
	Sum of squares	df	Mean square	F value	p-value prob > F	Sum of squares	df	Mean square	F value	p-value prob > F
Mean vs Total	516.35	1	516.35			146219	1	146219		
Linear vs Mean	93.73	3	31.24	19	<0.0001	47534.25	3	15844.75	23.61	<0.0001
2FI vs Linear	8.38	3	2.79	2.03	0.1599	1966.92	3	655.64	0.97	0.4358
Quadratic vs 2FI	15.36	3	5.12	19.94	0.0002	7318.41	3	2439.47	16.78	0.0003
Cubic vs Quadratic	1.08	4	0.27	1.08	0.4429	151.94	4	37.98	0.18	0.9433
Residual	1.49	6	0.25			1302.03	6	217		
Total	636.39	20	31.82			204492.6	20	10224.63		



(b)

Source	Tensile strength					Percentage elongation				
	Sum of squares	df	Mean square	F value	p-value prob > F	Sum of squares	df	Mean square	F value	p-value prob > F
Model	117.47	9	13.05	50.83	< 0.0001	56819.58	9	6313.29	43.42	< 0.0001
A-PVA	5.76	1	5.76	22.41	0.0008	5560.82	1	5560.82	38.25	0.0001
B-St	4.03	1	4.03	15.69	0.0027	93.74	1	93.74	0.64	0.4407
C-MA	83.94	1	83.94	326.91	< 0.0001	41879.69	1	41879.69	288.04	< 0.0001
AB	6.94	1	6.94	27.03	0.0004	121.32	1	121.32	0.83	0.3825
AC	0.24	1	0.24	0.92	0.3595	1113.48	1	1113.48	7.66	0.0199
BC	1.21	1	1.21	4.69	0.0555	732.13	1	732.13	5.04	0.0487
A ²	9.84	1	9.84	38.34	0.0001	5009.11	1	5009.11	34.45	0.0002
B ²	0.35	1	0.35	1.36	0.2709	3002.92	1	3002.92	20.65	0.0011
C ²	3.89	1	3.89	15.17	0.0030	85.5	1	85.5	0.59	0.4609
Residual	2.57	10	0.26			1453.97	10	145.4		
Lack of fit	1.44	5	0.29	1.28	0.3950	297.51	5	59.5	0.26	0.9187
Pure error	1.12	5	0.22			1156.46	5	231.29		not significant
Cor total	120.03	19				58273.55	19			

respectively (Table 7.1). The RSM based optimization of the recently fabricated PVA-St-CA-GI composite films affirmed a lower TS range of 9.92 to 0.54 MPa. From the response surface plots, it can be analyzed that the PVA fostered an enhancement in the TS. On the contrary, for an increment in the St and MA, the TS trends reduced. Further, such an effect has been prominent for the MA. The binary interactions affirmed that among all interactions, only PVA-MA interactions affirmed a positive influence upon the TS and with a very low significance level. Thus, all other interactions such as PVA-St and St-MA affirmed a negative influence upon the evaluated TS characteristics, where PVA-St interaction had a prominent significance level. Among all square terms, PVA and MA exhibited contrary influence with respect to their individual variable terms. The response surface plots can be analyzed as follows:

- a) PVA-St interaction: For lesser St content condition, the PVA increment favored the TS characteristics. On the contrary, for higher St concentration cases, after certain negligible enhancement during the initial phase, the TS reduced marginally with respect to the PVA increment. The St content increment detrimented the TS significantly for the scenario indicating high PVA content in the composite matrix. However, for the lower PVA content case, a marginal increment in the TS existed with respect to the St content increment. This is due to the reason that the addition of St to PVA detrimented towards the physical properties of the blend as PVA and St have poor compatibility with one another (Raj and Somashekar, 2004). Hence, an increment in both polymer concentrations in the composite matrix had a negative influence upon its physical stability. Therefore, either enhancement in PVA or St concentration is mandatory to achieve improvised physical stability and mechanical characteristics of the composite film.

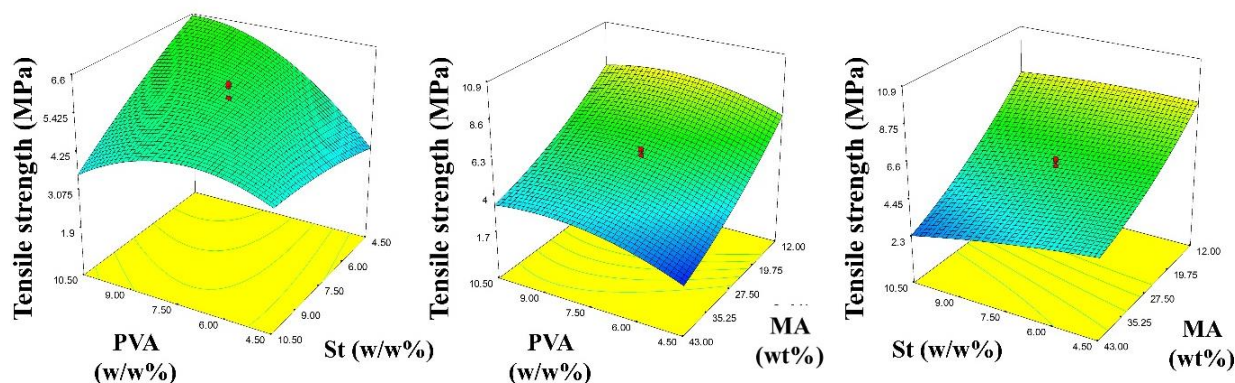


Figure 7.3: Response surface plots of tensile strength characteristics of PVA-St-MA composite films.

- b) PVA-MA and St-MA interaction: For the case of both PVA and St variations, the MA increment resulted in a reduction in the TS. However, for both higher and lower MA concentration cases, the PVA facilitated an increment in the TS. It was also noted that for the higher PVA and lower MA concentration case, the TS was highest and the trend was contrary to the trend apparent for the in-vitro degradation response and PVA-MA interaction scenario. The esterification reaction between MA and PVA enabled the full occupancy of the pertinent -COOH groups. Consequently, for the case of higher PVA and lower MA concentration, the MA did not possess free -COOH groups to further weaken the TS of the composite. Also, the St increment reduced TS but not to the extent being observed for the higher MA case.

7.5 Percentage elongation

7.5.1 Identification of best fit model

For the central composite design based RSM design targeting the model fitness of %E with respect to the independent variables, the ANOVA based best fit model analysis affirmed the relevance of quadratic expression. Thereby, the best fit model to represent %E is expressed as:

$$\begin{aligned} \%E = & +109.75 + 20.18A - 2.62B + 55.38C - 3.89AB + 11.80AC + 9.57BC \\ & - 18.64A^2 - 14.44B^2 - 2.44C^2 \end{aligned} \quad (16)$$

For the best fit model, model fitness parameters affirmed high significance (p -value < 0.0001 , F value 43.42 and R^2 value of 0.9750) (Table 7.3 b) and ensured upon the good degree of fitness.

Among all measured data sets, data set numbered 6 indicated the highest %E characteristics. The data sets corresponded to 7.5 w/w% PVA, 7.5 w/w% St, 53.17 wt% MA and 18.38 wt% Gl (Table 7.1).

7.5.2 Response surface plots

Figure 7.4 depicts the 3D response surface plots of %E with respect to the binary variations of constituent concentrations (PVA, St and MA). The experimentally obtained %E value range varied in the range of 190.85 – 6.06 % for a variation in the PVA, St and MA concentrations as 2.45 – 12.55 w/w%, 2.45 – 12.55 w/w% and 1.43 – 53.57 wt% respectively. From the model analysis, it can be seen that the %E enhanced with PVA and MA increments but reduced with the St increment. The binary interactions illustrated that the PVA-MA and St-MA interactions contributed positively towards %E. On the other hand, the PVA-St interactions inflicted a negative effect despite affirming a lower significance level for all binary combinations. Among all square terms, except for St, PVA and MA exhibited contrary effects with respect to their

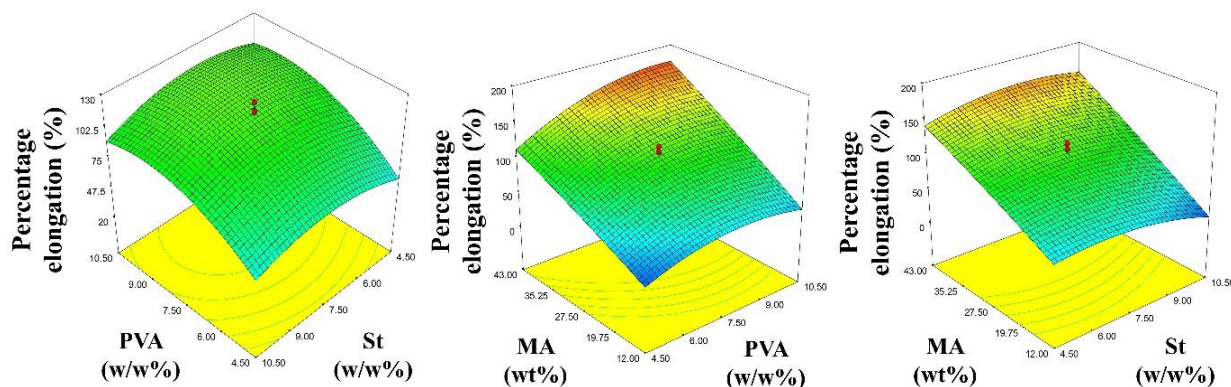


Figure 7.4: Response surface plots of the percentage elongation characteristics of PVA-St-MA composite films.

individual variable terms. This is in conjunction with the observation that the prominence existed only for the PVA and St squared terms. The %E response surface plots can be analyzed as follows:

- a) PVA-St interactions: Irrespective of higher or lower St concentrations, the %E increased significantly with an enhancement in the PVA concentration up to almost 9 w/w%. A further increment in PVA confirmed steady values. The influence of St concentration was negligible despite observing the fact that the initial St content increment facilitated a steady increase in the %E. Hence, it has been once again observed that the PVA promoted good mechanical stability and the St inflicted poor physical strength.
- b) PVA-MA interactions: PVA and MA content enhancement favored the %E trend. Among these, the MA had a prominent influence. Also, for higher MA cases, the increment in %E was significant with respect to an increase in the PVA concentration.
- c) St-MA interaction: The MA content based %E trends were similar to those being discussed for the PVA-MA interaction case. Similar to other organic acids such as CA, the plasticization property of the MA promoted the %E characteristics of the film.

However, a contrasting but negligible influence has been apparent for amplified variation of the St content.

7.6 Compositional optimality

Several available prior art indicated the wound dressing films to possess a minimal or acceptable value range for the characterization parameters. For a wound dressing applicable composite film, the physical characteristics have been affirmed in these prior art as (a) super absorbent film with $SI \geq 260\%$ (Ahmed et al., 2018) (b) hydrolytic in-vitro degradation based localized antibacterial agent (at least 48 % for 27 days incubation) (Hajian et al., 2017) (c) standard TS of 3.5 MPa (Basiak et al., 2018) and (d) %E of 12.5 % (Devi et al., 2012) that ensure stable shape characteristics even in the environment of wound exudates. Among all measured data being summarized for the MA based PVA-St film (Table 7.1), the best data refers to a composition of 7.5 w/w% PVA, 7.5 w/w% St, 27.5 wt% MA and 18.38 wt% Gl (central point data set with a serial no of 5) that yielded MA based film characteristics as 577.62 % SI, 62.46 % WL, 5.43 MPa TS and 115.73 % elongation. Further, all data being obtained through experimental efforts were subjected to numerical optimization using the best fit ANOVA based regression models. Consequently, the optimal data indicated by the software referred to a formulation that exhibited the best values for all characterization parameters but not a single property. For each property, the optimal value was significantly higher than the minimum acceptable value range being conveyed in the literature. Further, the numerical optimization was subjected to a bias to indicate the generation of optimal data sets within a specified range for all four responses or dependent variables. The specified bounds for such investigation were to consider the lowest value set based on the literature indicated values ($SI > 260\%$, $WL > 48\%$, $TS > 3.5\text{ MPa}$ and $\%E > 12.5\%$)

and the highest value was set to be the highest response values being obtained for the experimental data sets (among MA based PVA-St films).

The RSM based numerically optimized data set referred to 5.76 w/w% PVA, 7.41 w/w% St, 26.46 wt% MA and 18.38 wt% Gl for which the optimal responses have been obtained as 493.64 % SI, 71.58 % WL, 4.92 MPa TS and 88.61 % elongation. For the CA based PVA-St composite film, the best findings (numerical optimization using RSM approach and with similar criteria) being reported in chapter 5 refer to 7.5 w/w% PVA, 7 w/w% St, 28.6 wt% CA and 18.4 wt% Gl. For this film, the optimal responses have been indicated to be 300.48 % SI, 51.4 % WL, 4.98 MPa TS and 87.54 % elongation. Further, to achieve further improvement in the inexpensive film cost (based on retail cost of the deployed materials), additional bias was supplied to the design expert software. This referred to considering the lower limit as the already specified values and the higher limit was based on the optimal film constitution indicated for the CA based PVA-St composite film. Accordingly, the provided bias referred to the inclusion of additional compositional constraints as 4.5 – 7.45 w/w% for PVA, 4.5 – 6.96 w/w% for St, 12 – 28.57 wt% for MA. Further, with a similar methodology, the dependent variable range has also been constrained as 300.48 – 961.98 % for SI, 51.4 – 88.72 % for WL, 4.98 – 10.88 MPa for TS and 87.54 – 190.85 % for %E. In this regard, it shall be noted that the higher bounds for the responses have been set as the highest response values being obtained for all experimental data sets (among MA based PVA-St films). Further, the lower bounds for the case refer to the optimal response values being obtained for the CA based PVA-St films. For the case, the optimized formulation refers to 6.92 w/w% PVA, 6.67 w/w% St, 25.96 wt% MA and 18.38 wt% Gl. For the indicated data set, the optimal responses refer to 583.83 % SI, 65.47 % WL, 5.57 MPa TS and 99.53 % elongation.

In summary, two optimal data sets have been obtained from the RSM based numerical optimization approach. These referred to either a general optimization approach (Opt 1) or optimization supplemented with an additional constraint to reduce the retail cost of the deployed precursors (Opt 2). In other words, the onus is towards further reducing film constitution related materials cost in comparison with the CA based PVA-St film retail material cost.

7.7 Retail cost analysis

With respect to the retail cost of the materials being deployed, the optimized PVA-St-MA-GI composite films (225 cm² area) have been compared with the optimized CA based PVA-St formulation being reported in our earlier investigation (chapter 5). In this regard, it shall be noted that the MA has been expensive than the CA and comparatively possesses a lower number of –COOH groups. Hence, MA has poor crosslinking ability than the CA. Such variation is very likely to facilitate better characteristics of SI and WL (in-vitro degradation). However, the relevant challenge was to find appropriate (reasonably higher) mechanical properties by considering an affordable MA content. Table 7.4 of the chapter summarizes the cost comparison analysis of the best CA and MA based optimized films. The table reports two alternate formulations based on the optimal data sets being indicated in our earlier efforts.

Fortunately, the optimized MA based PVA-St film has been comparatively inexpensive with respect to the CA based optimized film. Despite indicating a devaluation of the physical properties, the cost related benefits are promising. The optimal MA based PVA-St film constitution (5.76 w/w% PVA, 7.41 w/w% St, 26.46 wt% MA and 18.38 wt% GI) has been 12.61 % inexpensive in comparison with the CA based optimal PVA-St film constitution (7.5 w/w% PVA, 7 w/w% St, 28.6 wt% CA and 18.4 wt% GI). Incidentally, the optimized PVA-St-

Table 7.4: A summary of optimal composition, response characteristics and cost parameters of PVA-St-MA-GI and PVA-St-CA-GI composite films.

Composition				Swelling index (%)	In-vitro degradation (%)	Tensile strength (MPa)	Percentage elongation (%)	Total cost (Rs)	Cost (Rs/cm ²)
PVA (w/w%)	St (w/w%)	GI (wt%)	CA (wt%)						
7.45	6.96	18.38	28.57	300.48	51.4	4.98	87.54	42.29	0.188
PVA (w/w%)	St (w/w%)	GI (wt%)	MA (wt%)						
5.76	7.41	18.38	26.46	493.64	71.58	4.92	88.61	36.95	0.164
6.92	6.67	18.38	25.96	583.83	65.47	5.57	99.53	40.36	0.179

Gl-MA film indicated respectively increments of 64.28, 39.26 and 1.22 % in SI%, WL% and %E, characteristics in comparison with the reported characteristics of the optimized PVA-St-Gl-CA film. Among all considered characterization parameters, only TS was reduced marginally by 1.2 % with respect to the TS being evaluated for the optimized PVA-St-Gl-CA optimized film. Further, without devaluation of any evaluated response characteristics, the reported alternate optimal MA based composition (6.92 w/w% PVA, 6.67 w/w% St, 25.96 wt% MA and 18.38 wt% Gl) was about 4.56 % inexpensive than the PVA-St-Gl-CA optimized film. For the case, in comparison with the optimal characteristics of the CA based PVA-St composite films, the SI, WL, TS and %E enhanced by about 94.3, 27.37, 11.85 and 13.7 % respectively.

7.8 Comparative analysis with the optimal prior art data

A comparative assessment of the design expert software based optimized formulation and best prior art data was considered. The outcome has been summarized in Table 7.5. It can be observed that in comparison with the best findings being summarized in chapter 5, the PVA composite film properties increased significantly (300.48 to 583.83 % for SI, 51.4 to 65.47 % for WL, 4.98 to 5.57 MPa for TS and 87.54 to 99.53 % for %E). Only one prior art (Yoon et al., 2006) was found to be relevant for the comparative assessment with the carried work in terms of the MA concentration effect on the physical properties of the PVA-St blended films. However, the authors did not elaborate upon the optimality of such concentrations. On the contrary, the best composition of wound dressing applicable MA based PVA-St composite film was interpreted by our research group using the data trends being reported by Yoon et al. (2006). The data suggested that for a fixed choice of PVA and St (each 2.22 w/w%) and MA (0.89 w/w%), the composite film exhibited 270 % SI, 38 % solubility, 25 MPa TS and 100 % elongation.

Table 7.5: A comparative summary of optimal process-product characteristics of PVA-St-MA composite films and relevant prior art data.

S. No.	Work	Drying temperature (°C)	Drying time (h)	Optimized aqueous precursor constitution (w/w%)				Swelling index (%)	In-vitro degradation (%)	Tensile strength (MPa)	Percentage elongation (%)
				PVA	St	MA	GI				
1.	This work	50	12	6.54	6.31	3.34	2.36	583.83	65.47	5.57	99.53
2.	Yoon et al. (2006)	50	12	2.22	2.22	0.89	-	270	-	25	100
				PVA	St	CA	GI				
2.	Objective 3	50	12	6.99	6.53	3.87	2.49	300.48	51.4	4.98	87.54

Except for TS, the optimal film characteristics being found in this work indicated superior characteristics for all other characterization parameters. Further, other prior art was not considered for the comparative assessment. This is due to the reason that the optimization of film constitution and subsequent fabrication was with respect to an alternate base polymer and for different applications. Further, it is interesting to note that, in comparison with the best findings reported in chapter 5 of the thesis, the MA based optimal PVA-St composite films possessed very high combinations of SI, WL and %E. Hence, the MA based PVA-St composite films can be concluded to perform excellently in comparison with those being reported in the earlier findings of our work.

7.9 Qualitative measurements of optimally fabricated composite films

7.9.1 Antibacterial activity

The antibacterial activity of optimized formulations of PVA-St-MA-GI films (optimized formulation based on criterion 1 named as Opt 1 and criterion 2 named as Opt 2) and a control sample (kanamycin, 10 $\mu\text{g/mL}$) have been investigated using *E. coli* and *L. monocytogenes*. The targeted formulations include that being obtained through the general optimization approach (Opt 1) and the formulation being achieved with an additional constraint associated with the retail cost of the deployed precursors (Opt 2). For both cases, a similar extent of antibacterial growth has been confirmed around the film surface area (Figure 7.5). Thus, it is apparent that a clear inhibition zone against both *E. coli* and *L. monocytogenes* has been affirmed by both optimally formulated films. Among both optimized formulations, the Opt 2 formulation (6.92 w/w% PVA, 6.67 w/w% St, 25.96 wt% MA and 18.38 wt% GI) exhibited marginally improved and promising results of antibacterial activity in comparison with the Opt 1 formulation (5.76

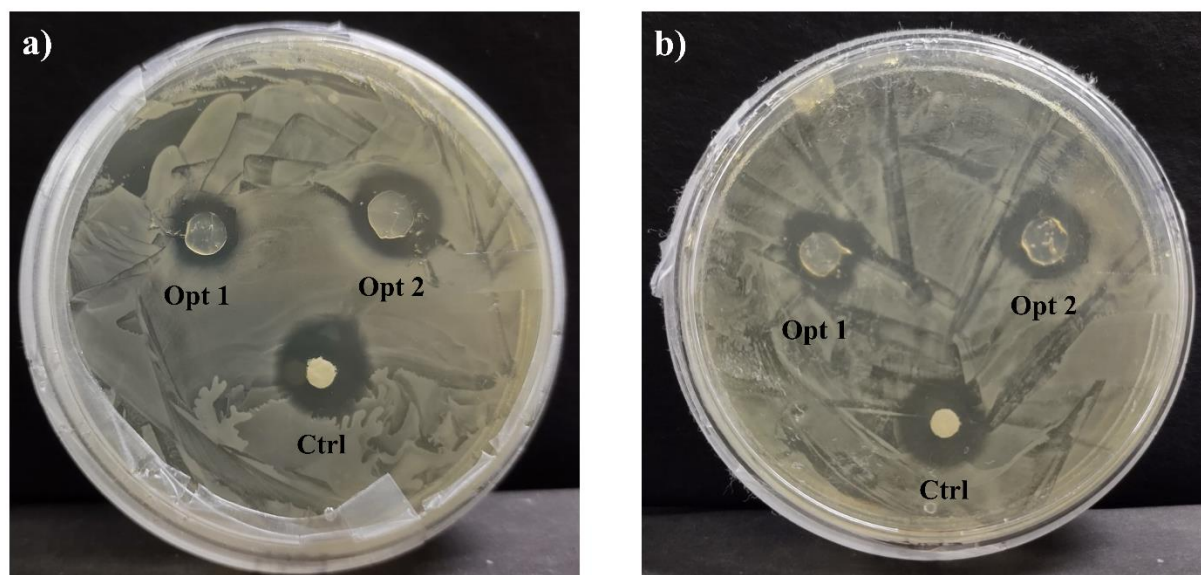


Figure 7.5: Photographs depicting antibacterial activity of optimally fabricated PVA-St-MA composite films with respect to (a) *E. coli* and (b) *L. monocytogenes*.

w/w% PVA, 7.41 w/w% St, 26.46 wt% MA and 18.38 wt% Gl). This has been especially for the case of *E. coli*. Similar to the functionality of other organic acids, the MA also facilitated an acidic environment that facilitates a breakdown of the synthesis route associated with the protein and cell membranes in the bacterial cell.

Further, it shall be noted that the optimal CA constitution based formulations exhibited far prominent positive antimicrobial activity against *L. monocytogenes* in comparison with the *E. coli*. However, this is not the case for the films being developed using MA based optimized formulations. In summary, in comparison with the CA, it can be confirmed that MA has been more effective against *E. coli*.

7.9.2 In-vitro biocompatibility

The commercial application of a wound dressing material requires the prior assessment of its cytotoxic potential characteristics. Materials with lesser cytotoxicity extent affirm upon higher cell growth potential and are thereby affirmed to have good in-vitro biocompatibility characteristics. This is often being evaluated as the percentage RGR using HEK cells in experimental investigations. The same has been addressed for the MA based optimally formulated PVA composite films (Opt 1 and Opt 2 formulations).

The PVA composite films being formulated with both alternate optimized compositions were soaked in the FBS supplemented DMEM media to facilitate 24 or 48 h of extraction. The extracted media were eventually diluted in 3 dilutions namely 5, 10 and 100 % volume proportions and were thereafter regarded to be the alternative treatment solutions against the HEK cells.

For both cases and almost for all diluted proportions of the extracted samples, the proliferative influence on the cell growth was affirmed. For the 24 h extract sample, significant positive cell growth was observed for a corresponding increment in the treatment or extract concentration. However, for the 48 h extract sample case, the variation or change in the cell growth with respect to the increment in treatment concentration was not significant. Also, it was observed that for the highest treatment concentration, the 24 h extract sample case exhibited better RGR in comparison to that being analyzed with the 48 h extract sample case. On the contrary, it can be also affirmed that after 48 h of extraction, higher MA content in the media facilitated greater WL of the PVA-St-MA-GI composite film. This is being attributed to the enhanced leaching of the film constituents that detrimentally influenced the cell growth and hence lower prolific activity. Also, for both 24 and 48 h of extraction time, the film being prepared with Opt 2 formulation

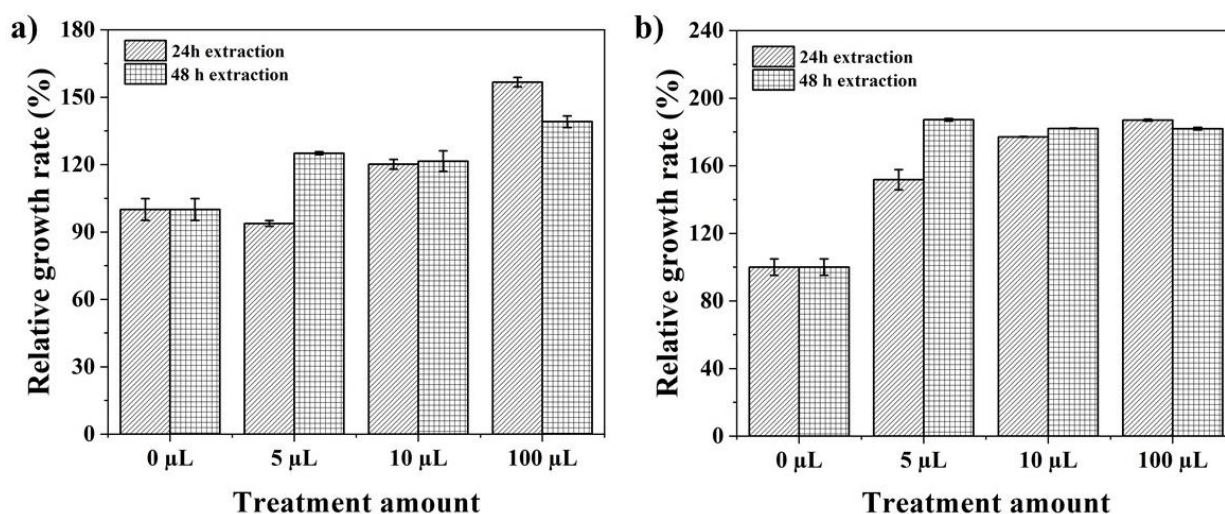


Figure 7.6: In-vitro biocompatibility plots associated to the optimally fabricated PVA-St-MA composite films after 24 and 48 h of extraction with HEK cells (a) Opt 1 and (b) Opt 2 formulation based films.

(6.92 w/w% PVA, 6.67 w/w% St, 25.96 wt% MA and 18.38 wt% Gl) exhibited higher RGR in comparison with the film being prepared with the Opt 1 formulation (5.76 w/w% PVA, 7.41 w/w% St, 26.46 wt% MA and 18.38 wt% Gl) (Figure 7.6). Moreover, for the Opt 2 formulation, it has been seen that the reduction in the proliferative activity after using 48 h of extraction has been significantly improved. On the contrary, the difference between the RGR (187.01 ± 0.83 and 181.95 ± 1.2 %) is found to be negligible using 24 and 48 h of extracted treatment from Opt 2 formulation case, in comparison with the Opt 1 formulation case (156.64 ± 2.12 and 139.14 ± 4.45 %) and the MA based RSM+%E formulation of PVA composite film case (215.59 ± 7.36 and 104.78 ± 8.08 %) in chapter 6.

7.10 Summary

A critical summary of the findings associated with the investigations being reported in this chapter can be presented as follows:

- The design expert RSM software facilitated a useful understanding into the wider concentration range based interaction between polymers (PVA and St) and MA. Despite being a weak organic acid with a lesser number of -COOH groups in comparison with the CA, the MA indicated its performance as a potential additive in due course of the fabrication of the PVA-St based wound dressing composite film. The wider concentration range of the three constituents (PVA, St and MA) in the investigated film formulations affirmed the achievement of optimal MA based PVA-St films with better characteristics and inexpensive formulation in comparison with the CA based optimal PVA-St wound dressing composite films.
- The consideration of additional constraint towards the further optimization of PVA-St-MA-Gl composite film in terms of retail precursor cost reduction facilitated the achievement of further optimal formulation in terms of better physical properties, qualitative effectiveness and lower retail cost of the fabricated film.
- Based on overall optimization, the best film constitution of the MA based PVA-St composite film referred to 6.92 w/w% PVA, 6.67 w/w% St, 25.96 wt% MA and 18.38 wt% Gl. The optimal film exhibited very high SI (583.83 %), in-vitro degradation (65.47 %), %E (99.53 %) and acceptable TS values (5.57 MPa). The optimized formulation also exhibited positive antibacterial effectiveness against both *L. monocytogenes* and *E. coli* and promoted relative cell growth (181.95 ± 1.2 %) using HEK cells.
- In terms of cost analysis, among the two optimally fabricated PVA-St-MA-Gl composite films, Opt 2 formulation was 9.21 % expensive than the Opt 1 formulation along with improvised physical and qualitative characteristics. However, it was 4.56 % inexpensive

than the previously reported CA based optimal PVA-St composite films in the thesis (chapter 5).

In summary, the MA based PVA-St composite films offer substantial promise towards wound dressing applications. This is one of the critical novelties being identified in this Ph.D. thesis.

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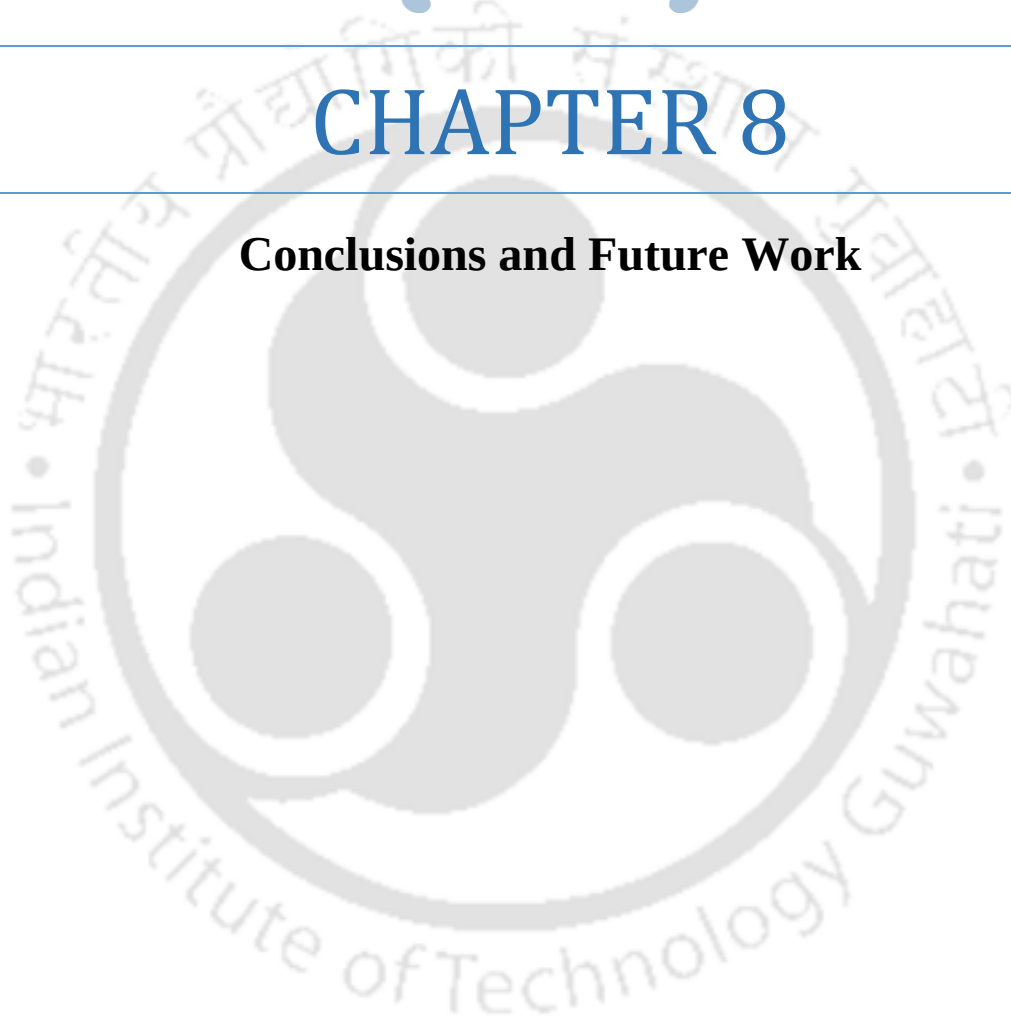






CHAPTER 8

Conclusions and Future Work





Conclusions and Future Work

In this chapter, section 8.1 elaborates upon few important inferences that can be deduced from experimental investigations of PVA composite hydrogel films. These have been presented in various sub-sections that devote towards alternate objectives of the Ph.D. thesis. Following this, section 8.2 summarizes the possible scope for further research in the field of PVA based composite hydrogel films for wound dressing applications.

8.1 Conclusions

The Ph.D. thesis addressed substantial investigations in the formulation and characterization of CA, TA and MA based PVA composite hydrogel films for wound dressing applications. For the CA based PVA composite films, the experimental investigations initially followed a simplistic experimental design approach that eventually improvised towards a statistical design based approach. Thereby, three major objectives have been set in a hierarchy for CA based films. Thereafter, the TA and MA based PVA composite films have been addressed based on the useful insights and inferences being deduced for the first three objectives of the Ph.D. thesis.

The following sub-sections delineate upon the critical objective and subjective findings of five major objectives being set for the Ph.D. thesis. Also the critical objective findings were tabulated in Table 8.1, along with the cost analysis of the best findings of the Ph.D. thesis along with the best findings of the appropriate prior art in the field of PVA composite films.

Table 8.1: A summary of optimal composition, response characteristics and cost parameters of CA and MA based PVA composite films addressed in the Ph.D. thesis and in the prior art.

S. No.	Work	Optimized aqueous precursor constitution (w/w%)			Swelling index (%)	In-vitro degradation (%)	Tensile strength (MPa)	Percentage elongation (%)	Cost (Rs/cm ²)
		PVA	St	CA	GI				
1.	Objective 1	7.08	7.08	5.04	5.32	59.77	2.54	38.55	0.214
2.	Objective 2	4.79	9.59	2.16	2.16	53.27	7.65	9.13	0.165
3.	Objective 3 ¹	6.99	6.53	3.87	2.49	51.4	4.98	87.54	0.188
4.	Objective 4, case 1 ²	6.99	6.53	3.87	2.49	64.79	5.14	147.86	0.188
5.	Shi et al. (2008)	3.0	1.0	0.8	0.8	-	44.0	178.9	0.055
6.	Wu et al. (2017)	7.27	7.27	2.59	5.46	-	45.54	-	0.209
		PVA	St	MA	GI				
7.	Objective 4, case 2 ³	6.99	6.53	3.87	2.49	66.56	4.88	103.68	0.194
8.	Objective 5	6.54	6.31	3.34	2.36	65.47	5.57	99.53	0.179

¹ The optimized formulation is based on the RSM optimization and the response value of the properties was referred through the software only.

² The RSM optimized PVA-St-CA-GI film was subjected to experimental evaluation and the obtained experimental value has been compared and reported based on the software referred value in the objective 3.

³ The RSM optimized PVA-St-CA-GI film formulation was replaced using MA instead of CA in the formulation and was subjected to experimental evaluation.

8.1.1 Optimality of CA concentration, drying temperature and time of CA based PVA composite films

- For the case of literature based fixed concentrations of PVA, St and Gl in the hydrogel films, the optimization of CA concentration, drying temperature and time were targeted as the first objective. For such parametric investigations, the optimal parametric values refer to 35.58 wt% CA concentration, 50 °C crosslinking temperature and 12 h drying time.
- The optimal characteristics of CA optimized PVA composite hydrogel membranes refer to 260.5 % SI, 67.26 % solubility, 59.77 % in-vitro degradation and good antibacterial activity against both *L. monocytogenes* (Gram-positive bacteria) and *E. coli* (Gram-negative bacteria).
- The CA optimized formulation of PVA-St-Gl-CA composite film exhibited super adsorbent ($SI \geq 260$ % said to be a super-absorbent (Ahmed et al., 2018)) and high WL or hydrolytic in-vitro degradation characteristics. However, the film possessed a comparatively lower elastic modulus. In comparison with the best available prior art (Shi et al., 2008, Birck et al., 2014), the optimized formulation also indicated low drying or crosslinking temperature in terms of a 35 % reduction in energy costs. However, the fabricated films with optimized CA content have been analyzed to possess a 10 % higher retail cost than the comparable composition reported by Wu et al. 2017 (Wu et al., 2017).

8.1.2 Synergy of PVA, St, Gl and CA in PVA composite films

- The synergy of PVA, St, Gl and CA have been addressed through the design expert software based experimental design approach. Thus, in this step, further numerical

optimization was not targeted. Based on experimental investigations, the optimal formulation corresponds to 5 w/w% PVA, 10 w/w% St, 15 wt% CA and 15 wt% Gl to achieve very high SI (338.37 %), acceptable in-vitro degradation (53.27 %), good TS values (7.65 MPa) and positive antibacterial effect against both Gram-positive and negative bacteria.

- The experimental optimal formulation associated with synergistic influence studies was significantly inexpensive with respect to the composition being reported as an optimal formulation in the former objective of the Ph.D. thesis. This is due to the formulation possessing a lower combination of PVA, Gl, and CA but not St (St being the most expensive material in the quaternary formulation).
- Response surface methodology based analysis indicated a highly non-linear dependence of SI and in-vitro degradation and moderate non-linear dependence of TS with variations in these four constituent concentrations. Among all binary interactions referred by the design software, the PVA-St combination significantly influenced all characterization parameters considered as dependent variables in the design. However, the influence of the PVA-CA combination on SI was most prominent, among all other binary interactions. The other two dependent variables, namely in-vitro degradation and TS, CA-Gl and PVA-Gl interaction respectively are the second best binary interactions to influence the same.

8.1.3 Compositional optimality of CA based PVA composite films

- The numerical optimization based analysis of RSM design based experimental data set for the CA based PVA composite films indicated 7.45 w/w% PVA, 6.96 w/w% St, 28.57 wt% CA and 18.38 wt% Gl as the optimal formulation. The films being prepared with

such composition exhibited very high SI (300.48 %), in-vitro degradation (51.4 %), acceptable TS (4.98 MPa), good %E (87.54 %), positive antibacterial effectiveness against both *L. monocytogenes* and *E. coli* and promoted cell growth (145.48 %) using HEK cells for 48 h of extracted treatment.

- The consideration of %E as an additional response variable profoundly altered the formulations being achieved through the numerical optimization of the response surface methodology approach. In due course of the consideration of %E as an additional response variable, almost similar characteristics have been obtained for the RSM based optimized formulation (RSM+%E) case and experimental data based optimized formulation (EXP+%E) case. However, for both cases that did not consider %E as an additional variable, a contrary trend existed in terms of the comparative evaluation of characteristics associated with experimentally optimized formulation case (EXP-%E) and RSM based optimized formulation case (RSM-%E). Hence, both RSM based experimental design approach and numerical optimization approach need to be adopted for the scenarios not involving %E as an additional response variable. Also, it was observed that, among all the constituents, Gl has negligible or minimal influence on all characterization parameters but not SI. Hence, the binary interaction terms associated with Gl possessed a very low *F* value for independent variables such as in-vitro degradation, TS and %E.

8.1.4 Comparative efficacy of TA/MA/CA based PVA composite films

- The fourth objective targeted the comparative efficacy of alternate organic acids, (CA, MA and TA) in the due course of optimally performing PVA composite hydrogel fabrication. The conducted characterizations affirmed MA to be the second best choice of

organic acid and after CA to achieve PVA composite films with superior performance characteristics. Due to its weakest crosslinking characteristics, the MA based PVA composite films with RSM+%E formulation possessed the highest SI values (739.29 %). However, the film's solubility (61.59 %) is not significantly higher in comparison with the CA (75.88 %) and TA (61.81 %) based PVA composite films. Also, for the same formulation MA based PVA films exhibited higher mechanical characteristics (TS: 4.88 MPa and %E: 103.68 %) than the TA based PVA films. In this regard, it shall be noted that the CA based PVA films possessed the highest mechanical characteristics (TS: 5.14 MPa and %E: 147.86 %). Also, for the 24 h extracted treatment case, in comparison with the CA based films possessing 149.55 % relative cell viability, the MA based PVA-St composite film exhibited the highest relative cell viability (215.59 %).

- Apart from CA, MA based PVA composite films were being analyzed to possess promising combinations of all evaluated characteristics. The consideration of MA as an alternate organic acid in the PVA composite film matrix indicated lower WL (27.96 %, on 1st day for RSM+%E formulation) during the initial time period in comparison with the CA based film (51.93 %, on 1st day for RSM+%E formulation). However, the WL of the MA based films gradually increased on a daily basis in due course of the 27 days experimental time period. Thereafter, the WL values reached similar values as those being obtained for the CA based PVA composite films (For RSM+%E formulation; MA based film: 66.56 % and CA based film: 64.79 %). Such slow and steady increment in in-vitro degradation characteristics is a desired feature in terms of the associated pathway of delayed drug release from a wound dressing product. Further, despite being a weak crosslinker, the MA induced promising mechanical characteristics. Also, the qualitative

analysis affirmed that the MA based films exhibited positive antibacterial effectiveness and cell growth for HEK cells. However, extended experimentation and optimization of PVA-St-MA-Gl blend are required due to the detrimental effect towards cell growth of the extract treatment sample being deduced with MA based films and after 48 h of time period.

8.1.5 Compositional optimality of MA based PVA composite films

- The numerical optimization based RSM design approach associated with the MA-based PVA composite hydrogel films refers to the optimal formulation as 6.92 w/w% PVA, 6.67 w/w% St, 25.96 wt% MA and 18.38 wt% Gl. The optimal film exhibited very high SI (583.83 %), in-vitro degradation (65.47 %), %E (99.53 %) and acceptable TS values (5.57 MPa). The optimized formulation also exhibited positive antibacterial effectiveness against both *L. monocytogenes* and *E. coli* and promoted cell growth (181.95 %) using HEK cells for 48 h of extracted treatment.
- The consideration of additional constraint towards the optimization of PVA-St-MA-Gl composite film in the final objective devoted towards the reduction of the retail cost of the deployed precursors. Thereby, the best composition was achieved that indicated better combinations of physical properties and qualitative effectiveness in comparison with the formulation being achieved with the general numerical optimization approach. In terms of cost analysis, among the two optimized PVA-St-MA-Gl composite films, the Opt 2 formulation (obtained with an additional constraint targeting a further reduction in the retail cost of the constituents) was 9.21 % expensive than the Opt 1 formulation (being obtained with similar numerical optimization strategy being followed for CA based film optimality. Also, the optimal formulation was 4.56 % inexpensive in comparison with the

optimized formulation of PVA-St-GI-CA composite film being obtained as the best in the Ph.D. thesis through numerical optimization of the RSM design based approach.

8.2 Future work

The following areas of work have been identified for consideration in the near future to further encourage customized subjective research in the vast field of synthetic polymer based bio-composite hydrogel films:

- Drying time optimization based on the physical characterization of the PVA-St-GI-organic acid composite films. Such an approach can facilitate the combinatorial reduction of drying time and temperature for the enhanced feasibility towards the massive fabrication of the mentioned films.
- The role of antibiotics and drugs into the blend can be investigated in terms of the drug release and WL characteristics of the PVA composite films. The HPLC based analytical characterization will be an integral part of such investigations. Such studies would facilitate a greater understanding of the role of drug release in both hydrolytic as well as real-world environmental scenarios.
- The real-world applications of the fabricated films can be targeted through additional characterization in terms of WVTR. Thereby, the performance and optimality of the composite films can be understood and better realized in real world scenarios.
- The adopted research methodology and optimization of quaternary formulations were devoted towards wound dressing applications. A similar approach and methodology can be targeted towards food packaging applications by considering characterization parameters such as oxygen transmission rate and antioxidant activity.

- To reduce the total number of statistically designed experiments, the thesis targeted a fixed choice of GL concentration in due course of MA based PVA composite films. This was based on the hypothesis that since the GL had negligible influence in the CA-based composite films, the same is applicable for the MA based films. However, considering the fact that CA has higher carboxyl groups than MA, it may be very much hypothesized that MA may not suppress the influence of GL in altering the desired characteristics of the PVA composite films. Hence, future research investigations can target combined optimality of all quaternary constituents including GL to obtain useful insights and validate the pertinent hypothesis.
- Other organic acids such as succinic, oxalic and maleic acids can be targeted for the fabrication of PVA composite films with superior performance characteristics and for wound dressing/food packaging applications.
- The role of hybrid polymers such as PVA and PEG, PVA and chitosan can be considered to achieve films with better characteristics.
- Scale up related studies associated with solution casting based fabrication of defect-free PVA composite films for wound dressing applications.
- Antibacterial test of best performing wound dressing compatible PVA composite film with wide varieties of Gram positive and Gram negative bacterial strains to further enhance the real world applications of the films.
- The thesis focused towards general characteristics of wound dressing targeted PVA based hydrogel films. However, several bio-evaluation tests need to be addressed in the near future. These include hemo-compatibility, cell migration, in-vitro wound closure %, wound scratching assay and other in-vivo tests. Further, several alternate formulations

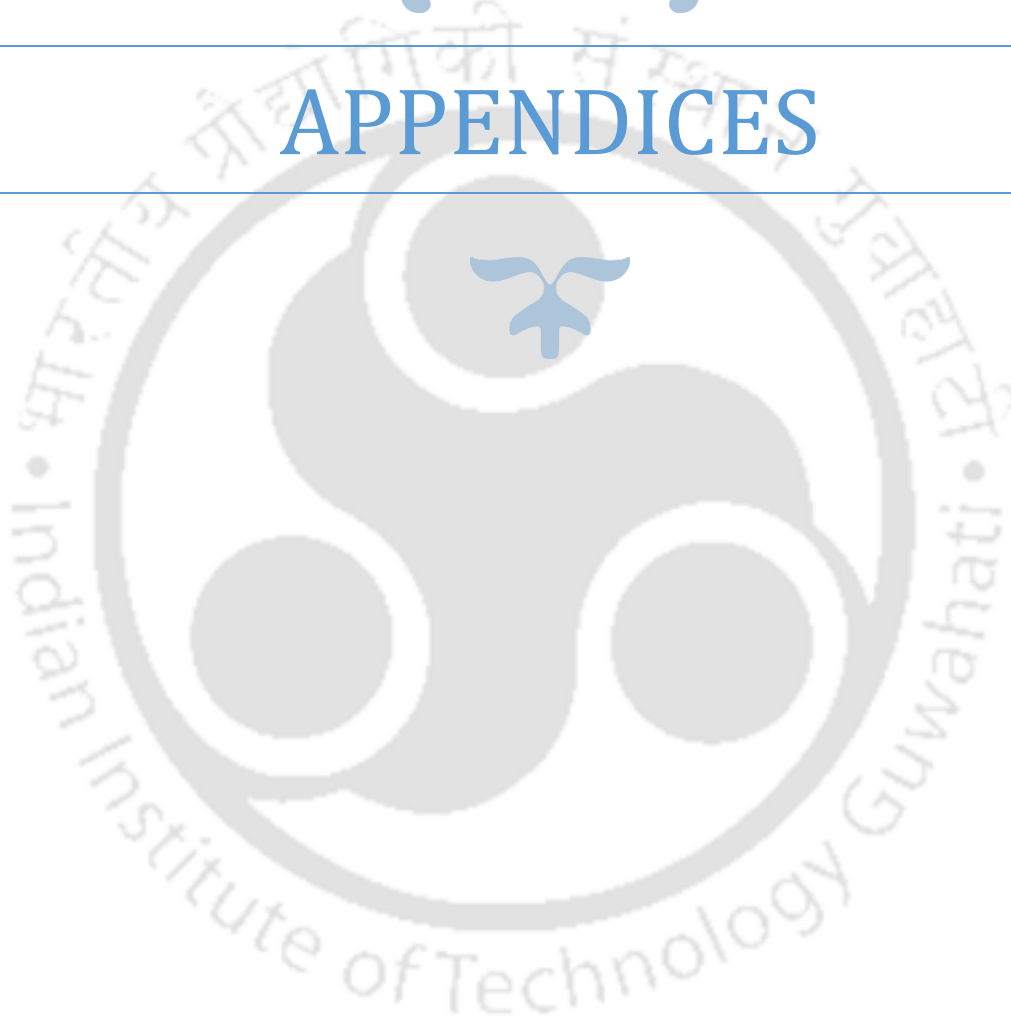
have been reported in this work as competitive defect free hydrogel film making formulations. Thus, in due course of bio-compatibility studies, it may be possible that the best formulation may have poor performance in terms of few bio-compatibility tests. Thereby, the alternate formulations reported in the thesis do also have their prominence for such scenarios.

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APPENDICES





Appendix A: Properties of Commercial Wound Dressing Materials

For various commercial wound dressing materials, the following tables A1-A6 summarize their major constituent polymers, promising characteristics/properties and cost. Among these tables, Tables A1, A2, A3, A4, A5 and A6 refer to hydrogel, hydrocolloid, alginate, foam, collagen and film type commercial wound dressing materials respectively.

Table A1: A summary of the characteristics and other properties of alternate hydrogel wound dressing materials.

Brand name	Manufacturer	Constituent	Properties/Benefits	Cost	References
TegaGel	3M Health Care Ltd	Calcium salt of alginic acid	Manage of a variety of exuding wounds (non-draining, minimally-draining and necrotic wounds), provides moist environment to enhance wound healing and assists autolytic debridement, wear time up to 7 days.	\$6.74/ 15g	Thomas 1992
Carrasyn	Carrington	Acemannan	Clear for easy visibility of the wound, non-oily gel donates moisture to wounds, used for natural healing process, more-viscous formulation for increased longevity, non-sterile and latex free.	\$5.51/ 85g	IR1
NuGel	Systagenix	Polyvinyl pyrrolidone, sodium alginate	Create moist healing environment for granulating and epithelializing wounds, manage chronic wounds throughout all stages of healing, gently and effectively rehydrates necrotic and sloughy wounds, the alginate increases the absorption, occlusive, used for dry, light and moderately exuding wounds, latex free.	\$8/ 15g	Ghadi et al. 2016
Exu dry dressing	Smith & Nephew	Outer layer & wound contact layer - High density polyethylene Inner Layers - Highly absorbent rayon/cellulose blend	One piece wound dressing made up of multiple layers, non-adherent, highly absorbent, reduce the risk of maceration and irritation, permeable, non-occlusive, can be used as a wet or dry dressing, secondary dressing required, antimicrobial format available and flexible.	\$8.54/ 2" x 3"	IR2

Brand name	Manufacturer	Constituent	Properties/Benefits	Cost	References
Flexigel		Anti Shear Layer - High density polyethylene Polyacrylamide matrix with embedded hydrophilic polysaccharide particles	Moisture vapor permeable, absorbs up to 5 times its own weight, soothing effect on superficial wounds, transparent, can use either side of dressing, easy application and prevent bacterial contamination.	\$52.05/4" x 4" (Box of 5)	IR3
Intrasite gel	Smith & Nephew	Modified carboxymethyl cellulose (2.3%), propylene glycol (20%)	Balanced gel formulation, debriding action (rehydration of hard necrotic tissue), absorbs slough and exudate without damaging fragile granulation tissue, creates a moist wound healing environment to allow re-epithelialization, Leads to reduced scarring, non-adherent, easy to use, secondary dressing required, antimicrobial format available, transparent and limited shelf life.	₹3,800/25g	Ghadi et al. 2016
Regranex gel		Sodium carboxymethylcellulose, 0.01% becaplermin	A recombinant human platelet-derived growth factor (rh PDGF-BB) formulation, FDA approved, non-sterile, applied on lower extremity diabetic neuropathic ulcers, 43% greater incidence of healing and 32% (six weeks) faster closer for neuropathic ulcers.	\$1,382.78/15g	Senet 2004
Geliperm	Geistlich Sons Ltd	Agar, polyacrylamide	Gel is able to absorb up to six times its own weight of wound exudate and cavities produced within the granular structure can also take up bacteria, pus, cell debris, by capillary action and wear time up to 3 days.	£9.00/10cm x 10cm	Ezra et al. 2009
Vigilon	Bard	4% insoluble crosslinked polyethylene oxide	Non-adherent, sterile, natural anesthetic effect cools and reduces pain, highly absorbent and ideal for skin tears, minor burns and radiation reactions.	\$932.95/4" x 4" (Box of 10)	Mandy 1983
AquaFlo	Covidien	Polyethylene glycol, propylene glycol	Provides a moist environment, allows customization (cut to size), translucent gel, cooling sensation helps reduce pain upon dressing change, provides wound protection, wear time up to 7 days and extremely absorbent.	\$5.71/3" Disk	Ghadi et al. 2016

Brand name	Manufacturer	Constituent	Properties/Benefits	Cost	References
Aquaform	Robert Bailey	Modified starch copolymer, glycerine, methylparaben (food grade), imidazolidinyl urea	Applied on dry or low exuding wounds, clear, viscous formula stays on the wound for longer, higher glycerol content helps with skin moistening and conditioning, rehydrates necrotic tissue, aids autolytic debridement and maintains a moist healing environment.	£27.54/ 8g x10	Timmons et al. 2008
Bionect	Fidia Farmaceutici S.p.A. - Italy	Sodium salt of hyaluronic acid (0.2%), sorbitol solution 70%, sodium dehydroacetate, methylparaben, propylparaben, carbomer 980, sodium hydroxide	Clear, colorless gel, act as a lubricant on the skin by restoring moisture and preventing friction or abrasion.	-	IR4
Curasol	Healthpoint	-	Clear, viscous, mildly absorbent, non-inflammatory, water soluble, fragrance free, non-greasy, protects from foreign contaminants, non-cytotoxic and provides appropriate moist environment.	\$491.59	IR5 & 6
CarraSorb	Riemser AG	Calcium alginate	Gel forming, non-woven, preservative free, non-toxic, absorptive and ideal for highly exuding wounds.	\$82.45/ 4" x 4" (Box of 10)	IR7
GRX wound gel	Alternative Exports	-	Gel based wound filler, provide a moist wound environment to stimulate granulation tissue formation, non-adherent, beneficial on all 4 healing stage, latex free.	\$5.51/ 85g	IR8
Purilon gel	Coloplast	Sodium carboxymethylcellulose, calcium alginate	Sterile, hydrating and absorbing properties, high cohesion even after absorption of debris and exudate which makes removal easy, provides effective autolytic debridement, maintains a moist wound environment, permeable, limited shelf life, antimicrobial format available, viscous gel texture counteract leakage and maceration.	₹315/ 15g	Ghadi et al. 2016

Brand name	Manufacturer	Constituent	Properties/Benefits	Cost	References
Granugel	Convatec	Pectin, sodium carboxymethylcellulose, propylene glycol	Facilitates autolytic debridement, moisturizes dry, necrotic, sloughy or granulating wounds, clear, viscous, absorb excess exudate and wear time up to 7 days.	£46.95/ 15g x 10	Williams 1996
Sterigel	Seton	Hemicellulose	Amorphous gel, unsuitable for heavily exuding wounds, particularly effective at rehydrating dry or necrotic tissue and golden brown colour.	-	Williams 1997

Table A2: A summary of the promising characteristics and properties of alternate hydrocolloid wound dressing materials.

Brand name	Manufacturer	Constituent	Properties/Benefits	Cost	References
Iodosorb (Cadexomer)	Smith & Nephew	Cadexomer iodine, iodine, PEG 300, PEG 1500	Bactericidal against MRSA and <i>P. aeruginosa</i> , broad-spectrum antimicrobial activity up to 72 h, removes exudate and debris.	₹11,400 / 40g	IR9
Sorbex	Medline	Cellulose	Highly absorbent, primary or secondary wound dressing, non-adherent outer layer, designed for ulcers, pressure sores, surgical incisions and 1st or 2nd degree burns, sterile and latex free.	\$49.99/4" x 6" (Box of 10)	IR10
Duoderm (polymer blend)	ConvaTec Ltd	Sodium carboxymethylcellulose, gelatin, pectin and adhesive polymers	Manage light to moderately exuding wounds, easy to use helps promote autolytic debridement and suitable for managing different stages of wound healing.	\$2143/ 4" x 4"	Hermans 1987
Granuflex E extra thin		Polyurethane	Semipermeable, impermeable to exudate and micro-organisms, used for lightly exuding wounds, coated with thin layer of adhesive (Granuflex) thus water proof, produce moist conditions and promote epithelialization without causing maceration.	-	Heffernan et al. 1994

Brand name	Manufacturer	Constituent	Properties/Benefits	Cost	References
Tegasorb			Create optimal moist environment, absorbent, transparent, water proof wipe-clean surface and wear time up to 7 days.	\$632.75	Williams 2000
Comfeel	Coloplast A/S	Polyurethane coated with sodium carboxymethylcellulose and calcium alginate	Semipermeable, transparent, bacteria and water proof top film, flexible to dress any part of the body and excellent support for closure of wounds.	\$3.04/ 2" x 2-3/4"	IR11
Debrisan (Dextranomer)	Pharmacia and Upjohn Ltd	Dextran	Sterile, semipermeable, bacteria and granular substances are removed with the secretions, cannot be applied to the dry and heavily exuding wounds.	-	Jacobsson et al. 1976
Dermafilm	DermaRite Industries, LLC	-	Impervious barrier to moisture, bacteria and contamination, mild adhesive surface area adheres to peri-wound skin but won't stick to wound bed, supports moist wound healing and autolytic debridement and can be applied to low exuding, non-infected shallow wounds.	\$27.58/ 6" x 6" (box of 5)	IR12
Restore Cx	Hollister Inc.	-	Sterile, thin, occlusive, flexible film backing provides moist healing environment and allow moisture vapor to pass, manage superficial wounds.	-	IR13
Biofilm	CliniMed Ltd	polyester fabric sheet, polyisobutylene (adhesive coating), Gel, pectin and CMC	Polyester fabric: Permeable, highly hydrophobic, resist penetration bacteria. Hydrocolloid base: dry dressing is virtually impermeable to water vapor, but absorbs water and swells to form a hydrophilic gel and the permeability increases.	-	Smith et al. 1992
Ultac	Sherwood Med	30% alginate	Absorbs exudate prevent maceration of the peri-wound skin, designed for lightly exuding to dry wounds and maintains optimal moist healing environment, latex free.	\$89.95/ 4" x 5" (Box of 5 rolls)	IR14

Table A3: A summary of the promising characteristics and properties of alternate alginate wound dressing materials.

Brand name	Manufacturer	Constituent	Properties/Benefits	Cost	References
AlgiSite	Smith & Nephew	Calcium salt of alginic acid rich in mannuronic acid	Soft and conformable (easily molded, folded or cut to any shapes), less amount of fibers released, easy to remove, fast gelling, allows gas exchange, provide moist wound environment, applied on wet wound, highly absorbent and adhere if used on very lightly exuding wounds, wear time up to 7 days.	₹2,959/2" x 2" (Box of 10)	IR15
Sorbsan	Pharma-Plast Ltd, Steriseal Division	Calcium salt of alginic acid, mannuronic and guluronic acid	Hemostatic, interacts with exudate to form a hydrophilic gel, painless to remove (can be washed away with normal saline without causing damage to newly forming tissue) and absorb large amounts of exudate, wear time up to 2-3 days.	£15.50/ 5cm x 5cm (Pack Size 10)	Williams 1994
Kaltostat	ConvaTec Ltd	Sodium and calcium salts of alginic acid in the ratio of 80:20	Primary dressing, manage moderately to heavily exuding wounds, wear time up to 7 days, gel-fiber matrix, maintains a moist wound environment, facilitates atraumatic removal, aids in the control of minor bleeding and usable on infected wounds.	\$61.2/ 2" x 2" (Box of 10)	(Attwood 1989)

Table A4: A summary of the characteristics of foam based commercial wound dressing materials.

Brand name	Manufacturer	Constituent	Properties/Benefits	Cost	References
Flexzan	Mylan Pharmaceuticals	Polyurethane	Semi-occlusive, adhesive, sterile, ultrathin, highly conformable, allows excess wound moisture evaporation prevent fluid accumulation, outer skin surface protects wounds from exogenous contamination and trauma while maintaining a moist wound healing environment.	\$639.04/ 2" x 3" (Case of 50)	IR16
Biopatch	Johnson & amp; Johnson	Chlorhexidine gluconate	Powerful antimicrobial and antifungal dressing, provides 360° protection from patients own skin's microflora around the insertion site up to 7 days, absorptive, non-irritating, non-toxic and latex free.	\$17.41/ 1" Disc x 4mm	IR17

Brand name	Manufacturer	Constituent	Properties/Benefits	Cost	References
Biatain	Coloplast	Polyurethane	Non-Adhesive, soft, conformable (extremely flexible), manage low to high wound exudate, low risk of leakage or maceration, ensures a moist environment for healing, wear time up to 7 days, semi-permeable, bacteria proof and water proof top film.	\$3.49/ 2" x 2.75"	Pritchard 1999
Cutinova	Smith & Nephew Healthcare Ltd	Polyurethane, polyacrylate	Superabsorbent, gel matrix, self-adhesive, hydro-selective, semi-transparent, sterile, semi-permeable membrane that acts as a carrier and provides a bacterial barrier, use on most types of wounds (best suited to low to moderately exuding wounds), wear time up to 7 days.	£22.5/ 5cm x 6cm (Pack of 10)	IR18
Reston	Health Care	polyurethane coated with hypoallergenic, acrylate adhesive	Self-adhering, lightweight, conformable padding, can be cut, layered and shaped, latex free.	\$69.99/ (Pack of 10)	IR19
Lyofom	Seton Healthcare Group plc	Polyurethane	Sterile, used on lightly to moderately exuding wounds, hydrophilic wound contact area promotes absorption to reduce risk of maceration, easy to apply and remove, permeable, maintains moist warm environment to the formation of granulation tissue and epithelialization and favor auto-debridement.	\$6.8/ 3" x 3.75"	Thomas 1993
Ivalon	Fabco - First Aid Bandage Company	Hydroxylated polyvinyl acetal	Soft, highly absorbent, fast wicking, fiber-free, extremely strong, smooth rounded edges ease insertion, atraumatic removal and custom shape or size can easily achieved.	-	IR20

Table A5: A summary of the characteristics and properties of collagen based commercial wound dressing materials.

Brand name	Manufacturer	Constituent	Properties/Benefits	Cost	References
Biopad	Angelini Pharma	100% pure native equine type I CC	Primary wound dressing with highest CC content in the market, accelerate closure of hard-to-heal wounds and enhance the wound healing process, thicker dressing construction, biodegradable gel formation, secondary	\$46.09/ 2" x 2" (Box of 3)	IR21

Brand name	Manufacturer	Constituent	Properties/Benefits	Cost	References
Cellerate RX gel	Wound Care Innovations	Hydrolyzed type I bovine CC	Provides moist environment for surgical sites and wounds.	\$241.78/28g	IR22
BCG matrix	Brennan Medical	CC and β -glucan	-	-	Ghadi et al. 2016
Biostep CC matrix	Smith & Nephew	CC	Highly conformable, unique dual-action MMP targeting and deactivation, easy to apply, absorbs a high level of moisture, wear time up to 6 days, need careful removal of secondary dressing, antimicrobial format available, flexible and limited shelf life.	\$217.99/2" x 2" (Pack of 10)	IR23

Table A6: A summary of the promising characteristics and properties of alternate film type commercial wound dressing materials.

Brand name	Manufacturer	Constituent	Properties/Benefits	Cost	References
Tegaderm	3M Healthcare	Guar gum	Breathable, sterile, transparent and water proof, and provide a barrier to external contaminants.	154.8₹ /strip	IR24 & Ghadi et al. 2016
Blisterfilm	Covidien	-	Sterile, transparent, single layer film with adhesive free window in the center, maintain optimal moisture levels, wear time 2-7 days, provide a high moisture vapor transmission rate and evaporates excessive fluid (a breeding ground for bacteria), while maintaining moisture in the wound tissue for optimal healing.	\$11.50/ 2" x 3" (Box of 20)	IR25
Poly skin II		Thin polymer film coated with	Allows moisture and oxygen vapor exchange for longer wear time and one handed aseptic delivery, helps prevent roll up, impermeable to external liquid and microorganisms, better adhesion in moist	\$0.49/ 2" x 2-3/4"	IR26

Brand name	Manufacturer	Constituent	Properties/Benefits	Cost	References
		a hypoallergenic adhesive	environments, easy to use two tab delivery system and wear time 2-7 days.		
Silon-TSR®	Jobskin®	Complex weave of biopolymers	Thin soft membrane, non-adherent, highly compliant, does not integrate into the wound during healing, reduces pain throughout the healing process and during dressing removal, easy to apply and can be repositioned during application.	£9/ 13cm x 25cm	IR27
Aluderm	Soehngen	Hydrophilic absorbent layer and hydrophobic Polypropylene as final layer	Strong absorbent (keep highly secreting wounds dry), do not stick to wound, hypoallergenic, does not release fibers, breathable, promotes skin formation and healing, physiologically safe, free of chemical binding materials.	€291/ 40 cm x 60 cm	IR28
Opsite plus	Smith & Nephew		Transparent, water proof, prevent blistering, low-adherent, highly absorbent pad, extended wear time, bacterial barrier, high moisture vapor transmission rate reduces risk of maceration, conformable and allow easy application.	£14.4/ 6.5cm x 5cm (Pack of 25)	Fear 2001
Cutifilm		Polyurethane	Sterile, hypoallergenic adhesive, bacteria proof, water proof, secure adhesion, low adherent pad, permeable to moisture vapor and oxygen, highly absorbent.	\$21.99/ 10cm x 8cm (Pack of 5)	IR29
Bioclusive	Johnson & Johnson Medical Ltd		Transparent, self-adhesive, breathable, semi-permeable, maintain moist wound environment, serves as both primary and secondary dressing for dry or lightly exuding wounds, barrier to bacteria and to viruses 27 nm in diameter or larger, flexible and conformable.	\$1.99/ 2-3/8" x 2-3/4"	IR30
Release		Ethyl-methyl acrylate	Low adherent thus easily removed from the wound, management of clean and suitable for light to moderately exuding wounds.	£20.58/ 9.5cm x 9.5cm (Pack of 40)	IR31

Brand name	Manufacturer	Constituent	Properties/Benefits	Cost	References
C-View	Aspen Medical	Polyurethane, coated with acrylic adhesive	Transparent, semi-permeable, water proof, non-absorbent, suited for little and no exuding wounds, better retention, self-adhesive, easy to apply as well as remove, easily cut to dress difficult wound sites, provides a bacterial barrier and latex free.	£24/ 12cm x 12cm (Pack of 10)	Morris 2001
Flexipore	Activeheal	-	Biodegradable, porous adhesive surface absorbs wound exudate, external surface act as a barrier to microorganisms and very high water vapor transport rate.	£9/ 6cm x 7cm	Somers et al. 1992
Mepore	Mölnlycke Health Care	Polyester, coated with acrylic adhesive (polyolefin)	Water proof, backing film protects wounds from contamination, suitable for lightly exuding wounds, self-adhesive, low adherent, breathable to prevent maceration, low friction to clothing and bed linen, easy to remove and flexible.	\$0.26/ 2.5" x 3"	IR32
Skintact	Robinson	-	Sterile, dual sided, low adherent, doubled sided for ease of use, two layers of perforated film with an inner absorbent layer and highly conformable.	£1.62/ 5cm x 5cm (Pack of 10)	IR33

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- IR2: <https://www.smith-nephew.com/professional/products/advanced-wound-management/exu-dry-wound-dressing/>
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Appendix B: Benefits of Alternate Additives Deployed in Commercial Wound Dressing Materials

For various potential additives used as a wound dressing material, the following Tables B1-B3 summarize their benefits. Among these tables, Tables B1, B2 and B3 refer to antibiotics, synthetic and organic type additives respectively.

Table B1: A summary of deployed antibiotics in wound dressing materials and their benefits.

S. No.	Product	Antibiotic additives	Benefits	Author ¹
1.	PVA hybrid hydrogel film	Erythromycin	- Against <i>Escherichia coli</i> over 24 h shows significant results. - No effect against <i>Staphylococcus aureus</i>.	Tavakoli et al., 2017
2.	PVA-acacia gum hybrid hydrogel	Erythromycin	- Starting release rate has been higher than the later stages. - PVA controls the release in second phase and decreases risk of infection.	Fathollahipour et al., 2013
3.	PVA-dextran hydrogel	Gentamicin	- Little influence on water vapor transmission rate. - Significantly improve wound healing. - More swellable, flexible, and elastic wound dressing.	Hwang et al., 2010
4.	PVA-sodium alginate hybrid hydrogel	Minocycline	- Clear zone of inhibition created and it was directly proportional to the concentration of minocycline. 99.9 % antibiotic was released within first 15 min, termed as 'burst release of drug'.	Kaur et al., 2019
5.	PVA hydrogel	Ampicillin	<i>E. coli</i> has a hard resistant against membrane compositions with or without ampicillin. - Effective against <i>S. aureus</i>.	Fahmy et al., 2015
6.	Chitosan membrane	Tetracycline hydrochloride and silver sulfadiazine	- Water-soluble TH promotes more release than water-insoluble AgSD. - Strong antibacterial activity against both of <i>E. coli</i> and <i>S. aureus</i>. As a water-soluble drug, TH spread out easily, so its inhibition zones were larger than those of AgSD. - Cell viability was significantly stronger from day 1 to day 3.	Ma et al., 2017

¹ All the references referred in table B1, B2 and B3 are given in chapter 1

Table B2: A summary of synthetic additives being used for the modification of wound dressing materials and their benefits.

S. No.	Product	Synthetic additives	Benefits	Author ¹
1.	PVA-St-Cs hydrogel membrane	nano ZnO	- Decreases water absorption, porosity but increment in mechanical property like, tensile strength and percentage elongation. - Exhibited antibacterial activity against <i>E. coli</i>, <i>S. aureus</i> and <i>Pseudomonas aeruginosa</i> with cell viability of more than 87 %.	Baghaie et al., 2017
2.	PVA-gellan patch	Silver	- With high Ag content matrices assume a quite unpleasant brown color. - High antimicrobial activity seems to be independent on the Ag content.	Cencetti et al., 2012
3.	Cs-PVA hydrogel	Ag nanoparticle	- Both <i>P. aeruginosa</i> and <i>S. aureus</i> were significantly inhibited. <i>P. aeruginosa</i> was more susceptible than <i>S. aureus</i>. With increasing Ag increase inhibition diameter. - Higher concentrations are more effective for wound size reduction. - For regeneration of tissue PCsAg30 group is better.	Nguyen et al., 2019
4.	Cs-PVA hydrogel nano-composite	Ag-functionalized titanium dioxide nanoparticle	- Effective swelling and antimicrobial activity against <i>E. coli</i> and <i>S. aureus</i>. - Successful cell growth.	Rinehart et al., 2016
5.	PVA-gellan patch	Borax	- Pronounced dehydration rate and lower silver release profiles, with a lower initial burst. - Borax crosslinked samples with high molecular weight PVA enhance the antimicrobial activity.	Cencetti et al., 2012
6.	PVA dual delivery hydrogels	Borax	- Up to 1 wt%, increases the storage and loss moduli and complex viscosity. - With decreasing the Borax concentration release increased.	Rezvan et al., 2018
7.	PVA hybrid hydrogel film	Sodium tetraborate (borax)	- Fast erythromycin release with low borax density, while increment up to 1% controlled drug release. Hence, lower degradation and enhancement of mechanical properties. - Better antibacterial activity against <i>S. aureus</i> and <i>E. coli</i>. - Exhibited higher than 90% cell viability.	Tavakoli et al., 2017

S. No.	Product	Synthetic additives	Benefits	Author ¹
8.	PVA-St hydrogels	Boron and glutaraldehyde	- BA improve TS much effectively than GA. Application of both BA and GA together improve TS further more and reduce swelling. - SP-BA had no antimicrobial activity. - SP-GA-BA indicated moderate antibacterial activity against <i>E. coli</i>, <i>S. aureus</i>, <i>P. aeruginosa</i> and <i>Candida albicans</i> in comparable with erythromycin, whereas SP-GA acted against <i>Bacillus subtilis</i> and <i>C. albicans</i>. SP-GA-BA is more pronounced on Gram-negative than on Gram-positive bacteria.	Bursali et al., 2011
			- In SP-citric acid or SP-glycerol blend addition of GA increases TS and decreases %E, swelling degree and solubility. - For all the properties, in SP-CA blend with increment of GA change is sharp. Whereas, in case of SP-GI blend the decreasing or increasing trends are visible but negligible.	
9.	SP blend films	GA	- In SP-citric acid or SP-glycerol blend addition of GA increases TS and decreases %E, swelling degree and solubility. - For all the properties, in SP-CA blend with increment of GA change is sharp. Whereas, in case of SP-GI blend the decreasing or increasing trends are visible but negligible.	Yoon et al., 2006

Table B3: A summary of organic additives being deployed for various wound dressing materials and their benefits.

S. No.	Product	Organic additives	Benefits	Author ¹
1.	PVA hybrid hydrogel film	Manuka honey	- Dissolution of honey increases porosity. - Release occurs from 4 to 15 h. At 24 h, the negligible honey concentration results in lower bio-adhesion properties. - Enhanced antibacterial activity against <i>S. aureus</i> as compared to <i>E. coli</i>.	Tavakoli et al., 2017
2.	PVA hydrogel	Hyaluronic acid	- With increasing HA, gel fraction and TS decreased; whereas swelling, %E, protein adsorption and hydrolytic degradation increased. - HA content less than 20 % showed high cell viability^o and no toxicity. - Showed antimicrobial activity against <i>Candida albicans</i>.	Fahmy et al., 2015
3.	PVA-PEG hydrogel film	<i>Centella asiatica</i>	90% drug was released at 12 h. - Prevents microbial growth against both <i>P. aeruginosa</i> and <i>S. aureus</i>. - Demonstrated cell viability of 97.83 % for HDF and 98.74 % for HaCaT cell.	Ahmed et al., 2017

S. No.	Product	Organic additives	Benefits	Author ¹
4.	PVA dual delivery hydrogel	Curcumin	Diffusion through pluronic micelles is very slow and half of the drug release takes about 10 days.	Rezvan et al., 2018
5.	PVA hydrogel film	Aloe vera	- Increasing AV resulted increasing porosity, hydrolytic degradation, WVTR and surface roughness. - Water absorption capacity and mechanical properties was highest by 30% AV content. Also provided fibroblast proliferation and viability.	Hajian et al., 2017
6.	SP films	Citric acid	- DS, TS decreases and %E increases with increasing CA content. - CA less than 20 wt%, best against toxicity. But using up to 30% CA, 80% cell viability.	Shi et al., 2008
7.	SP blend films	CA or glycerol	- DS, TS decreases and solubility (S), % E increases with increasing CA and GL content. - CA was better than GL for TS, %E and DS. Whereas, for S, GL is more preformed.	Yoon et al., 2006
8.	SP blend films	CA or Gl or sorbitol	- DS, TS decreases and S, % E increases with increasing CA, SO and GL content. - For TS decrement and %E increment, CA was best and SO 2nd best. For higher DS and S, GL is best.	Park et al., 2005
9.	SP blend films	Succinic acid	- DS, %E (almost negligible) decreases and S, TS increases with increasing. - SA and Gl blend improve %E.	Yoon et al., 2006
10.	SP blend films	Gl or maleic acid or tartaric acid	- DS, TS decreases and S, % E increases with increasing TA, MA and Gl content. - Effect of the additives for DS SA>Gl>MA>TA, and for S, TA>MA>SA>Gl. - For TS and %E, TA>MA>Gl. - SA and Gl blend improve TS.	Yoon et al., 2006
11.	SP blend films	Gl, SO, SA, MA, TA and CA	- TS decreases and % E increases with increasing CA, TA, MA, SO and Gl content, except for SA. For TS and %E, CA>TA>MA>SO>Gl. - Modification of TS by addition SA with Gl or SO. For TS, SOSA>GISA>SO>Gl. - Similarly, blend of Gl or SO with SA, improve %E. SO>Gl>SOSA>GISA>SA.	Yun et al., 2006

S. No.	Product	Organic additives	Benefits	Author ¹
			DS decreases with increasing all the additives concentration. Effect are like CA>TA>MA>SO>SA>Gl.	
12.	Cs membrane	Gl	- Increasing Gl content (Up to 0.5% Gl) %E increased and TS decrease. But excessive Gl destroy matrix. - Contact angle decreased and mass ratio, WVTR, swelling rate increased with increasing Gl.	Ma et al., 2017
13.	Carrageenan based hydrogels	Glycerin and SO	- For lower content of glycerin (1.5%), increment of SO, swelling ratio dropped. - Samples with higher SO and lower glycerin were the stiffest and vice versa.	Kozłowska et al., 2018



Appendix C: ANOVA based Analysis of Experimental Investigations

The data significance of the experimental investigations have been confirmed through the one-way ANOVA analysis for all relevant responses (SI, solubility, WL, TS, %E and relative cell growth characteristics). The data presented for various formulations in chapter 3 (for change in drying temperature and CA concentration) and chapter 6 (for change in alternate organic acids in various optimized PVA composite film formulation; i.e., EXP-%E, EXP+%E, RSM-%E, RSM+%E) of the Ph.D. thesis have been already addresses with statistical analysis. For each relevant cases, the one-way ANOVA based data analysis summary has been presented in Tables C1-C6, as example. The tables affirm high F values and low p -values for the obtained data. Only for tensile strength characteristics, in Table C4, lowest F value was obtained for RSM+%E formulation of PVA composite film. This is due to the variation obtained for MA based RSM+%E formulation of PVA composite film. Hence, the change in tensile strength characteristics between CA/MA based PVA composite film for the same formulation is not significant.

Table C1: ANOVA analysis data of swelling index characteristics for variation in alternate organic acids with respect to RSM+%E formulation of PVA composite film.

Alternate organic acids	Trail 1	Trail 2	Trail 3	Mean	Standard deviation	SE of mean	F value	p -value
CA	297.58	300	308	301.86	5.45	3.15	3870.82	4.64 E ⁻¹⁰
TA	341.28	352.38	358.52	350.73	8.74	5.05		
MA	738.64	734.41	744.83	739.29	5.24	3.03		

Table C2: ANOVA analysis data of solubility characteristics for variation in alternate organic acids with respect to RSM+%E formulation of PVA composite film.

Alternate organic acids	Trail 1	Trail 2	Trail 3	Mean	Standard deviation	SE of mean	F value	p-value
CA	74.19	72.63	80.8	75.88	4.34	2.5	26.29	0.00107
TA	63.37	60.12	61.93	61.81	1.63	0.94		
MA	62.5	60.22	62.07	61.59	1.21	0.7		

Table C3: ANOVA analysis data of in-vitro degradation or weight loss characteristics (a) for variation in alternate organic acids with respect to RSM+%E formulation of PVA composite film on 27th day and (b) for variation in days with respect to MA based RSM+%E formulation of PVA composite film.

(a)

Alternate organic acids	Trail 1	Trail 2	Trail 3	Mean	Standard deviation	SE of mean	F value	p-value
CA	60.98	68.25	65.15	64.79	3.65	2.11	58.23	1.1759E ⁻⁴
TA	94.74	89.23	96.45	93.47	3.77	2.18		
MA	65	64.1	70.59	66.56	3.51	2.03		

(b)

No. of days	Trail 1	Trail 2	Trail 3	Mean	Standard deviation	SE of mean	F value	p-value
1	26.53	27.36	30	27.96	1.81	1.05	90.68	4.16581E ⁻⁹
2	43.75	43.44	47.27	44.82	2.13	1.23		
3	47.47	54.84	55.26	52.53	4.38	2.53		
7	66	65.42	62.39	64.6	1.94	1.12		
14	67.09	63.96	64.38	65.15	1.7	0.98		
27	65	64.1	70.59	66.56	3.51	2.03		

Table C4: ANOVA analysis data of tensile strength characteristics for variation in alternate organic acids with respect to RSM+%E formulation of PVA composite film.

Alternate organic acids	Trail 1	Trail 2	Trail 3	Mean	Standard deviation	SE of mean	F value	p-value
CA	5.82	5.01	4.58	5.14	0.63	0.36		
TA	3	2.85	3.09	2.98	0.12	0.07	4.86	0.05551
MA	5.9	5.55	3.2	4.88	1.47	0.85		

Table C5: ANOVA analysis data of percentage elongation characteristics for variation in alternate organic acids with respect to RSM+%E formulation of PVA composite film.

Alternate organic acids	Trail 1	Trail 2	Trail 3	Mean	Standard deviation	SE of mean	F value	p-value
CA	153.09	139.91	150.58	147.86	7	4.04		
TA	41.78	42.51	47	43.76	2.83	1.63	235.87	1.98089E ⁻⁶
MA	111.58	99.12	100.33	103.68	6.87	3.97		

Table C6: ANOVA analysis data of relative cell growth characteristics for variation in alternate organic acids with respect to RSM+%E formulation of PVA composite film and 24 h extracted sample.

Alternate organic acids	Trail 1	Trail 2	Mean	Standard deviation	SE of mean	F value	p-value
CA	150.9	148.19	149.55	1.92	1.36		
TA	180.79	170.4173	175.63	7.29	5.15	59.88	0.00382
MA	220.8	210.39	215.59	7.36	5.2		



Appendix D: Thickness and Dimensions of Dried PVA Composite Hydrogel Films

Based on various experimental investigations, the average thickness and dimensions of alternate dry PVA composite hydrogel films have been summarized as follows in Tables D1-D4. The relevant variations refer to variations in drying temperature, CA concentration, quaternary constituent concentrations (PVA, St, Gl and CA), organic acid choice and ternary constituent concentration (PVA, St and MA). For all cases, the dried films were prepared as 21.5 cm x 10.5 cm films (225 cm²). Optimized drying temperature for data presented in Tables D2-D4 refers to a drying temperature of 50 °C

Table D1: Drying temperature and CA composition dependent thickness data of dry PVA-St-Gl-CA composite hydrogel films (21.5 cm x 10.5 cm).

Composition		Thickness (mm)
Drying temperature (°C)	CA concentration (g)	
40	0.5	0.084
	1	0.101
	2	0.157
	3	0.201
	0.5	0.129
50	1	0.177
	2	0.206
	3	0.182
	0.5	0.098
60	1	0.103
	2	0.146
	3	0.165

Table D2: Film thickness data of composite hydrogel films prepared at 50 °C and variant choices of quaternary constituent (PVA, St, CA and Gl) concentrations.

Composition				Thickness (mm)
PVA concentration (w/w%)	St concentration (w/w%)	CA concentration (wt%)	Gl concentration (wt%)	
7.5	7.5	27.5	27.5	0.136
7.5	7.5	27.5	27.5	0.104
10	5	15	40	0.112
7.5	7.5	27.5	2.5	0.129
7.5	7.5	2.5	27.5	0.103
5	5	15	40	0.075
7.5	7.5	27.5	27.5	0.065
5	10	40	40	0.1
5	10	15	15	0.121
7.5	12.5	27.5	27.5	0.129
10	10	15	15	0.105
10	10	40	15	0.143
10	10	15	40	0.161
10	5	15	15	0.091
5	5	15	15	0.078
2.5	7.5	27.5	27.5	0.184
5	10	15	40	0.076
10	5	40	15	0.084
12.5	7.5	27.5	27.5	0.116
7.5	7.5	52.5	27.5	0.121
7.5	7.5	27.5	52.5	0.142
7.5	2.5	27.5	27.5	0.104
7.5	7.5	27.5	27.5	0.102
5	10	40	15	0.113
5	5	40	40	0.079
7.5	7.5	27.5	27.5	0.132
5	5	40	15	0.181
10	5	40	40	0.106
7.5	7.5	27.5	27.5	0.124
10	10	40	40	0.166

Table D3: PVA composite film thickness data of alternate organic acids based films (21.5 cm x 10.5 cm) being prepared at 50 °C.

Composition		Thickness (mm)
Organic acids	Case	
CA	EXP-%E	0.121
	EXP+%E	0.111
	RSM-%E	0.13
	RSM+%E	0.098
TA	EXP-%E	0.116
	EXP+%E	0.083
	RSM-%E	0.114
	RSM+%E	0.154
MA	EXP-%E	0.161
	EXP+%E	0.136
	RSM-%E	0.057
	RSM+%E	0.086

Table D4: Dry composite hydrogel film thickness data of PVA-St-MA-GI films prepared at 50 °C for variant choices of ternary constituent concentrations and fixed GI concentration (18.38 wt%).

Composition			Thickness (mm)
PVA concentration (w/w%)	St concentration (w/w%)	CA concentration (wt%)	
12.55	7.5	27.5	0.093
7.5	7.5	27.5	0.108
7.5	7.5	27.5	0.082
4.5	10.5	12	0.089
7.5	7.5	27.5	0.098
7.5	7.5	53.57	0.142
7.5	2.45	27.5	0.041
7.5	7.5	1.43	0.141
2.45	7.5	27.5	0.171
10.5	4.5	12	0.054
4.5	4.5	43	0.159
10.5	10.5	43	0.139

Composition			Thickness (mm)
PVA concentration (w/w%)	St concentration (w/w%)	CA concentration (wt%)	
4.5	10.5	43	0.175
4.5	4.5	12	0.134
7.5	7.5	27.5	0.057
7.5	7.5	27.5	0.062
7.5	7.5	27.5	0.058
10.5	4.5	43	0.102
10.5	10.5	12	0.133
7.5	12.55	27.5	0.151



PUBLICATIONS





International Refereed Journals

- 1) Das, A., R. Uppaluri and C. Das (2019). "Feasibility of poly-vinyl alcohol/starch/glycerol/citric acid composite films for wound dressing applications." International journal of biological macromolecules 131: 998–1007.
- 2) Das, A., R. Uppaluri and C. Das (2020). "Compositional synergy of poly-vinyl alcohol, starch, glycerol and citric acid concentrations during wound dressing films fabrication." International journal of biological macromolecules 146: 70–79.
- 3) Das, A., S. Bhattacharyya, R. Uppaluri and C. Das (2020). "Optimality of poly-vinyl alcohol/starch/glycerol/citric acid in wound dressing applicable composite films." International journal of biological macromolecules 155: 260–272.

Articles under Preparation

- 1) Das, A., M. Saha, M. Gupta, L. Rangan, R. Uppaluri and C. Das, "Comparative efficacy of citric acid/tartaric acid/malic acid additive based PVA/St composite films."
- 2) Das, A., M. Saha, M. Gupta, L. Rangan, R. Uppaluri and C. Das, "Optimality of poly-vinyl alcohol/starch/malic acid blended composite films for wound dressing applications."

Conference Presentations

- 1) Das, A., R. Uppaluri and C. Das (2019). "Synergy of PVA, starch, citric acid and glycerol concentrations and their optimality with RSM for wound dressing application." Reflux'19, IIT Guwahati.





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Feasibility of poly-vinyl alcohol/starch/glycerol/citric acid composite films for wound dressing applications

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ABSTRACT

This work addresses the competence of PVA/starch/citric acid (PVA/St/CA) based composite films for wound dressing applications. Literature reported composition of PVA, starch and glycerol were adopted and the optimality of crosslinking temperature (40 to 60 °C) and citric acid concentration (0.5 to 3 g) have been investigated during solution casting based composite film fabrication. The prepared polymer films have been characterized with swelling index, solubility based biodegradability, gel fraction, mechanical strength, water vapor transmission rate and antimicrobial effectiveness. The optimized film (composition) were analyzed to possess excellent swelling ($260.5 \pm 2.9\%$) and in-vitro degradation (45.5 ± 1.8 – $59.8 \pm 0.4\%$ degradation, during 14 days) characteristics. Further, the film possesses enhanced combinations of water vapor transmission rate and antibacterial activity with Gram-negative (*Escherichia coli*) and Gram-positive (*Listeria monocytogenes*) bacteria. With all these promising characteristics, the low temperature (50 °C) and long duration (12 h) fabricated PVA-St-CA films have been inferred to be a potential candidate for wound dressing applications.

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1. Introduction

Ideal characteristics of wound dressing materials include ability to provide moist environment to avoid wound dryness, protection from any kind of contamination, absorption of wound exudates, stimulation of cell growth factors, adequate mechanical strength and flexibility and biocompatibility/biodegradability. However, due to the inability to have a generic dressing material that caters to customized healing requirements, a myriad of synthetic dressing materials are accessible in the medical and pharmaceutical industry [1]. Till date, based on biocompatibility, biodegradability and ability to enhance re-epithelialization, several natural polymers such as polysaccharides, proteoglycans and proteins and synthetic polymers have been extensively investigated for their applicability as wound dressing materials.

Many of the above mentioned materials have significant limitations in terms of widespread applicability, processing capability, fabrication cost and cost of the materials. While alginate has excellent fluid absorption characteristics (upto 20 times its own weight) and collagen dressings enhance healing (by bringing the wound edges together), they are highly expensive and difficult to handle and process [2,3]. Similarly, chitosan possesses excellent biocompatibility and biodegradability but is expensive due to the tedious extraction process [3]. On the other hand, starch is water-soluble, inexpensive and excellent natural biodegradable material due to its constitution [2]. Consisting of alpha-(1,4)

glycosidic bonds within in the amylose and amylopectin polymeric structures, starch exhibits both amorphous and crystalline functionalities. The amylose regions of its structure and branching points of amylopectin contribute towards amorphous properties and detrimental mechanical properties (lower strength and elongation and higher brittleness) [4]. To overcome such limitation, chemical modification of starch is often targeted that involves etherification, oxidation, hydrolysis and crosslinking. The chemical modification allows introduction of newer functional groups to alter physicochemical properties of the starch. Precisely, structural changes are targeted to the abundantly available hydrophilic hydroxyl groups by facilitating intra or inter-molecular bonds at random positions. Crosslinking enables chemical bonding based reinforcement of hydrogen bonds to enhance mechanical and thermal properties, water stability and pH resistance of the starch [5]. The bond interlinking during such crosslinking refers to inter-molecular linkages of primary (C_6-OH) or secondary (C_2-OH) or tertiary (C_3-OH) hydroxyl groups with the functional groups of crosslinking agents such as phosphorous oxychloride, sodium trimetaphosphate (STMP), sodium tripolyphosphate (STPP), epichlorohydrin, formaldehyde, boric acid etc. [6]. While crosslinking is useful to enhance mechanical properties to some extent, crosslinked starch does not have adequate mechanical strength to serve as a wound dressing material. On the other hand, synthetic polymers such as polyvinylpyrrolidone, polyethylene glycol and polyvinyl alcohol can also be targeted additionally to further engineer and optimize blended properties of chemically modified starch for wound dressing applications. Despite being non-toxic, water-soluble, biocompatible and having excellent wetting

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Compositional synergy of poly-vinyl alcohol, starch, glycerol and citric acid concentrations during wound dressing films fabrication

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ABSTRACT

This work addresses response surface methodology (RSM) design based investigations to obtain useful insights on the compatibility and synergy of quaternary formulations [variant macromolecular concentrations of starch (5–10 w/w%), polyvinyl alcohol (5–10 w/w%), citric acid (15–40 wt%) and glycerol (15–40 wt%)] and to yield wound dressing films with appropriate combinations of swelling index (SI), in-vitro degradation during 27 days and tensile strength (TS). The design studies inferred that while SI and in-vitro degradation followed quadratic expressions with respect to variations in the quaternary compositions, the TS was relatively less complex with bilinear and linear terms of the independent variables. Based on experimental investigations, optimized film (composition: 5 w/w% PVA, 10 w/w% St, 15 wt% CA and 15 wt% GI) exhibited excellent SI (338.37%), acceptable in-vitro degradation (53.27%) and gel fraction (GF 34%), higher TS (7.65 MPa) and water solubility (66%) but poor elongation (9.13%). Further, the optimal composition based polymer composite film possessed promising antibacterial activity with gram-negative (*Escherichia coli*) and gram-positive (*Listeria monocytogenes*) bacteria to indicate its competence in terms of a durable and inexpensive biocompatible wound dressing film.

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1. Introduction

Abundant consumption of synthetic polymers possesses serious ecological problems. Given the expensive and hazardous nature of recycling and disposal methodologies of synthetic polymers, a smooth transition in the polymer economy hints towards a growing emphasis on biodegradable and renewable natural polymers [1]. Despite being biodegradable, these renewable polymers are expensive [2,3] to accommodate in the existing economy and hence a transitory phase is inevitable that attempts to achieve composites prepared with natural and synthetic polymers [4]. Such composites offer viable solutions to enhance the biodegradability of synthetic polymers. In the field of biocomposite films for biomedical applications, conventional and frontier research emphasizes the achievement of inexpensive, easy to fabricate, highly bio-degradable films with acceptable combinations of characterization parameters [2,4].

Among various available natural polymers, compared to chitosan (CS), collagen, gelatin (Gel) and alginate, starch (St) is the most affordable and plausible for the fabrication of synthetic polymer-based composite materials and biocomposites due to ease of production from sustainable natural biological resources such as corn, potato, wheat

and rice [4,5]. In addition, starch is a non-toxic constituent of a biocomposite [6]. However, with high water solubility and insignificant physicomechanical characteristics, starch has fundamental limitations in terms of poor processability characteristics and shape stability in fluids. Therefore, native starch is difficult for direct utility and needs to be considered by blending it with appropriate synthetic materials [7]. In this regard, relevant techniques that are usually adopted include gelatinization, cross-linked phosphorylation, and hydroxypropylation [4]. These techniques facilitate effective integration and crosslinking of starch into polymer composite films to potentially enhance the desired properties for commercial applications [8].

On the other hand, among various available synthetic biodegradable polymers for biomedical applications, polyvinyl alcohol (PVA) is highly inexpensive and affordable in comparison with poly(lactic-co-glycolic acid) (PLGA), polycaprolactone (PCL), Polyglycolide (PGA) [9,10]. Compared to these expensive synthetic polymers, polyethylene glycol (PEG) is inexpensive but non-biodegradable [11]. However, PVA facilitates better degradation of biocomposites. Also, PVA is an FDA approved biomaterial and its isotactic material preferentially undergoes degradation with the stereo-chemical configuration of the constituent hydroxyl groups [12]. PVA-St composite biomaterials are hydrophilic, non-toxic and easy to process and also became popular as a scaffold supporting material for tissue engineering. In summary, compared to all other synthetic polymer-based composite films, the PVA-St composites possess

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Optimality of poly-vinyl alcohol/starch/glycerol/citric acid in wound dressing applicable composite films

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ABSTRACT

This work addresses response surface methodology (RSM) design based investigations to obtain optimality of quaternary formulations [variant macromolecular concentrations of starch (St, 5–10 w/w%), polyvinyl alcohol (PVA, 5–10 w/w%), citric acid (CA, 15–40 wt%) and glycerol (Gl, 15–40 wt%)] associated to wound dressing films. Appropriate combinations of the swelling index (SI), weight loss (WL%) during 27 days, tensile strength (TS) and percentage elongation (%E) have been considered during such studies. The optimized composition was achieved through RSM optimization and exhibited very good water absorption (300.5% SI) and flexibility (87.5%E), and acceptable in-vitro degradation (51.4% WL) and TS (5 MPa) values, which are significantly better than reported data. Further, the film constitution indicated amplified antibacterial effectiveness against both Gram-positive (*Listeria monocytogenes*) and Gram-negative (*Escherichia coli*) bacteria and enhanced cell growth (145.5%) to thereby infer upon the potential associated with its application as a viable wound dressing film.

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1. Introduction

Wound dressing substantially accentuates the mitigation of disruptions in the epithelium continuity [1]. Traditional dressings such as gauzes are detrimental due to mechanical debridement of tissues and induce painful wound trauma during dressing operations. Further, given the ability of exogenous bacteria to penetrate up to 64 layers of gauze, they are concluded to be nonocclusive [2]. The development of transparent wound dressing films facilitates a moist environment for healing and promotes autolytic debridement along with impermeability for bacteria. However, due to their inability to imbibe wound exudates, such dressings are applicable for wounds with minimal exudates [1]. To circumvent this issue, polymer-based composite hydrogel films have been proposed to serve wounds possessing a higher amount of exudates [1,3]. Such hydrogels do not have good mechanical characteristics and lose their occlusivity to bacteria after being swollen with exudates [1].

With the integration of several constituents that enormously contribute towards their diverse functionalities, polymer composite hydrogel films possess complex intriguing structures. These films facilitate the enhancement of the desired property, mitigation of undesired characteristics and thereby accommodates alternate pathways towards bettering the real-world applications [3]. The available prior art indicates that customized polymeric blends have been targeted for the

preparation of novel wound dressing materials with emphasis upon the specific role of the polymers. Devi et al. fabricated fibrin/chitosan (CS) composite sheets by varying their constitution in the hydrogel films. In these films, since chitosan detracts, tensile strength (TS) of the hydrogel films, sodium alginate with variant proportions has been added to enhance mechanical characteristics of the composites [4,5]. Similar detrimental trends in mechanical characteristics have been reported for increment in CS by Baghaie et al. for polyvinyl alcohol (PVA)-Starch (St)-CS hydrogels. These composite materials possess good water vapor transmission rate (WVTR), antibacterial effectiveness and 95% wound healing [6]. Fan et al. inferred that PVA based CS/gelatin (Gel) hydrogels exhibited improved coagulation characteristics for samples prepared with enhanced Gel concentration. The optimized hydrogels possessed good combinations of pH-sensitivity, swelling ability, water evaporation rate but poor TS (2.2 MPa) [7]. Adopting five constructive freeze-thaw (F-T) cycles and variation in both PVA and PEG concentrations, Ahmed et al. optimized hydrogels film based on the response surface methodology (RSM) approach. The optimized film containing *Centella asiatica* has been characterized to possess a 453.3% swelling index (SI), 98.7% cell viability and enhanced antimicrobial limit and TS [8]. Poonguzhali et al. fabricated a nano search reinforced CS/Polyvinylpyrrolidone (PVP) membrane with enhanced healing effect and collagen formation [9].

In summary, the available prior art for hydrogel wound dressing composite films indicates the utility of alternate natural polymers (such as CS, St, alginate, Gel) after appropriate blending with synthetic polymers such as PVA, PEG, and PVP. Among these synthetic polymers,

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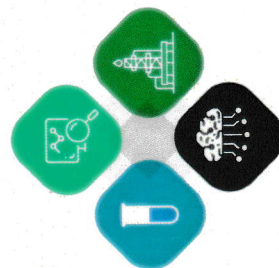
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1.	Das, A., R. Uppaluri and C. Das (2019). "Feasibility of poly-vinyl alcohol/starch/glycerol/citric acid composite films for wound dressing applications." International journal of biological macromolecules 131: 998–1007.
2.	Das, A., R. Uppaluri and C. Das (2020). "Compositional synergy of poly-vinyl alcohol, starch, glycerol and citric acid concentrations during wound dressing films fabrication." International journal of biological macromolecules 146: 70–79.
3.	Das, A., S. Bhattacharyya, R. Uppaluri and C. Das (2020). "Optimality of poly-vinyl alcohol/starch/glycerol/citric acid in wound dressing applicable composite films." International journal of biological macromolecules 155: 260–272.

List of Publications in Conference Proceedings	
1.	Das, A., R. Uppaluri and C. Das (2019). "Synergy of PVA, starch, citric acid and glycerol concentrations and their optimality with RSM for wound dressing application." Reflux'19, IIT Guwahati.

After your PhD, Are you taking up any job/research position? If yes, please provide details. No.

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