Simultaneous Extraction and Recovery of Catechins using Liquid Membrane

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Simultaneous Extraction and Recovery of Catechins using Liquid Membrane

Thesis

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requirements for the degree of

DOCTOR OF PHILOSOPHY

by

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Dedication

To My Parents

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CERTIFICATE

It is certified that the work contained in the thesis entitled "**Simultaneous Extraction and Recovery of Catechins using Liquid Membrane"** by **Mriganka Sekhar Manna** has been carried out under our supervision and that this work has not been submitted elsewhere for a degree.

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Abstract

Liquid membrane (LM) technology has drawn attention of the research community since the early 90s and it has increased manifold in recent years. This thesis aims at exploring the efficacy of LM technology for the separation and pre-concentration of catechins from green tea leaves. A suitable LM that can extract the catechins is identified through equilibrium study. Experimentation with various solvents and carrier agents reveal that the sunflower oil-tributyl phosphate (TBP) and *n*-decane-trybutyl phosphate (solvents) are two favorable combinations for recovery of the catechins. The performance of various LM based processes *viz.* in the area of bulk liquid membrane (BLM), flat sheet supported liquid membrane (FS-SLM) and hollow fiber supported liquid membrane (HF-SLM) in the recovery of catechins using the identified membrane liquid (ML) is then investigated.

The components of ML *i.e.* the solvent and the carrier were selected in two phase equilibrium studies. The distribution coefficient (D_1) of catechin between the membrane phase and the aqueous feed phase provides the catechin extraction feasibility of the membrane phase. The stripping agents also selected through two phase equilibrium studies finding the distribution coefficient (D_2) of catechin between aqueous stripping phase and the membrane phase. The various physico-chemical parameters like pH of aqueous solutions, concentration of carrier in the membrane phase, concentration of the catechin and the stripping agents in feed phase and stripping phase respectively, experimental temperature *etc*. were primarily evaluated in two phase equilibrium studies. The optimum parameters obtained in two phase equilibrium studies were later verified and improved in three phase transportation studies. The three phase transportation studies involve the diffusion step in between extraction and re-extraction or stripping of the catechin at the respective interfaces of the liquid membrane.

This doctoral project aims at studying simultaneous extraction and recovery of catechins using LM techniques. After the initial investigation on the merits of LM based techniques to separate the synthetic (+) catechin in two phase equilibrium study and simplest BLM configuration, the heart of the study was involved in two configurations of Supported Liquid Membrane (SLM). Transportation of catechin was accomplished in FS-SLM and HF-SLM prior to its recovery in aqueous solution of 0.4 M ethanol. In addition, catechins were recovered and enriched through their iron complexation through FS-SLM module. Influences of various parameters on recovery of catechins through LM techniques were studied for efficient recovery in ethanol solution as well as for the iron complexation. Iron-catechin complex was characterized by various analytical instruments. Optimized transportation technique was employed in the transportation of various catechins present in real extract of green tea leaves of Assam, India. The very general problem of instability of SLMs was studied to improve the life time of the SLM towards its commercial application. All the criteria for best stability obtained in FS-SLM studies were employed for the recovery of catechins in HF-SLM. Various parameters related to mass transfer were determined in HF-SLM. The cleaning protocol of HF-SLM was experimentally found out for the reusability of same membrane module for the treatment of real extract of various catechins from green tea leaves.

Keywords: Catechins, Liquid membrane (LM), Bulk liquid membrane (BLM), Supported liquid membrane (SLM), Tributyl phosphate (TBP). Iron complex of catechin

Summary

The present research work aims at an unexplored yet having potential scope of research. It provides a systematic approach to implement LM based technology for the recovery of catechins from green tea leaves. Therefore, the overall aim of this thesis is to explore the efficacy of LM based technology for the separation and purification of catechins. Further, metal (iron) complexation of the tea polyphenols (catechins) was integrated with the LM based separation process. To achieve this overall aim, the thesis finds the following measurable objectives:

- Identification of suitable carrier and solvent-carrier combination
- Exploration of vegetable oil as the solvent of ML
- Identification of the best operating conditions for various configurations of LM
- Production of metal complex of catechins
- Characterization of the iron-catechin complex

The Thesis has been organized in eight chapters that include:

Chapter-I: Introduction and Literature Review

Chapter-II: Materials and Methods

Chapter-III: Recovery of Catechin through BLM

Chapter-IV: Recovery of Catechins through FS-SLM

Chapter-V: Recovery of Catechins through HF-SLM

Chapter-VI: Recovery and Enrichment of Catechins through Iron-complexation using FS- SLM

Chapter-VII: Detailed Stability Analysis

Chapter-VIII: Conclusions

A brief description of each of the chapters is furnished below:

Chapter-I: Introduction and Literature Review

This chapter presents an introduction of the aim of the research along with elaborate literature review on the relevant issues. It is intended in this thesis to explore the possibility of using LM for transportation of bioactive compounds, catechins from their plant sources. Hence, the need of separation of catechins from their sources is elaborated. The sources of catechins, existing processes and possibility of an alternative technique for their recovery in the commercial scale are addressed. Principle of LM, its applications, features and classifications are elaborated along with its demerits in this chapter. A thorough literature review on the earlier works related to the solvent extraction processes using various conventional solvents are incorporated. In addition, the advantages of LM application over the solvent extraction for the recovery of catechins are introduced. The enrichment of bioactive catechins through the metal (iron) complexation has been discussed. Finally, the importance and objective of the present research work are highlighted.

Chapter-II: Materials and Methods

This chapter discusses in detail the three types of LM set-up *viz.* BLM, FS-SLM and HF-SLM designed to carry out this research work. The experimental procedures followed in each case are described. This chapter also gives information on the materials used in various experiments along with the sources from where they were procured. The analytical instruments used for this research work are also summarized here.

Chapter-III: Recovery of Catechin through BLM

The chapter presents an experimental study on the simultaneous extraction and recovery of synthetic catechin through BLM. Various environmentally benign solvents, *viz*. vegetable oils, have been employed as ML along with various transport-enhancing carrier agents to identify the best carrier-solvent combination that would yield optimum performance of the BLM. Tributyl phosphate (TBP) (carrier) in sunflower oil (SFO) (solvent) is found to be the

best among the tested combinations. Initially two phase (feed-membrane) equilibrium studies have been carried out in order to study the effects of operating conditions, *viz.* pH, temperature, initial catechin concentration in feed phase and carrier concentration on the equilibrium distribution. Based on these results, simultaneous extraction and recovery of the catechin are carried out in a BLM using ethanol as the stripping agent and the optimum operating conditions are re-defined accordingly. A comparison between environmentally benign vegetable oil and the hazardous conventional solvent has been studied for their performances as solvent of ML.

Chapter-IV: Recovery of Catechins through FS-SLM

This chapter presents the results and discussion on the transportation of catechins through FS-SLM prior to their recovery in ethanol. "TBP dissolved in *n*-decane" is used as the membrane phase of FS-SLM. Various physico-chemical parameters were tuned for optimized transportation of catechin. Initially, catechin hydrate dissolved in Milli-Q® deionized water (*i.e.* synthesized extract) was used as feed phase. Subsequently, a case study with real plant extract was also carried out at the optimized process conditions. Issues regarding transportation of catechins from real extracts of green tea leaves and that from synthesized solution of catechin hydrate are discussed elaborately in this chapter.

Chapter-V: Recovery of Catechins through HF- SLM

This chapter focuses on the recovery of bioactive catechins from tea leaves (*Camellia Sinensis*) extract through HF-SLM module. The flow rates of streams and differential pressures across the membrane surfaces are optimized for achieving a stable LM. The established model equations for the solute transportation in LM technique are employed to explain the experimental results. The operational conditions for transportation of catechin from its synthesized solution were optimized and they were subsequently verified for the transportation of various catechins, in aggregate, from real extracts of green tea leaves. The problem of membrane fouling during the recovery of catechins from real extract of green tea

leaves are duly addressed in this chapter and techniques for eliminating those problems are discussed. A membrane cleaning protocol is chalked out experimentally. Combination of membrane modules for once-through transportation of catechins is formulated prior to the commercial application of the recovery process using HF-SLM.

Chapter-VI: Recovery and Enrichment of Catechins through Iron-complexation using FS-SLM

This chapter introduces the idea of metal complexation of catechins and their recovery integrated with FS-SLM technique. Seven metal ions (Al^{3+}, Cu^{2+}, Ni^{2+}, Mg^{2+}, Mn^{2+}, Zn^{+2} and Fe^{3+}) are examined as probable complexing/precipitating agents for catechin in a two-phase stripping set-up and Fe^{3+} is selected on the basis of its best capacity for catechin complexation and precipitation. Owing to the property of precipitation of iron-catechin complex inside stripping section, the pre-concentration and recovery of the target solute become very easy. As a consequence of complex precipitation, equilibrium of stripping reaction shifts towards the right which augments the continuous operation of extraction and recovery of catechin manifold. Parameters influencing the recovery of the catechins as complex are optimized. This chapter also presents the various analytical methods for the characterization of iron-catechin complex.

Chapter-VII: Detailed Stability Analysis

This chapter presents an extensive experimentation on the stability of the SLMs. Various reasons for the instability of the SLMs are considered and the related parameters are experimentally investigated. The experiments are accomplished in the simplest configuration of SLM *i.e.* in FS-SLM. An electrolyte (NaCl) is added to the aqueous phases to increase the interfacial tension between membrane phase and both the aqueous phases. Surfactant with appropriate Hydrophilic-Lipophilic Balance (HLB) is added to the membrane phase to increase its hydrophobicity that resulted in lesser loss of solvent into the aqueous phases.

Various combinations of aqueous/organic phases and characterization of emulsion droplets formed thereby are analyzed, and that yielded the explanation of reduction of loss of ML. Minimum critical displacement pressures for the ML to come out of the pores of the support membrane were evaluated experimentally for various support materials based on their physical properties such as pore diameter, thickness, tortuosity and also the irregularity of the shapes of the pores.

Chapter-VIII: Conclusions

This chapter summarizes the inferences drawn from the present research work and provides recommendations towards future direction for recovery of bioactive and/or medicinal compounds (other than catechins) from other plants and herbs.

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CHAPTER-I

Introduction and Literature Review

CHAPTER-I

Introduction and Literature Review

It was introduced in this chapter the scope and objectives of the research work presented in this thesis along with an elaborate literature review on the relevant issues. It was intended in this thesis to explore the possibility of using liquid membrane (LM) for transportation of bioactive compounds, viz., catechins, from their plant sources. Hence the need of separation of catechins from their sources was elaborated. The sources of catechins (i.e. herbs and plants), existing processes for their recovery along with their shortcomings and possibility of an alternative technique for recovery of catechins in the commercial scale were addressed. Principle of LM, its applications, features and classifications were elaborated along with its demerits in this chapter. A thorough literature review on the earlier works related to the solvent extraction processes using various conventional solvents were incorporated. In addition, the advantages of LM application over the solvent extraction for the recovery of catechins were discussed. Finally, the importance and objective of the present research work were highlighted.

1. Introduction

Herbs and plants, which are otherwise used as *ayurvedic* medicine from time immemorial, are major source of organics that have immense medicinal value. Herbal medicines are becoming popular these days due to their low or no side effects. Even otherwise, rural and tribal people use herbal plant extract as an inexpensive alternative to the more conventional allopathic genre. The south-Asian countries, in particular India, are very rich in various medicinal herbs and plants. In India the north-eastern state of Assam is famous for tea (*Camellia Sinensis*) plantation which is a

rich source of catechin(+C), a type of polyphenol. Other sources of catechins include (but are not limited to) berries, olive oil, chocolate / cocoa, coffee, pomegranates, popcorn, fruits and other vegetables. Catechin is a pharmacological substance that is effective for reduction of cholesterol and hypertension, microbial activity and protection against cardiovascular disease and cancer. In recent times, the researchers have been inspired to design novel polyphenol-based or polyphenol-inspired drugs as better anti-cancer agents [1]. Catechin is also effective as anti-oxidant in human cell [2]. Oxidation reactions produce free-radicals that can start chain reactions, which in-turn cause damage or death to the human cells. Anti-oxidants are reducing agents which terminate these chain reactions and immune the human body. Proper functioning of the immune system against the diseases like cancer, arthritis, autoimmune disorders, cardiovascular diseases, aging *etc.* are dependent upon appropriate consumption level of dietary antioxidants [3].

However, catechin compounds exist in trace quantity in their herbal sources (such as green tea leaves) and this is the major bottleneck for their recovery. Large amount of solvent is required for recovery of even a trace quantity solute in the feed. Hence, the traditional solvent extraction process is neither economically viable nor logistically tangible. Moreover waste solvent would cause acute environmental concern. On the other hand, LM based separation technique integrates two solvent extraction processes in series with minimal usage of solvent. LM is a homogeneous, thin film of liquid (membrane phase) interposed between two other liquid phases, *viz.* feed (or source) phase and receiving (or strip) phase [4-7]. The membrane liquid (ML) or the organic phase must be immiscible with the feed/receiving phases. Feed phase typically contains solute that need to be transported across the thin film of LM to the receiving phase. The transport of solute across the LM occurs due to the difference in solubility and diffusivity in the liquid film as well as the concentration gradients in the phases.

1.1 Liquid membrane

Nernst and Riesenfeld developed the concept of LM long back in 1902 [8]. Two extraction/re-extraction processes occur at both sides of LM at interfaces between membrane phase and either of the source and receiving phases. A carrier agent is often added in the organic phase which forms a solute-carrier complex in the organic phase. The solute or solute-carrier complex transports from feed side interface to the strip side interface by diffusion mechanism. Addition of carrier agent augments the transportation of solute. The ML is chosen in such a way that the phase is immiscible with the feed/stripping phase. Feed phase typically contains solute that need to be transported across the thin film LM to the receiving phase. The transport of solute across the LM occurs due to the difference in solubility and diffusivity in the liquid film as well as the concentration gradients in the phases. Selective separation of the solute is possible if ML is chosen wisely so that only the target solute is soluble and diffusive in the membrane phase. When the solute is not soluble at all or less soluble in the selected solvent, carrier/extractant is added in the solvent to increase the solubility of the solute by solute-carrier complexation. In this case the ML or the organic phase is the combination of a solvent and a carrier. The increased solubility is manifested by the enhanced distribution coefficient (D_1), defined as the ratio of catechin in membrane phase and the catechin in feed phase after the equilibrium is reached. The carrier itself is not used as the solvent as it is relatively high viscous, and has higher molecular weight and is more expensive compound. The transport through LM is highly dependent on the viscosity of the membrane phase as diffusivity according to Stokes Einstein equation, is inversely proportional to viscosity [9] *i.e.*

$$D = \frac{kT}{6\pi\eta r} \tag{1.1}$$

where, k is the Boltzmann constant, η is the viscosity of the organic phase and r is the molecule radius. Hence, the viscosity of the membrane phase should be low for higher transport. The addition of high viscous carrier in the LM increases the viscosity of ML, thereby decreases the effective diffusivity ($D_{eff.}$) of solute-carrier complex in the membrane. But if the distribution coefficient increases manifold with addition of carrier, the overall transport efficiency is increased as well [10]. From the perspective of separation of catechins from aqueous extract, which is the central theme of this thesis, aqueous/organic/aqueous configuration of LM is followed.

1.1.1 Mechanism of separation through LM

Three different phases are involved in the system of LM. Target solute is transported from feed phase to the stripping phase via the membrane phase. Extraction and/or re-extraction may occur due to feeble hydrogen bonding or by strong chemical reaction between solute and carrier. When the solute is transported by the simple solution-diffusion mechanism by virtue of difference in solubility (chemical potential) of the solute among the phases, the transportation is termed as the passive transport. It is driven by the growth of entropy of the system and does not require any chemical energy. The transport mechanisms in LM based separation can be categorized into two major types, *viz*. passive transport and active transport.

1.1.1.1 Passive transport

Passive transport is a movement of ions/atoms/molecules across membranes that is driven by the growth of entropy of the system and does not require any chemical energy. The four main kinds of passive transport are solution diffusion, facilitated diffusion, filtration and osmosis. Solution diffusion is the phenomenon by which components from a high concentration zone moves towards lower concentration zone due to concentration gradient. Diffusion continues until this

gradient is eliminated [11]. Transport of solute *A* from feed phase to membrane phase occurs by higher solubility or diffusivity of solute *A* in the membrane phase as shown in Fig. 1.1. The rate of mass transfer in this case is low and depends on the solubility of solute in the LM as well as strip phase.

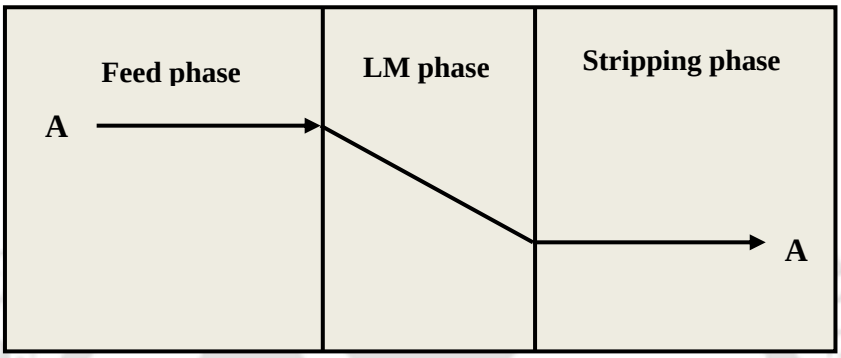


Figure 1.1: Ordinary diffusive transport of component, *A* through LM

Facilitated diffusion (or carrier-mediated diffusion) is the movement of molecules across the cell membrane via special carrier agents embedded within the LM. Solute molecules bind with its specific carrier agents, and the complex move through the membrane. Facilitated diffusion is also a passive process as the solutes move down the concentration gradient without using energy. To increase the rate of mass transfer or efficiency of the LM separation, a carrier agent is added to the membrane phase. The carrier should be soluble only in LM and should have the ability to form complex reversibly with a specific solute. The mechanism is represented schematically through Fig. 1.2. This is called the uniport mechanism because a single component is transferred through the LM. Here the transport of component *A* is enhanced by the presence of the carrier molecule *C*. The carrier *C* forms a complex *AC* at the feed/membrane interface. Complex *AC* then diffuses through the membrane due to concentration gradient across the membrane and

releases the solute *A* at the membrane/strip interface. The free carrier *C* then diffuses back to the feed/membrane interface due to concentration gradient and the cycle continues. In this case two processes occur simultaneously. Part of component *A* is transported by free diffusion (*i.e.* solution diffusion mechanism) whilst other part is transported due to the formation of solute-carrier complex that enhances the solubility of the solute *A* in the membrane phase. Hence the transport rate is increased. One basic feature of carrier mediated transport is that the complexation reaction must be reversible. Otherwise solute transport would stop when all the carrier molecules would have formed complex with the solute. Secondly, the affinity between the carrier and solute should not be very strong or very weak. A strong complex, *i.e.* one exhibiting high affinity between the carrier and solute may result in slow release at the membrane/strip interface while a weak complex, *i.e.* one exhibiting low affinity between the carrier and solute would yield limited facilitation. Therefore, there should be optimum bond energies of this reversible complex. This bond energy is recommended to be in the range of $1 - 5 \times 10^4$ kJ/kmol [9].

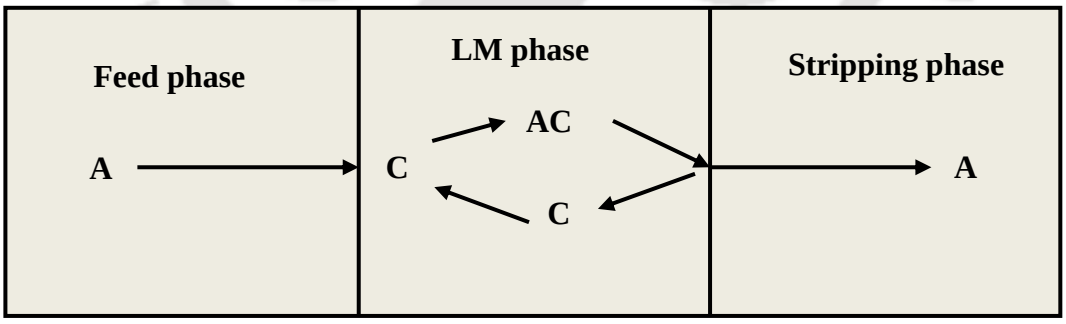


Figure 1.2: Mechanism of carrier mediated or facilitated transport in LM with mobile carrier

1.1.1.2 Active transport

In active transport, the movement of a substance across a cell membrane occurs against its concentration gradient (from low to high concentration). This is known as uphill transport. Kedem *et al.* [12, 13] has proposed a more general definition. According to him, active transport is accomplished only by the cross-coupling of the flux of species, *i* with that of other species or the chemical reaction, and the driving force is supplied by the free energy change of the coupled processes [14]. There are two types of active transport: primary and secondary. Primary active transport, also called direct active transport, directly uses chemical energy (such as from adenosine triphosphate or ATP) to transport all species of solutes across a membrane against their concentration gradient. Uptake of glucose in the human intestines is an example of primary active transport. Secondary active transport, on the other hand, allows one solute to move downhill (along its electrochemical potential gradient) in order to yield enough entropic energy to drive the transport of the other solute uphill (from a low concentration region to a high one). This is also known as coupled transport, as opposed to non-coupled or uniport transport where transport of a single component is facilitated. This research work basically deals with non-coupled transport and hence the coupled transport is not described here.

1.1.2 Types of transport of solute in LM based separation

There are three basic types of transport systems, *viz*. Cation Transport, Anion Transport and Neutral Transport, each of which has its own mechanisms and carrier types. In each of these types, it has to be noticed that regardless of mechanism of their complex formation, charge-neutrality must be maintained. This research work is related to the transport of neutral solute coupled with carrier. The mechanism is described in sub-section 1.1.1.1 and represented schematically through Fig. 1.2. Depending on the solubility of the solute in the solvent, the total

transport of the solute may be from two ways, *i.e.* by only carrier mediated or combination of solution-diffusion mechanism and carrier mediated. The mechanism of facilitated or carrier mediated and non-coupled transport which is also an example of transport of neutral solute can be described by the following steps [9, 15]:

(i) Diffusion of solute from the bulk of the feed phase to the feed-membrane interface.

(ii) Complexation between the carrier, *C* and the solute, *A* at the feed/membrane interface.

(iii) Diffusion of solute-carrier complex across the membrane.

(iv) De-complexation at the membrane/ strip (receiving) phase interface.

(v) Diffusion of free carrier back to the feed/membrane interface.

(vi) Diffusion of solute from membrane/strip interface to the bulk of the strip phase

The important properties of the carrier are necessary for the efficient transport. Firstly the complexation reaction of carrier with the solute will be reversible. Secondly, the solute-carrier interaction will not be too high to occur de-complexation. There should be optimum bond energies of this reversible complex. This bond energy is recommended to be in the range of 10 to 50 $kJ.mol^{-1}$ [9]. Both the chemical reaction and the mass transfer occur simultaneously in the facilitated transport. There are two types of carrier mediated transport *viz.* non-coupled transport and coupled transport. Non-coupled transport is the transportation of a single species (only solute) whereas coupled transport is the transport of other species like H^+ or Cl^-along with the solute. This research work basically deals with the non-coupled transport and hence the coupled transport is not presented here.

1.1.3 Carrier

Carriers attribute both to selectivity and efficiency of transport in the LM based separation process. Small amount of carrier is sufficient for the above purpose because of the small solvent

inventory associated with the membrane especially in case of SLM and also because of their non-volatile nature. Carriers are characterized by [8]

- Selectivity to the solute and reversibility of binding with the solute
- Moderate binding energy
- Non-binding with a solvent
- Moderate viscosity
- Lack of ability to coalesce and
- Non-toxicity

Carriers are categorized into three major types *viz*. acidic, alkaline and neutral, primarily on the basis of their functional groups. Selection of carrier is done on the basis of the nature of the solute. Acidic carriers have COOH, P(OH), SO_3H or chelating groups and are capable of extracting the cations. The alkaline carriers are used for the extraction of the anionic metal complexes. Amines (such as trioctylamine) are ideal examples of alkaline carriers. Neutral carriers are generally used as cationic carriers in liquid membranes for selective transport of different metal ions or they make hydrogen bonding with un-ionized solute to form the complex. Organic phosphoryl compounds and macrocyclic molecules are the most widely used neutral carriers for metal transportation [16]. One important example of the use of a neutral carrier is observed with the transport of phenol from aqueous feed solution of 2.0 pH by tributyl phosphate (TBP) and trioctyl phosphine oxide (TOPO). The macrocyclic neutral carriers carry the metal ions by encapsulating them within their cavity [17-19]. The extraction efficiency of such neutral carriers depends on the size of their cavity and the size of the inserted ions. Typical neutral carriers used in various LM processes are listed in Table 1.1.

Table 1.1: Use of neutral carrier in LM

Neutral carrier	Application in the extraction of	Ref.
Di-benzo -18-crown-6	Na^+, Ur(VI)	17-20
Di-cyclohexyle-18-crown-6	Hg (II)	18
Cryptana (2,2,1) [N2O5]	Ag(I), Cu(II) and Zn(II)	20
Cryptand(2,2,2) [N2O6]	Ag(I), Cu(II) and Zn(II)	20
Tributyl phosphate (TBP)	Phenol, uranyl nitrate, plutonium ion(Pu^+)	47
Trioctyl phosphine oxide (TOPO)	Phenol	48

1.1.4 Types of LM

LMs are of three types based on their configurations: bulk liquid membrane (BLM), emulsion liquid membrane (ELM) and supported liquid membrane (SLM). According to the geometry SLM is again of three types, flat sheet, hollow fiber and spiral wound membranes. The other types are electrostatic pseudo LM and contained liquid membrane. Fig. 1.3 shows about the various configurations of LM. This research work is more concerned with BLM and two types of SLMs *viz.* flat sheet supported liquid membrane (FS-SLM) and hollow fiber supported liquid membrane (HF-SLM). BLM is studied for initial estimation of the various parameters related to mass transfer and transport feasibility of solute. As SLM is the most promising for commercial applications, it is studied in detail. In the following sub-sections, BLM and SLMs have been introduced in brief.

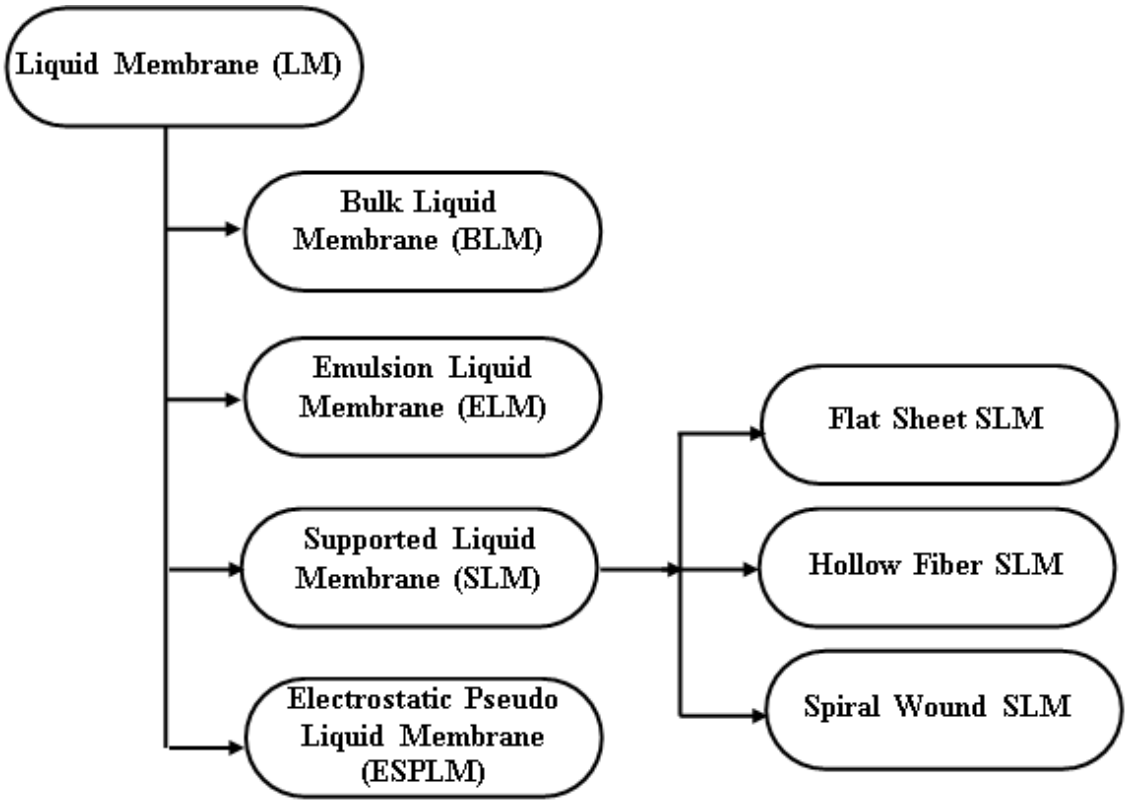
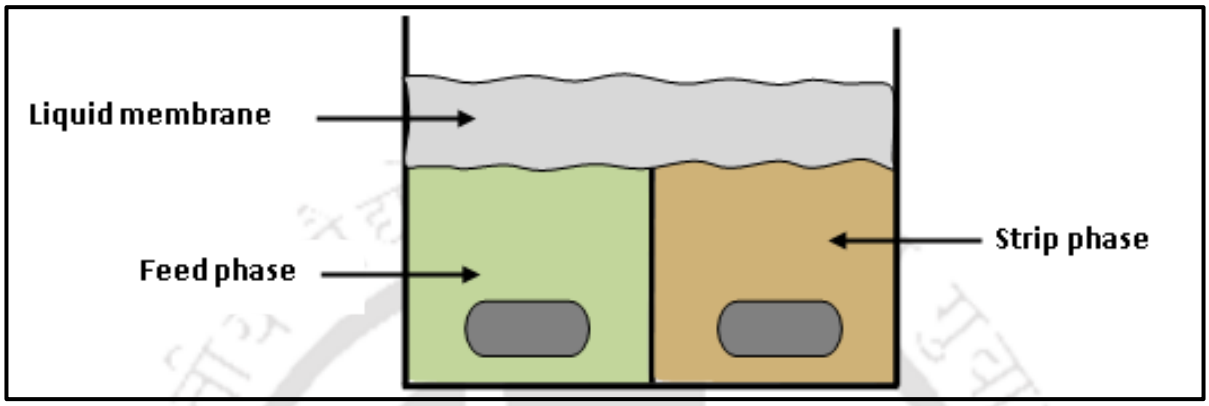


Figure 1.3: Family of liquid membranes

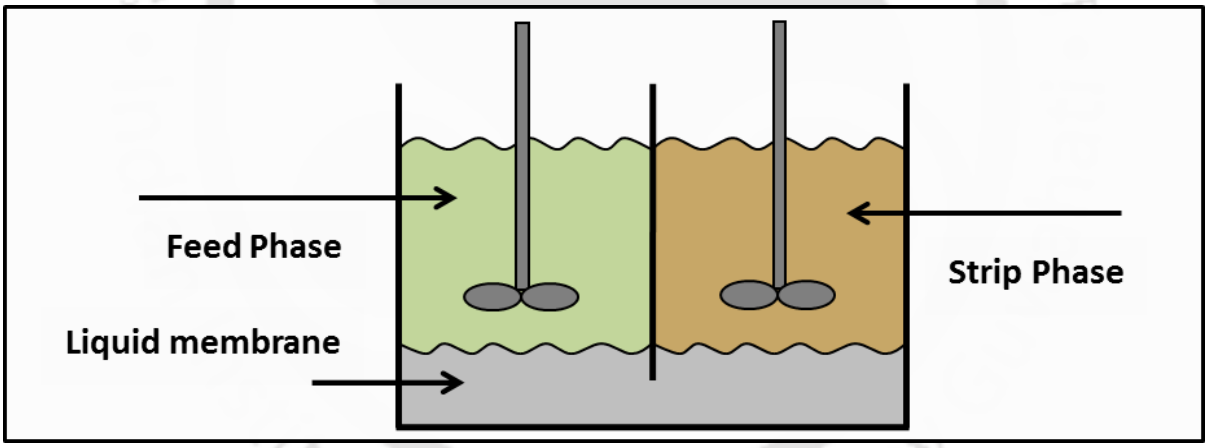
1.1.4.1 Bulk liquid membrane (BLM)

BLM is the simplest of all configurations of LMs. In a BLM setup, two aqueous phases are separated with a solid barrier (*e.g.* a glass wall) in a vessel and the membrane phase, in bulk volume, shares an interface with both these aqueous phases as shown in the schematic representations in Fig. 1.4(a-b). The Fig. 1.4 (a) shows the case when membrane phase is lighter than the feed and strip phases and Fig. 1.4 (b) shows the other case when membrane phase is heavier than feed and strip phases. Separation study with BLM is very important and relevant because, it is the simplest of all configurations of LM and it provides the understanding of the separation feasibility of a LM for any system of concern. However, up-gradation of the laboratory scale BLM to a pilot/commercial scale happens to be practically ineffective mainly

due to very low mass transfer area per unit volume as well as for the longer diffusion path of the solute or solute-carrier complex.



(a)



(b)

Figure 1.4: Schematic of BLM: (a) for lighter ML (b) for heavier ML

1.1.4.2 Supported liquid membrane (SLM)

A solid micro-porous polymeric membrane holds the ML in its pores by capillary force. The typical thickness of the supports are in the range of 25-170 µm (for polymeric membranes) and the average diameters of the pores are in the range of 0.075-0.45µm [20]. Thus, a SLM consists

of three main parts (i) support material (ii) diluent (solvent) and (iii) carrier. The membrane phase is prepared by dissolving the carrier agent into the solvent and immobilized into the pores using the wetting characteristic of the ML and by capillary forces. The porous support material serves as a framework or supporting layer for the membrane phase. The porous support can be inorganic or organic (polymer) with compatible chemical properties and mechanical stability. The novelty of the SLM techniques lies in the fact that the diffusion path (L) of the solute can be designed to be very small provided that the stability of membrane is not compromised. According to the Fick's law, rate of diffusion of a solute through a medium (liquid) is inversely proportion to the diffusion path. Hence, to increase the rate of solute diffusion, thickness of the LM should be kept as minimum as possible. All these factors result in the increase in the solute flux. However, the success of SLM techniques depends on the judicious selection of support, ML and the stripping agent for solute of interest. Important properties of ML in view of membrane stability are viscosity of ML, interfacial tension between membrane phase and the aqueous phases, solubility of ML in aqueous phases, *etc*. In addition, the support material needs to be compatible with the membrane phase. The compatibility is of two types *viz.* physical and chemical compatibility. Physical compatibility includes thickness, pore size, shape of the pores, tortuosity *etc.* On the other hand, relative hydrophobicity of ML and the support material falls in chemical compatibility.

Ideally, the pores should be of identical in size and cylindrical in shape. But, very often, they are away from this uniqueness and regular shape. Moreover, the supports need to be of higher porosity so that the effective surface area for the solute diffusion is high. Therefore, the porosity, thickness, shapes and sizes of the pores of the supports as a whole contribute to the performance

of solute transportation. The effective characteristic is measured as the tortuosity (τ) of the support which is defined as follows [6]:

$$\tau = \frac{1 + V_p}{1 - V_p} \tag{1.2}$$

Where V_p is the volume fraction of the polymeric framework and again defined as:

$V_p = 1 - \varepsilon$, where ε is porosity of the support membrane.

Based on the geometry of the supports, SLM is in general of three types *viz*. FS-SLM, HF-SLM and spiral wound. FS-SLM, as the name implies, uses support material in sheet form. It is simple in structure and of low cost but has very low mass transfer area per unit volume as well. A schematic of FS-SLM is shown in Fig. 1.5.

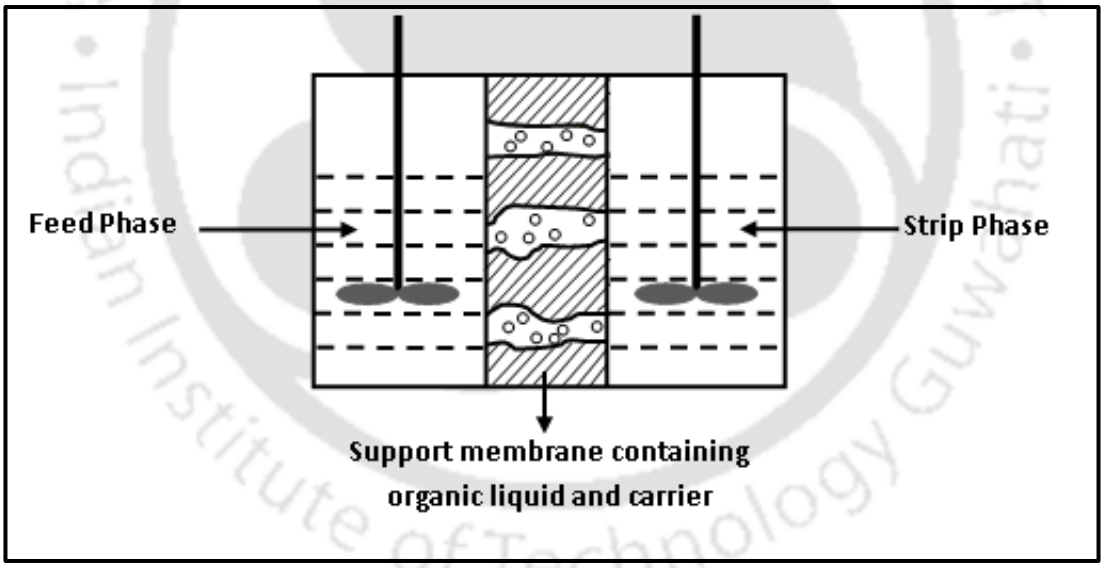


Figure 1.5: Schematic of FS-SLM

HF-SLM is just like a shell and tube heat exchanger, thus compact with increased mass transfer area per unit volume [21]. The typical mass transfer area to volume ratio of commercially available HFM module is 2930 $m^2.m^{-3}$ [22]. HF-SLM has an outer shell and there are large

number of thin porous fibers in the shape of tubes (ID: 0.5-1.0 mm) inside the shell [23]. The pores of the fibers are immobilized with the ML. The feed phase and strip phase are separately passed through either fiber and shell side or through shell side and fiber respectively along the length. A schematic representation of HF-SLM is shown in Fig. 1.6.

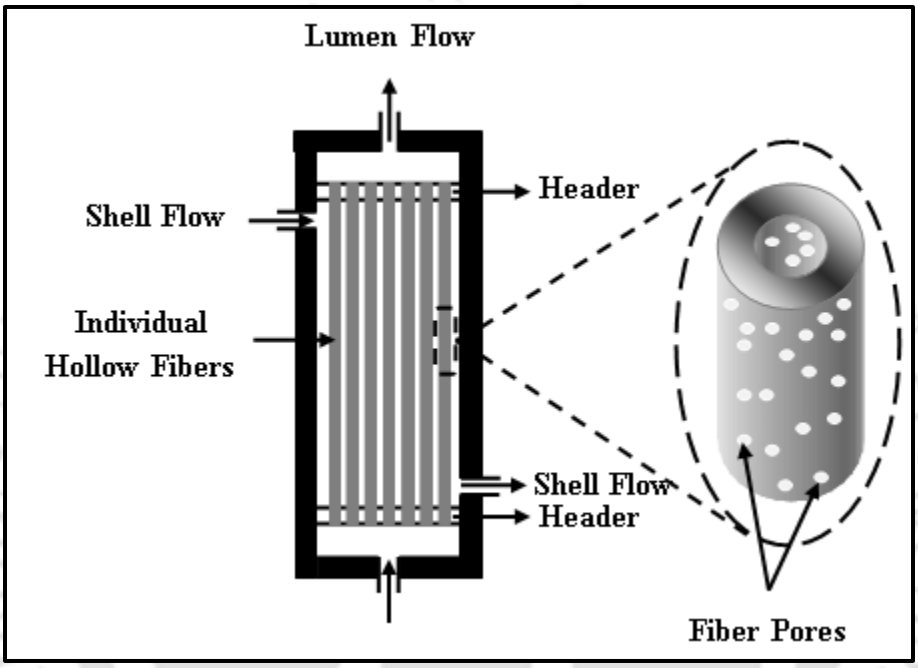
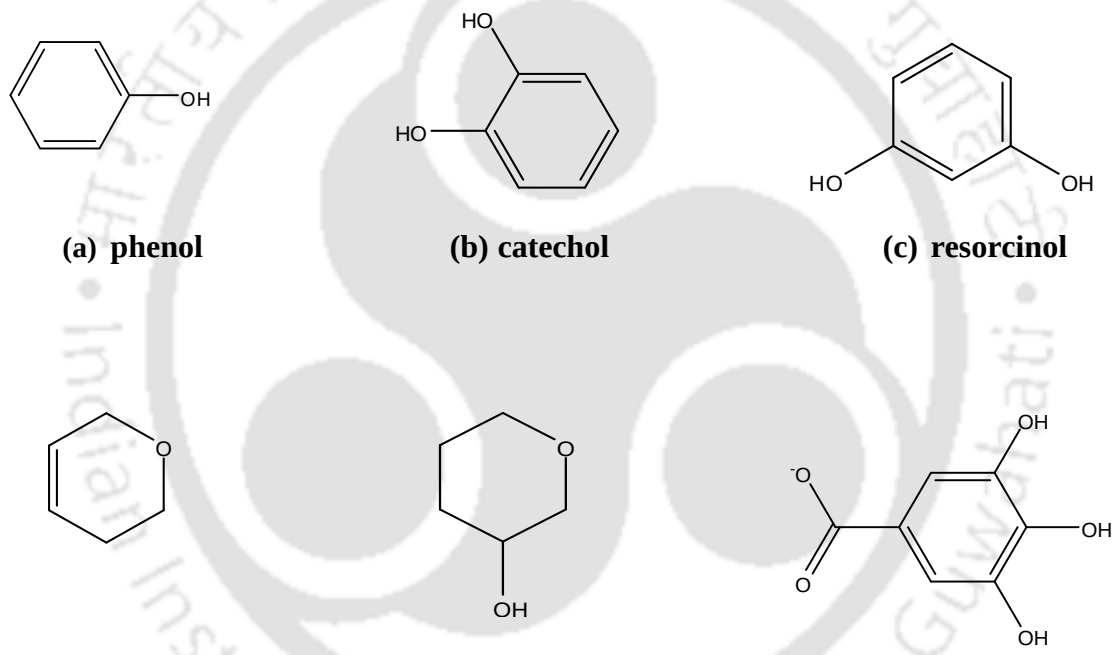


Figure 1.6: Schematic of HF-SLM

1.2 Solute

Catechins are secondary metabolites of tea plants. The polyphenols fall in the group of flavanol and in generalized group of flavonoid. They are extremely privileged type of polyphenols and are the important building blocks in pharmaceutical industries due to their chemoprotective and cardioprotective effects. They are also antioxidant and antimicrobial. Catechin possesses two benzene rings (called the ring$-A$ and the ring$-B$ and a dihydropyran heterocycle (the ring$-C$) with a hydroxyl group on carbon 3 as shown in Fig.1.7 [24-26]. The ring–A is similar to a

resorcinol moiety while the ring–*B* is similar to a catechol moiety. There are two chiral centers on the molecule on carbons 2 and 3 as shown in Fig. 1.8. Therefore, it has four diastereoisomers. Two of the isomers are in *trans*-configuration and are called catechins and the other two are in *cis*-configuration and are called (-)epicatechins. The different epimers are distinguished using chiral column chromatography [27, 28]. The structure of various catechin compounds are listed below with functional constituents:



(a) phenol **(b) catechol** **(c) resorcinol**

(d) dihydropyran **(e) dihydropyran with one –OH at carbon 3** **(f) gallate group**

Figure 1.7: Various constituent molecules of various catechins

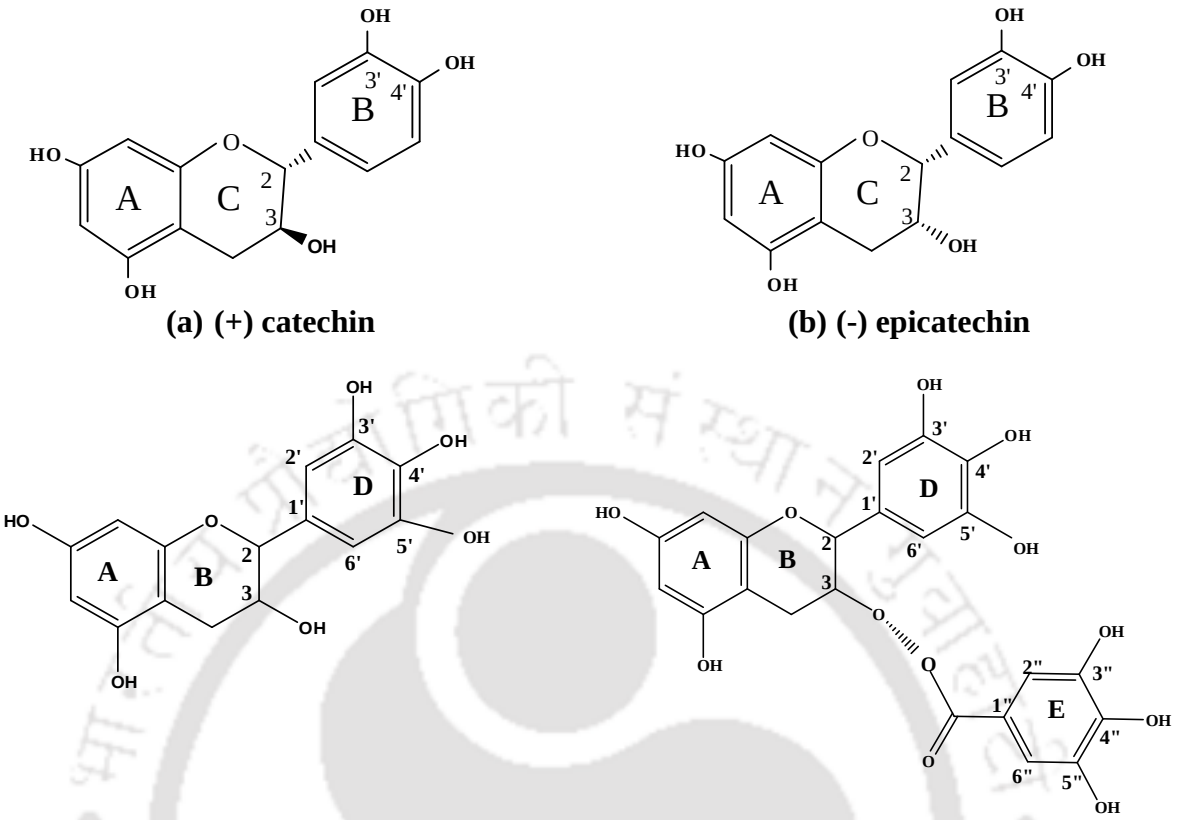


Figure 1.8: Various catechins with functional groups

Referring to no particular isomer, the molecules that have been shown in Fig. 1.8 can just be called catechin compounds or just catechins. Catechins exhibit various physical and chemical properties. Some important physical and chemical properties are:

- Water solubility
- Ethanol solubility
- Instability in water solution for longer time
- Stability in dried condition
- Tautomerism

- Self-transformation and reaction with metals

1.3 Applications of LM

Application of LM based technique includes various fields like wastewater treatment, separation of heavy metals, organic chemical separation from wastewater, purification of fermented products and the recovery of bioactive compounds from plant sources.

1.3.1 LM for general applications

Since N.N. Li invented LM (ELM) as separation technique in 1968, it is considered as an effective tool for a wide variety of applications [29]. Thereafter a number of researchers applied this technology in diverse areas such as separation of gases, wastewater treatment, separation of chemical compounds *etc*. Richard D. Noble and his co-workers have summarized these works elsewhere [30]. The review of the literature in the past two decades shows that LMs have been used for separation of metals such as Cu, Cd, Fe, Pt, Ag, Au, Ni, Sr, As, Hg, Cr, Co, Pb, Zn *etc.* [19, 31-37], alkali metals such as sodium, lithium, cesium *etc.* [38], organic and inorganic acids such as acetic acid, nitric acid *etc*., biochemical compounds such as amino acid, antibiotics *etc.* [39, 40], aromatics such as benzene, toluene *etc*. [41, 42], pharmaceutical products such as diclofenac, penicillin-G, cephalosporin-C *etc*. [43-45] and for removal of phenols from wastewater [46-48]. Matsumoto *et al.* [41] presented a SLM separation method for the selective separation of aromatic hydrocarbons (*viz*. benzene, toluene and xylene) using room temperature ionic liquid (IL) (imidazolium based) as the membrane phase. They observed that benzene, toluene and *p*-xylene were successfully transported through SLM. The selectivity of aromatic hydrocarbons was greatly improved by the use of IL.

One of the demerits of solvent extraction is the utilization of large amount of hazardous solvents. On the other hand, in SLM processes very small quantity of solvent is needed. Strict environmental regulations have recently shifted the research activities towards the development of efficient LM based on environment friendly solvents. The conventionally used solvents in LM techniniques are toxic, flammable and volatile in nature [23]. The solubility of these solvents in water leads to health hazards. Hence, researchers tried environmentally as well as physiologically benign solvents in ML. Venkateswaran *et al.* [49] reported SLM based separation of copper using vegetable oil such as coconut oil as diluent. They reported coconut oil as a novel and stable diluent for the separation of Cu^{2+} from copper plating wastewater and achieved about 60-70% removal of copper. The extraction was less than that reported by Lazarova *et al.* [50] (99%) and Castro *et al.* (100%) [31]. Muthuraman and Palanivelu [51] studied the transport of textile dyes through vegetable oil based SLM. They investigated the efficiency of various vegetable oils like palm oil, sunflower oil and coconut oil as diluent in the separation of dyes from waste water. They reported coconut oil as novel diluent for the separation of dyes. In similar type of work, Mahmoud *et al.* [52] extracted textile dye for waste water treatment. Chakrabarty *et al.* [6] used coconut oil as the ML for separation of Hg (II). Vegetable oils are comparatively expensive and food items. Nevertheless, researchers tried the environmentally benign vegetable oils to replace the conventional hazardous organic solvents in SLMs as their requirement is very less.

Shukla *et al.* [53] used TBP for transportation of uranium (IV) from wastewater through FS-SLM. Rathore *et al.* transported plutonium through the liquid membrane (LM) of 30 % (*v/v*) TBP in *n*-dodecane immobilized in the micro-spores of hollow fiber membrane module (Accurel PP) from aqueous acidic wastes. They achieved 70 % recovery of plutonium selectively from feed

solution containing 8 mg.dm^{-3} plutonium and other interfering wastes like 15 mg.dm^{-3} uranium, gross β^- = 49.33 mCi.dm^{-3}, gross γ = 15.73 mCi.dm^{-3} and 3 M HNO_3. The stability of the liquid membrane and the reproducibility of the transportation efficiency were found to be excellent in the impregnation mode by use of higher aliphatic hydrocarbon (*n*-dodecane) as solvent and optimizing flow rates of the feed and strip streams. In both the cases TBP worked as the neutral carrier in making TBP-metal nitrate complex in presence of nitric acid in the feed phase. Zidi *et al.* [46] used SLM technique for transportation of phenol where TBP (20%) in kerosene (as solvent) was immobilized in 0.2 μm pore size polyvinylidene fluoride (PVDF) membrane support and thereby achieved ~75% efficiency in recovery.

Pancharoen *et al.* studied the separation of heavy metals from the wastewater in a single [54] or successive two hollow fiber membrane (HFM) modules [55] for either a single metal or selective separation of multiple metals using synergistic extractants [22], respectively. The synergistic effect on arsenic removal was observed by adding Cyanex 471 in Aliquat 336 resulting in the synergistic coefficient of 2.8. Substantial amount of work has been accomplished in HFM module for separation of various metal ions [54-56] . However, very few literatures are available on transportation and recovery of bioactive and/or medicinal compounds in HF-SLM. Chen *et al*. [39] analyzed the kinetic behavior of reactive extraction and stripping of aspartic acid in two different hollow fiber liquid membrane contactors. A steady-state mass transfer model was examined with the experimental results and rate controlling step was identified by comparing the relative resistances of each mass transfer step. Syska *et al.* [43] performed the experimental and theoretical studies on simultaneous extractive removal and stripping of penicillin-G, a novel antibiotic in two successive HFM contactors. Individual and overall mass transfer coefficients in both modules were found and it was observed that the simultaneous extraction /stripping are

limited by the rate of the reverse chemical reaction or by the diffusion of the complex through the membrane in the second module.

SLM is regarded as the promising techniques for recovery of trace amount but value−added bioactive species along with its other applications like separation of heavy metal ions from aqueous solutions, removal of contaminants from waste water, gas separation *etc.* [5, 6, 43, 46, 47]. But, commercial application of this technique is very limited due to very less longevity of the LM. The very general problem of SLMs (both of FS- and HF-) is the integration of the ML in the pores of polymeric support to withstand the various forces generated during operation. Mutual dissolution of the three liquid phases comprising the system and the loss of organic membrane with time due to evaporation are other reasons for membrane instability. The instability of SLMs affects in several ways. The flux declines with time due to the loss of ML and affects the overall transportation of solute [57, 58]. At the same time, the complete recovery of lost ML in the aqueous phases is difficult. ML loss from the support pores may cause direct channeling of the feed and stripping solutions that leads to the mixing of the phases [59]. Moreover, ML mixing to the aqueous phases, especially to the stripping phases deteriorates the product quality. The disadvantage of less longevity of LM system demands for detailed introspection of the physico-chemical parameters of the LM components. In addition, various forces functioning on LM should be adjusted in order to increase the longevity of LM so that it can be operated in commercial level.

Researchers investigated the factors and mechanisms influencing the stability of SLM since it was established as the advanced alternative technique of conventional solvent extraction. Danesi and Rickert reported three probable reasons of instability of SLM [60, 61], (i) solubility of the organic phase in aqueous solutions, (ii) progressive wettability of the pores of polymeric support

which is induced by interfacial tension (γ) away from critical interfacial tension of membrane support, (iii) and most importantly the differential pressure existing between both sides (aqueous phases) of the LM caused by stirring and/or pumping of the flow streams.

Takahashi *et al*. [62] studied the stabilities of two types of SLMs, *viz*. flat sheet SLM and hollow fiber SLM, in terms of leakage of water across the membrane. Various reasons for the instability of membrane were reported by them. They argued that SLM system would be more stable when solvent is of higher interfacial tension and of lower surface tension than the critical surface tension of the polymeric solid support. Thus, aliphatic hydrocarbons, which are chemically more inert to polymeric solids and of higher boiling points, are suitable as solvents. The imbibition rate (q), that is caused by the solubility of aqueous and organic phases, progressive wettability, vaporization of solvent, forces exerted due to hydraulic pressure *etc.* as a combinatorial effect, can be expressed by the Rideal-Washburn equation [63]:

$$q = \frac{r_i \gamma cos\theta}{4\mu\delta} \tag{1.3}$$

Therefore, the stability of the ML in the pores will be higher if radius of pore (r_i) and interfacial tension (γ) are low whereas contact angle (θ), viscosity of solvent (μ) and thickness of membrane (δ) are high. The same problem of progressive wettability also arises (by an aqueous solution) with polymeric support that absorbs an organic solvent [64]. Once the organic liquid is absorbed by the polymer, the adhering water solution would progressively spread throughout the membrane in accordance with buoyancy, capillary action and pressure forces; a rate of leakage-curve is obtained in course of time. This has to be attributed to hydrodynamics and thermodynamic properties of membrane liquid (ML) and the support material [56].

On the other hand, a polymeric support can be well wetted with an organic solvent of surface tension σ which is smaller than the critical surface tension σ_c [65]. ML is held in the pores by

capillary forces. The critical displacement pressure, P_c that the ML can withstand depends upon the structure (size and shape) of pores and the interaction (chemical compatibility) between ML and the material (polymer) of support; as implied by the Young-Dupre equation [66].

$$P_c = \left({}^{2\gamma}/_{r_i} \right) . \cos\theta \tag{1.4}$$

A support-ML combination with high interfacial tension (γ) and smaller pore size (radius r_i) can withstand higher pressure. The value of $cos\,\theta$ depends on the smoothness of the surface of support material as well as the shape of the pores [20]. The interfacial tension (γ) and the contact angle (θ) have the opposite effect on the stability of LM, as implied by the Eqns. (1) and (2). Hence, these two parameters need to be optimized in order to achieve more stable LM. The loss of organic phase *i.e*. ML is least when shape of pore is cylindrical. Larger it deviates from cylindrical shape, more is the loss of organic phase [20].

Several hydrodynamic conditions, caused by the stirring and flow of streams, were analyzed and reviewed by Chandrasekhar [67], Walstra [68] and Gopal [69]. They discussed various types of instability that may occur in SLMs. The instability at the aqueous/organic interface arises when the phases move in different tangential velocities. This instability occurs in FS-SLMs because membrane phase is a stationary phase and the aqueous phases are in circulatory motion. This instability is called the Kilvin-Helmholtz instability. Another instability *viz*. Rayleigh-Taylor instability arises when lighter phase is accelerated due to movement of heavier phase. Instability may also arise from turbulence and the difference of densities of the LM and the aqueous phases because of buoyancy forces. Takahashi *et al.* [62] experimentally observed that the longevity of hollow fiber SLM becomes shorter as the module was altered from horizontal configuration to the vertical one.

Recently the following major techniques were attempted to improve the stability of SLM, such as:

- Continuous re-impregnation of the pores of support with organic phase which is present as an emulsion in one of the aqueous solutions
- Formation of barrier (layers) on the surface of polymeric support either by physical deposition or by interfacial polymerization
- Stabilization of SLM by surface coating using plasma polymerization

In the first technique the aqueous phases get polluted with the membrane phase. Moreover, there are other limitations such as the loss of ML and requirement of another technological step for the separation of the ML from the aqueous phases. Other two techniques suffer from reduced flux due to increased resistance and the reduced interfacial area for transportation of solute. All three techniques mentioned above did not succeed yet for commercialization. Hence, the optimization of the physico-chemical properties of the concerned phases and the functional forces related to the stability of SLM are important areas to explore further for commercialization of SLM techniques. In this study, we have explored the stability of SLM through characterization of the solvents, carrier and polymeric supports as well as optimization of other operating parameters *viz*., rate of stirring, use of electrolyte and surfactant.

1.3.2 LM for recovery of bioactive compounds

The most important antibiotics such as penicillin, cephalosporin *etc.* and their derivatives have been separated from the hydrolysates and reaction broths by LM using Aliquat 336 as carrier and *n*- heptane- kerosene (1:1) as diluents in ELM [44]. Wang and Juang [40] have used acidic extractant, di-(2-ethylhexyl) phosphoric acid (D2EHPA) for the extraction of amino acids in ELM. Maoela *et al.* [70] have described the identification and quantification of catechin in ethyl

acetate extracts of two medicinal plants *viz. C. mellei* and *C. quadrifidus* using cyclic and square wave voltammetry. They reported that the oxidation potential of catechin ranges from +171.0 mV to +631.6 mV and the content of catechin in medicinal plants, *C. mellei* and *C. quadrifidus* were 5.0 ppm and 4.7 ppm, respectively. They validated the presence of catechin by other analytical techniques such as HPLC, UV-VIS, NMR and FTIR. Dimitrov*et al*. [71] recovered tropane alkaloids, the bio-active substances from deadly nightshade (*Atropa Belladonna L.*) roots by integration of solvent extraction and liquid membrane separation techniques.

However, very few literatures are available on transportation and recovery of bioactive and/or medicinal compounds in hollow fiber supported liquid membrane. Coelhoso *et al*. [72] concentrated lactate in NaCl solution used as stripping phase through two hollow fiber membrane modules in series. They evaluated the diffusivity of lactate-carrier complex and the overall mass transfer coefficient with variation of carrier concentration in the membrane phase. Sirkar *et al*. [73] transported the pharmaceutical product, deltiazem from aqueous feed to aqueous stripping phase of L-malic acid through hollow fiber contained liquid membrane. The product was recovered as deltiazem malate and the rate of transportation was found to be controlled by the feed side mass transfer coefficient due to instantaneous reaction in the stripping side.

1.4 Metal complexation of catechin

In their quest for better anti-cancer agents, the researchers have been inspired to design novel polyphenol-based or polyphenol-inspired drugs containing various functionalized materials that take advantage of the unique physicochemical properties of the phenol functional group [1]. Polyphenols have high reactivity with other phenolics or metal compounds that yields compounds having sensorial properties like color and taste. The biological activity of green tea depends upon their bioavailability at the site of action. But, polyphenols generally lack the quality to be pharmaceutical drugs due to lack of adherence to Lipinski's stringent "rule of 5" [74]. However, the quality of the drugs (ligands) depends up on the complementarity of these ligands and corresponding receptors [75, 76]. Molecular and thermodynamic basis of catechin (epigallocatechin 3-gallate, EGCG) binding to skin keratin has been reported to regulate their bioavailability that reflects the binding mechanisms as hydrophobic assembly and aromatic interaction followed by hydrogen bonding [77]. Metal-catechin complexes have strong bioactivities that include even prevention of cancer [78, 79]. Synthesized metal complexes are more effective than their elemental form as the lipophilicity of a drug is increased through the formation of metal complex and action of the drug is enhanced due to effective permeability of the drug into the site of action [80]. Haemoglobin is a well-known metal complex which is basically an iron-complex that transports the oxygen to the live cells of animal body. Cisplatin, carboplatin and oxaliplatin are the well-known metal-based drugs widely used in treatment of cancer [80]. Other metal complexes have proven results too, in the treatment of diseases like diabetes, ulcer, rheumatoid arthritis, inflammatory and cardiovascular diseases. Substantial amount of work on the metal complexation of the catechin has been studied and complexation is confirmed by analytical methods like CPMAS13C NMR, FT-IR and chemical analyses [81-83].

As per the chemical coordination mechanisms of catechin, the electron donating catechol 3', 4' dihydroxyl groups (at ring−B, catechol moiety) make the coordinate bond with the ferric ion (Fe^{+3}) to form the complex. The coordination of ferric ion occurs with the oxygen atoms of two catechol groups (−OH) in ring−B. These two hydroxyl groups are favorable as they are in *ortho*-position and bond length is minimum. The other two hydroxyl groups (−OH) in ring−A will not form complex as they are in *meta*-position. The phenoxyl group (−OH) attached to the ring−C does not form the coordination bond with metal ion as per the coordination chemistry. Moreover, medicinal applications of essential metals in our diet in varying quantities have been realized in terms of their biological activity as complexes. Hence, catechins being the important polyphenols can strongly make complex with metal ions which are regarded as the enriched bioactives [84]. Substantial amount of work on the metal ion complexation of the catechins has been studied. The complexation is confirmed by analytical methods like CPMAS13 C NMR, FT-IR and chemical analyses [82-84]. Kumamoto *et al.* [85] studied the effect of pH and metal ions on antioxidative activities of catechin. Tyagi *et al.* [84] reported the interaction of iron with and catechin based on electrochemical study. They tried to approach the biomimetic conditions of iron chelation by catechins. Chen *et al.* [81] also studied the interaction of aluminium (metal) with catechins through analyses by FT-IR, XRD and ESR spectra of precipitates of metal-catechin complex. Theses, bivalent and trivalent metal ion complex of catechins are important species for some biological functions such as gene expression, apoptosis, enzyme regulation and neurotransmission [86, 87].

1.5 Conventional processes of catechins extraction vs. LM techniques

Solvent extraction is the conventional technique for the separation of various bioactive compounds. It is widely used in pharmaceutical industries for separation, purification or pre-concentration of bioactive components such as antibiotics and amino acids [88, 89]. In the context of separation of medicinal and pharmaceutical products, various researchers have reported that the secondary metabolites namely polyphenols, alkaloids, triterpenoids *etc*. that are present in herbs and plants, have potential medicinal values and they can be extracted by organic solvents [71, 90-94]. SLM technique is regarded as a promising up-gradation of the conventional solvent extraction process with the specific advantage of simultaneous extraction and stripping/recovery of the target solute. The other established techniques that are used for separation and purification of trace amount of solute from their dilute solutions in various research laboratories and also in some industrial applications include chromatography, adsorption, ion-exchange, crystallization, precipitation and coagulation. The said techniques are either too slow to be scaled up to the industrial application or they are not cost effective at all due to expensive regeneration process and high heat/electricity requirement. Some time they are not viable due to environmental pollution issues. Solvent extraction, on the other hand, is an energy saving process for separation purposes. Separation, purification or pre-concentration of bioactive compounds such as amino acids and antibiotics are carried out by solvent extraction method (from hydrolysates or fermentation broths) in food technology and pharmaceutical industries [40, 44, 70, 71, 91]. In recent energy crisis and stricter environmental regulations, use of large amount of solvent in liquid–liquid extraction process is not advisable. So, LM technique may be a suitable alternative. However, more research is needed to overcome the inherent drawbacks of LM processes like instability and low flux of transport to implement such technology in

industrial application. Catechins are generally extracted by the conventional organic solvents such as ethyl acetate, acetic acid, ethanol, acetone *etc.* in sequential extraction steps [70, 71, 91]. Jin *et al.*[91] found five different catechin derivatives, *namely* catechin (+C), epicatechin (-)EC, epicatechingallate (-)ECG, epigallocatechin (-)EGC and epigallocatechingallate (-)EGCG, in green tea leaves by extracting them using ethyl acetate. Goto *et al.* [95] studied the effects of various experimental conditions that may affect the efficiency of extraction of catechin from green tea leaves. Govar *et al*. [96] recovered catechins from green tea leaves by extraction using superheated water followed by successive solvent partition with chloroform and ethyl acetate. The identification and quantification of various catechin compounds in extract of green tea leaves and black tea leaves are accomplished by various authors [24, 85, 88, 89, 97-100]. They used sophisticated analytical instruments such as HPLC with UV-detector, MS-electrospray, HPCE *etc.* Indian black tea contains a total of 14.99 mg catechins((+)C, (-)EC, (-)ECG, (-)EGC and (-)EGCG)) per gram of dried raw green tea leaves of which quantity of catechin, (+C) is 3.4 mg and that of epicatechin, (-)EC is 4.86 mg [91]. During the production of black tea, enzymatic oxidation of the catechin leads to loss of quality of the catechins and formation of brown products such as theaflavins (TF) and thearubigins (TR) [28, 91].

Conventional solvent extraction is involved in several steps and multiple equipments with utilization of substantial amount of hazardous solvents for the recovery of catechins from green tea leaves [70, 91]. Supported Liquid membrane (SLM) process is an alternative recovery process for this purpose requiring very less amount of solvent and/carrier utilization with others advantages of selectiveness, lower carbon foot print, lower investment *etc*. Moreover, micro-porous hollow fiber membrane (HFM) module is regarded as one of the suitable alternatives for

this purpose that explores a new high efficiency equipment for solute transportation with the best advantage of high ratio of surface to volume of the module [101].

1.6 Scope of LM for extraction and recovery of catechins

From the extensive literature survey on separation and purification of bioactive compounds it can be concluded that the secondary metabolites namely polyphenols, alkaloids, triterpenoids *etc.* that are present in herbs and plants have potential medicinal values and they can be extracted by organic solvents [70, 71, 91, 102]. However, no attempt has been taken till date to explore the SLM processes for recovery of medicinal species, like catechins, from the bio-active botanicals *i.e*. green tea leaves. In spite of having tremendous scope of SLM in pharmaceutical applications, little work has been reported in literature till date. From the review of the literature reported so far it is evident that catechins are never explored for recovery in LM technique by the research community. Because of available source of raw materials it is highly possible to set up a pharmaceutical industry based on the target research output. Again, LM based processes based on green or environment friendly solvent such as ionic liquid, vegetable oil *etc*. are gaining more importance now a days for the reasons discussed in section 1.3.1. But, only a little information is available in the literature on the performance of such solvents as LM.

1.7 Importance and objective of the research work

The present research work aims at a systematic approach to implement LM based technology for the recovery of catechins from green tea leaves. Therefore, the overall aim of this thesis is to explore the efficacy of LM based technology for the separation and purification of bioactive catechins. To achieve this overall aim, the thesis finds the following measurable objectives:

- Identification of a suitable organic solvent (membrane phase) that can extract catechins from their aqueous solution by a two phase equilibrium study
- Identification of a suitable carrier agent or more precisely a suitable solvent-carrier combination that enhances the transport of solutes from one aqueous phase (source/feed phase) to the other (strip/receiving phase) through the intermediate membrane phase, by a three phase BLM study
- Identification of an environmentally benign solvent (vegetable oil) that could be used in BLM in order to minimize the environmental pollution
- Identification of the best operating condition in terms of concentration and pH of both aqueous phases, initial concentrations of feed phase, concentration of strip phase, carrier concentration and operating temperature which would yield best transportation of catechins in a BLM unit
- Verification of the above conditions for application to catechins recovery using SLM and selection of support material for a stable SLM configuration
- Study for the enhanced stability of SLM
- A comparative study of BLM, FS-SLM and HF-SLM and their various merits and demerits in recovery of catechins
- Case study *i.e.* catechins separation from aqueous extract of green tea leaves in both type of SLMs at the best operating condition obtained as above
- Enrichment of the bioactive catechins through their metal complexation and simultaneously enhancement of catechins recovery by complex precipitation in the stripping phase

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CHAPTER-II

Materials and Methods

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Materials and Methods

The information on the materials used in various experiments along with the sources from where they were procured was provided in this chapter. The analytical instruments used during this research work were also summarized here. Three types of liquid membrane set-up in detail, viz. bulk liquid membrane (BLM), flat sheet supported liquid membrane (FS-SLM) and hollow fiber supported liquid membrane (HF-SLM) designed to carry out this research work were also discussed in this chapter. The experimental procedures followed in each case were described.

2.1 Chemicals and reagents

Various materials used in the recovery of catechins along with their sources are summarized in this section. All reagents used in this work were of GR (Guaranteed Reagent) grade and were used as they were. Aqueous solutions were prepared with Milli-Q® de-ionized water (Millipore, USA). Synthetic extract of catechin hydrate ($C_{15}H_{14}O_6.H_2O$) was procured from National Chemicals, Vadodara, Gujarat, India. Solvents *n*-decane, *n*-dodecane, cyclohexane, *i*-octane, carrier tributyl phosphate (TBP), and stripping agent ethanol, *i*-propanol were procured from Merck, Germany. Good quality refined vegetables oils (Fortune, Adonis Wilmar Limited) *viz.* mustard oil, coconut oil, soya bean oil and sunflower oil (SFO) were procured from local market. The detailed specification and the compositions of SFO have been provided in Appendix-I. Various metal salts (nitrate and chloride) and other reagents were procured from Merck, India. The polymeric membrane support materials, polyvinylidenedifluoride (PVDF), polytetrafluoroethylene (PTFE), nylon 6, and polyethersulfone (PES), were procured from Pall

Life Science Corporation, India. Their physical properties and characteristics have been tabulated in the next section. The hollow fiber membrane (HFM) module was also procured from Pall Life Science Corporation, India. Standards of four catechins namely catechin ((+) C), epigallocatechin ((-) EGC), epicatechingallate ((-)ECG) and epigallocatechingallate ((-) EGCG) were procured from Sigma Aldrich.

2.1.1 Working solutions for "synthetic extract" of catechin

Stock solution (1000 $mg.L^{-1}$) of catechin was prepared by dissolving 212.4 mg of catechin hydrate ($C_{15}H_{14}O_6.H_2O$) in 200 mL of Milli-Q de-ionized water. The feed phases, in both the equilibrium and solute transportation studies, were prepared from the stock solution by dilution with deionized water up to the desired concentration. On the other hand, the organic phases were prepared by dissolving appropriate amount of the carrier in various pure solvents.

2.1.2 Preparation of "real extract" from green tea leaves and analysis of catechins

The real extract was prepared by extracting 1g of ground green tea leaves (Assam, India) in 130 mL deionized water at 60˚C for 10 h under continuous stirring at 600 rpm. The extract was initially filtered by 5.0 µm pore size filter paper (Advantec, Japan). Various catechins in the extract were identified and quantified in Reversed phase HPLC analysis with the help of the four catechin standards namely catechin (+C), (−)EGC, (−)ECG and (−)EGCG. The detail of the procedure for the identification and quantification of catechins of real extract is described in the next section.

2.2 Analytical instruments

2.2.1 Identification and quantification of catechins

UV-vis. spectrophotometry: A UV-vis spectrophotometer (Perkin Elmer, Model: Lambda 35) was used for measurement of concentration of synthetic extract of catechin. Samples collected during experimentation were first centrifuged (Eltek, TC 800 D) in order to obtain a clear solution prior to measurement of catechin concentration in UV-vis spectrophotometer at 279 nm wavelength. The concentration of a sample was measured with the help of pre-generated calibration curve and its equation for standard catechin (Fig. 2.1).

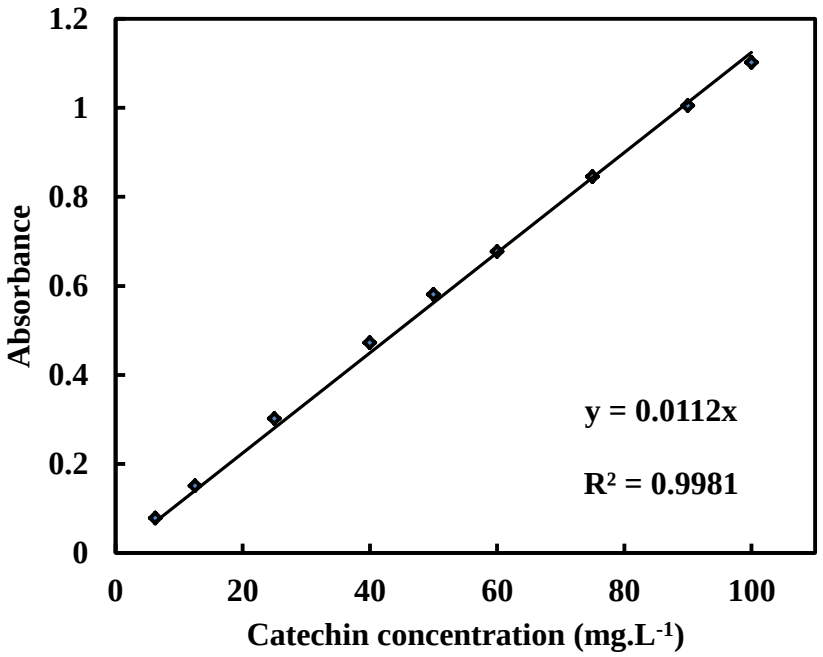


Figure 2.1: Calibration curve for analysis of catechin in UV-vis spectrophotometer

***High pressure liquid chromatography*:** The method described by Jin *et al.* [1] for analysis of catechins by HPLC method was used in this HPLC analysis with some modifications. Individual catechins in the tea leaves extract and each of the standards were analysed using a Shimadzu LC-

20AD HPLC (Tokyo, Japan) equipped with a ternary pump delivery system and UV detector. Each sample (10 µL) was filtered by using Acrodisc syringe filter (0.45µm supor, Pall Corporation) and injected into the column (Hypersil ODS, 250 mm × 4.6 mm × 5µm, Alltech, Deerfield, IL) via a rheodyne valve (20 µL capacity, Shimadzu, Tokyo, Japan). The eluting phases comprising of binary system of A (water/acetic acid, 100/0.1 vol %) and B (acetonitrile/acetic acid, 100/0.1 vol%) were applied in the ratio of 70:30 (A:B) at a flow rate of 1.0 mL.min.$^{-1}$. Identification of each catechin was confirmed by comparison of retention time and chromatography of the authentic standards (Fig. 2.2) and quantification was accomplished with calibration equations derived from each of the standards reported in Table 2.1.

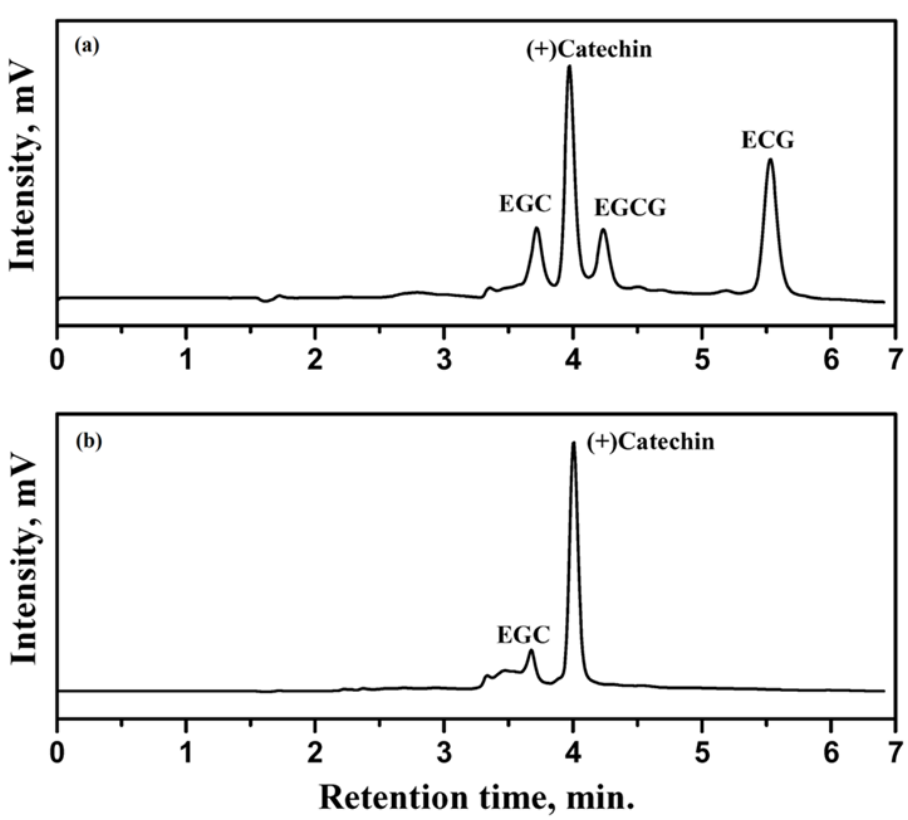


Figure 2.2: HPLC chromatography of catehin and other three derivatives (a) standard catechins from Sigma Aldrich (b) catechins from tea leaves extract

Table 2.1: The calibration equations of four catechins

Catechin compounds	Retention time in HPLC (min.)	Equations	R^2
EGC	3.715	*$Y=0.5111\times10^4$ X - 1.792 $x10^4$	0.999
(+)Catechin	3.968	$Y=1.4084\times10^4$ X - 1.336 $x10^4$	0.999
EGCG	4.231	$Y=0.7861\times10^4$ X - 1.375 $x10^4$	0.998
ECG	5.525	$Y=2.4045\times10^4$ X + $0.4639x10^4$	0.999

*Y= area x 10^{-5} (μV.s)
X=concentrations of various catechins ($mg.L^{-1}$)

2.2.2 Characterization of catechin complex

X-ray diffraction (XRD) analysis: The precipitates were examined by XRD using Cu Kα radiation on a D 8 Advance (Bruker, Germany) X-ray defractometer equipped with a secondary graphite monochromator, operating at 40 kV and 40 mA at a step-scan of 0.05˚ 2θ/s. The X-ray patterns were recorded in the 2θ range of 3–40˚.

Fourier transformation infrared absorption spectrometry (FT-IR) analysis: One milligram of the Fe-catechin precipitate dried in a vacuum desiccator was ground and mixed thoroughly with 200 mg of oven-dried KBr powder of FT-IR grade (Merck KGaA, Germany). The sample powder was placed in a die and compressed into transparent disk. The infrared spectra were recorded by using an IR Affinity-1 (Shimadzu corp.) infrared spectrophotometer.

Scanning electron microscopic (SEM) and electron dispersive X-ray (EDX) analysis: The vacuum-dried Fe-catechin complex powder was characterized by scanning electron microscopy

(SEM) combined with energy dispersive X-ray (EDX) analysis in order to characterize the surface morphology and to examine global chemical compositions of the complex, respectively.

2.2.3 Other instruments

For the measurement of pH a CP 901 digital pH meter and/or a EUTECH 510 digital pH meter was used and U-tube viscometer (Model: Stanhope-Seta, Type: A) was used for the measurement of viscosity. The interfacial tensions between LM and the aqueous phases were measured by tensiometer (Kruss Germany, K9). The particle sizes of emulsified droplet in the aqueous phases were measured by Zeta potential (Delsa™ Nano Common, model: Beckman Coulter).

2.3 Experimental studies

Tw phase equilibrium studies are carried out in order to select a suitable organic phase (solvent and/or carrier) that ensures high separation factor for transferring catechins from aqueous phase to the organic phase. It also provides the preliminary operational conditions for such separation. The three phase transportation studies, on the other hand, are required to find out the feasibility of LM based transportation process.

2.3.1 Two phase equilibrium distribution of catechin

The pH of aqueous solution of catechin was maintained by adding HCl/NaOH, as and when needed. Twenty mL of both organic and aqueous phases were mixed in 250 mL Erlenmeyer flask and the mixture was well-stirred in a shaking incubator at a certain temperature and stirring speed for sufficient duration so that the two phase suspension reaches the equilibrium. The mixture is allowed to remain undisturbed for 12 h in order to separate the phases and aqueous phase was thereby collected from the bottom and centrifuged at 3000 rpm. The clear sample was tested in UV-vis spectrophotometer. Catechin concentration in aqueous phase was determined

with the help of a pre-generated calibration curve (Section 2.2.1). The quantity of catechin transferred to the membrane phase is calculated by the mass balance, with fair assumption that no catechin was lost during experimentation. All the experiments were performed thrice to ensure the repeatability of the experimental results and the results are shown with standard error bar. The above protocol is repeated with all solvents and/or solvent/carrier combinations and for all ranges of temperature and stirring speed.

2.3.2 Three phase experimental studies with BLM [2, 3]

The laboratory scale BLM setup for the three phase experiments is shown in Fig. 2.3. The set up consists of cuboidal shaped glass container (75mm × 75 mm × 63 mm) with magnetic stirrers. The cell is divided into two compartments by a thin glass plate of thickness 5 mm. In order to ensure no channeling between the compartments, a blank test was carried out by keeping colored (crystal violet) solution in one compartment and water in the other. Both liquids were stirred continuously for 24 h by magnetic stirrers and the intensity of the color of liquids in both compartments was then measured with UV-vis. spectrometer at 584 nm. There was no change in the absorbance which confirmed no channeling between the compartments. The results of leakage test are incorporated in Appendix-II.

Two different BLM configurations can be set up depending on the density of the membrane phase. This research work deals with membrane phase which is lighter than the aqueous phases and the BLM set-up is made accordingly. The feed and the strip phases are placed in respective compartments in such a way that the level of these liquids remain well below the top of the separating wall. The membrane phase liquid is then poured from the top in such a way that the height of the membrane phase clears the top of the glass wall and thus creates a bridge between the feed and strip phases for possible diffusion of solute through the membrane phase. Care is

taken so that there is no leakage/accidental mixing of these phases. Aqueous phases were continuously stirred by magnetic stirrer and stirring speeds (rpm) were controlled by voltage regulators. Sufficient care had been taken to prevent unwanted mixing of feed and strip phases. Moreover, the stirrer speed was regulated in such a way that neither it allows formation of any emulsions at the feed/membrane interface nor it disturbs the membrane interface.

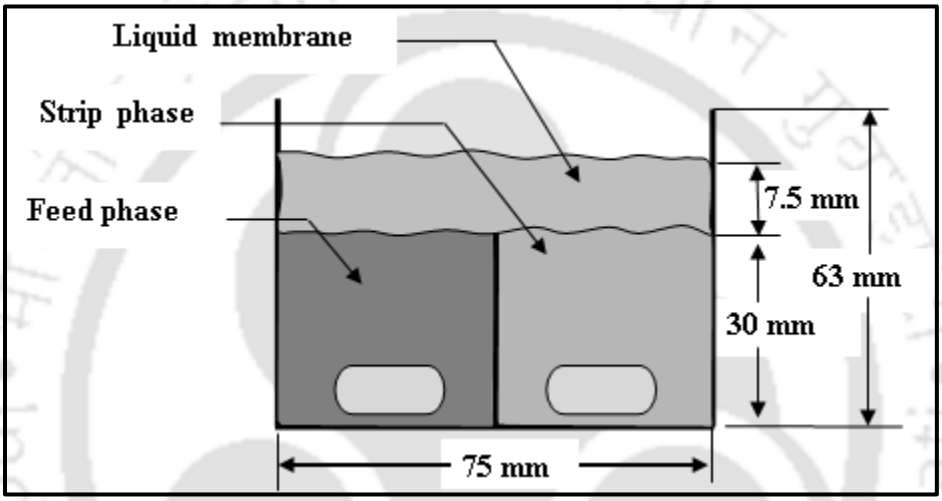


Figure 2.3: Schematic of BLM set up

The three phase experiments were carried out in the BLM set-up as described above. Area of membrane/aqueous interface was 19.5×10^{-4} m^2 at both sides. Catechin was transferred through these interfaces. The volumes of feed phase as well as receiving phase were 50 mL each and the volume of LM phase was 30 mL. Continuous stirring of aqueous phases ensured the solution to be well mixed and the bulk concentration to be uniform throughout. Three mL sample of both aqueous phases was collected periodically for determination of catechin concentration in UV-vis spectrophotometer.

2.3.3 Three phase experimental studies with FS-SLM

2.3.3.1 Set-up

The FS-SLM apparatus consisted of two cylindrical vessels (internal diameter 55 mm and height 95 mm) connected with pipes which are joined by flanges. A flat-sheet support membrane along with ML impregnated in its pores is placed in between the flanges as shown in Fig. 2.4. Two mechanical stirrers (Make: Remi; Model: RGQ 121/D) were used for stirring of aqueous phases in both the vessels whose effective volume is 130 mL each. The whole membrane disc was of 47 mm in diameter and the contact area of the membrane with each aqueous phase was 11.3×10^{-4} m^2.

2.3.3.2 Solid membrane support

Various polymeric membranes were used as support for the organic phase such as polytetrafluoroethylene (PTFE), Nylon 6, polyvinylidenefluoride (PVDF) and polyether sulphone (PES). The pore diameter of the support membrane was taken from the supplier and the porosity of supports was taken from the literature. The thickness of the support materials was measured by thickness measurement instrument (Litematic, VL-50).

Table 2.2: Characteristics of polymeric membrane supports

Support material	Pore size, d (μm)	Thickness, L (μm)	Porosity (ε)	Tortuosity (τ)	(ετ/L) (μm^{-1})
PTFE	0.2	77.2	0.51	2.92	0.019
PVDF	0.2	88.5	0.45	3.4	0.017
Nylon, 6	0.2	102	0.40	4.0	0.015
PES	0.2	107	0.5	3.0	0.014

2.3.3.3 Preparation of FS-SLM

The membrane liquid (ML) was immobilized into the pores of the flat sheet polymeric membrane. First the support material is immersed into the ML for at least 24 hours. It was then taken out and the excess liquid was wiped out gently from the flat surface by good quality non-fibrous tissue. It was fitted between two flanges connected to feed and strip compartments as described before.

2.3.3.4 Experimental procedure

Both aqueous phases were stirred using mechanical stirrer in order to reduce concentration polarizations of solute at the interfaces. Before the start of experiments including that of fed-batch system, required amount of electrolyte (NaCl) was dissolved either one or both aqueous phases in order to enhance the stability of LM. The rationale of the stability has been explained in the later chapter. Feed and strip phase samples (about 3 mL) were periodically taken out and catechin concentration was determined using UV-vis spectrophotometer (Perkin Elmer, Model: Lambda 35) at 279 nm wavelength (for catechin). The concentrations of individual catechins were measured by HPLC in case of experiments with real extract of green tea leaves. All experiments were carried out in triplicate and results are shown with standard error bars.

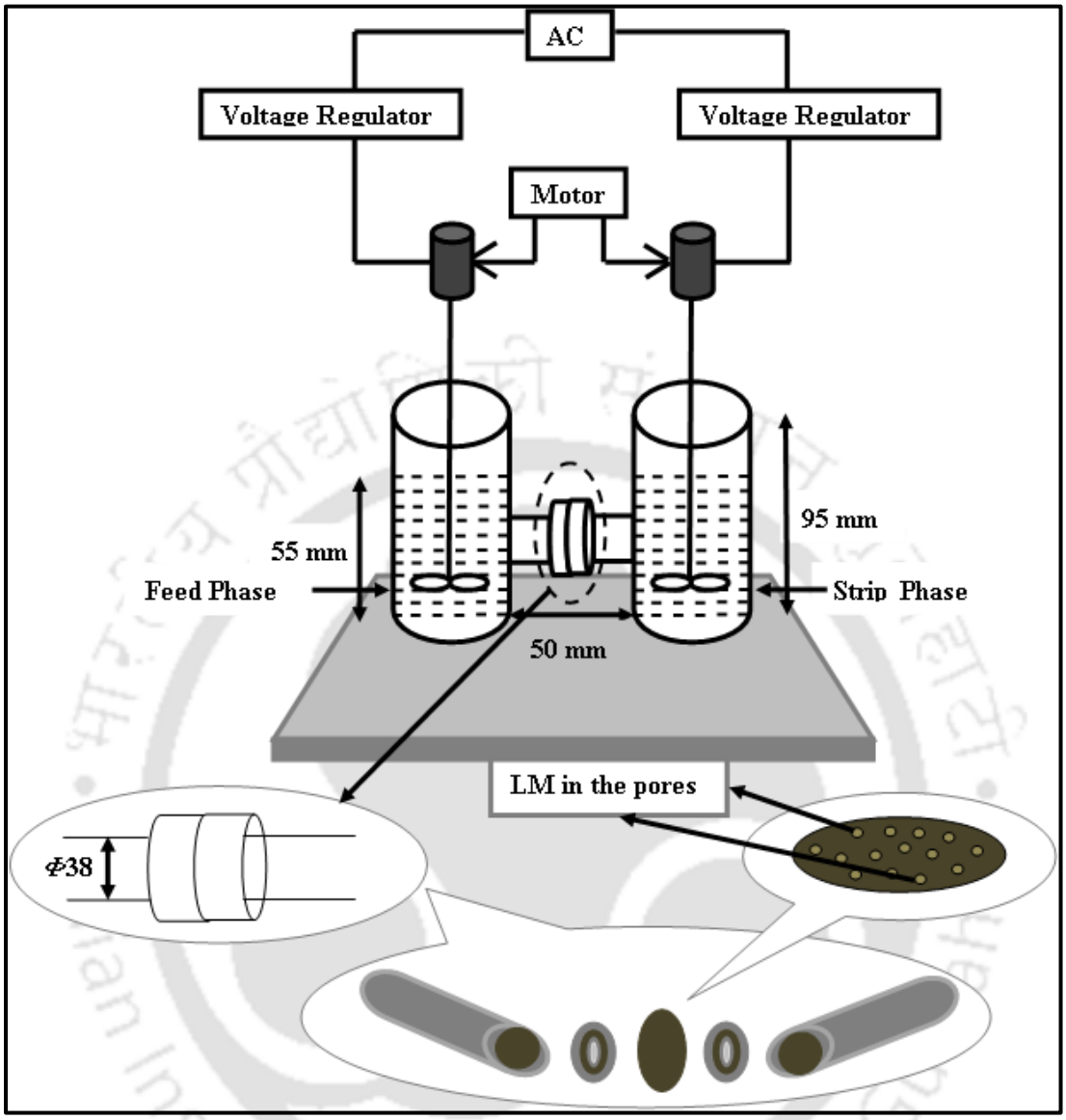


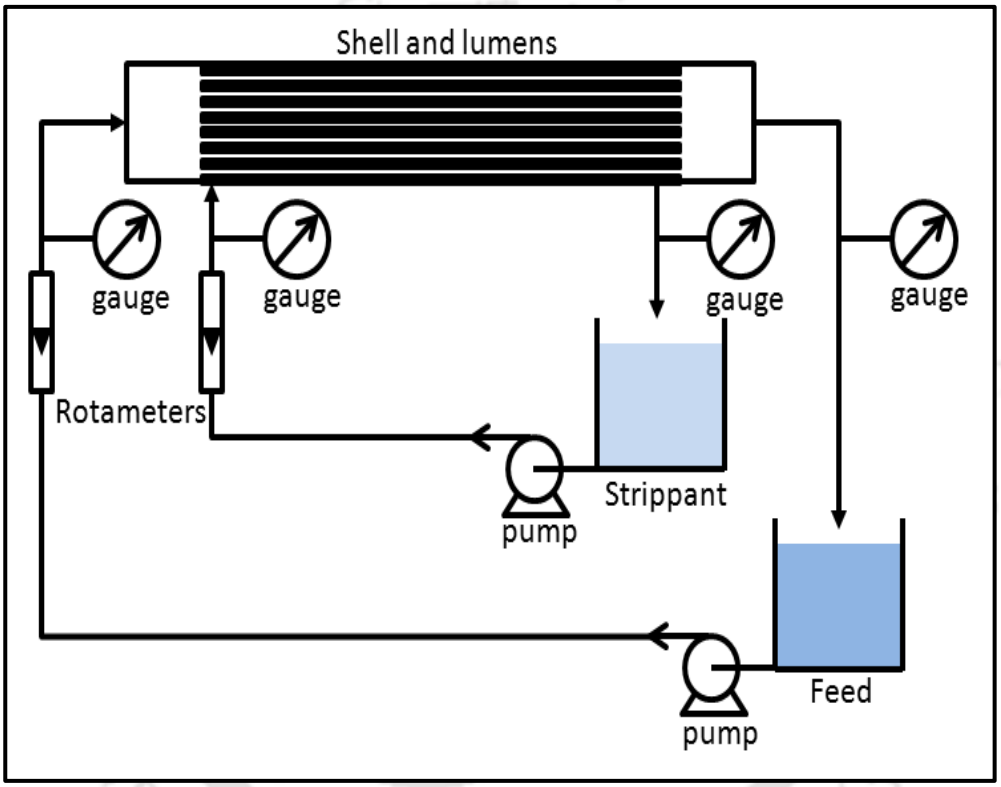
Figure 2.4: Schematic of FS-SLM set-up

2.3.4 Hollow fiber membrane (HFM) module

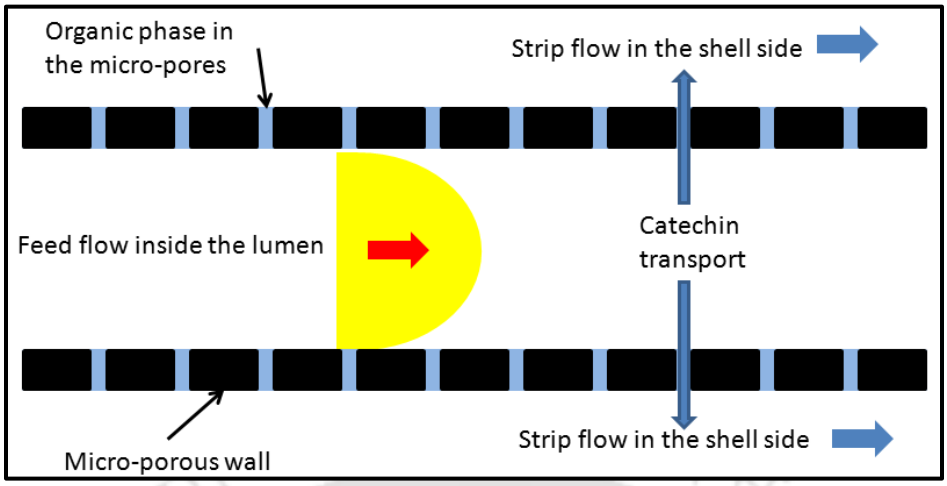
2.3.4.1 Set-up

The single module HF-SLM system is shown in Fig. 2.5(a), whereas, a single hollow fiber with LM in the pores and flow direction of the streams is shown in Fig. 2.5(b). The apparatus is composed of a hollow fiber module, two peristaltic pumps, two rotameters with variable flow

rate controllers, and four pressure gauges fitted in the inlet and outlet of feed and strip streams. The hollow fiber module is micro-porous polyethersulphone (PES) fibers woven into fabrics. Various physical properties and characteristics of the HFM module have been reported in Table 2.3.



(a)



(b)

Figure 2.5: (a) Schematic of HFM set up (b) Schematic of the single hollow fiber

Table 2.3: Characteristics of hollow fiber membrane

Shell characteristics	
Material	Polypropylene (PP)
Length	20×10^{-2} m
Inner diameter, $2r_i$	2.05×10^{-2} m
Outer diameter, $2r_o$	2.54×10^{-2} m
Fiber characteristics	
Material	Polyethersulphone (PES)
Number of fibers, N	2800
Effective length, L	20×10^{-2} m
Inner diameter, $2r_i$	2.2×10^{-4} m
Outer diameter, $2r_o$	3.0×10^{-4} m
Fiber thickness, δ	4.0×10^{-3} m
Effective surface area, A	6.0×10^{-2} m^2
Effective area/volume	0.1 $m2.m^{-3}$
Average pore size, d_p	0.2 µm
Porosity, ε	0.5
Tortuosity, τ	3.0

2.3.4.2 Experimental procedure

Hydrophobic polyethersulphone (PES) membrane having specifications (Table 2.3 and Table 2.4) was used as supplied. The module was mounted horizontally and the two flow streams were circulated co-currently. The ML was circulated in the lumens of the module for 25 minutes at a

flow rate of 10×10^{-2} $m^3.min.^{-1}$. Lumen side outlet was blocked. All the pores of the hollow fibers were filled with the ML within 20 minutes after the start of circulation however the circulation was continued for another 5 minutes. Each time 50 mL of ML was collected through the shell side outlet that confirmed the filling up of the pores by LM. Subsequently, the excess liquid membrane is flushed out through both passes of the module by circulating ultra-pure deionized water. The actual experimentation now begins with re-circulation of feed and the strip solutions by means of calibrated peristaltic pumps. Based on characteristics of lumen and shell side (Table 2.3 & Table 2.4) of the HFM, various parameters (flow rates and hydraulic pressure differential) were fixed (Table 2.4). Equal linear flow velocity was maintained both in lumen and shell side until both the sides were filled by the respective aqueous phases to maintain minimum pressure differential across the immobilized ML. This technique prevents the liquid membrane to be washed out during the streams initialization in the passes of the module. Once both sides were filled up by the flowing streams the feed side flow rate was changed as per the objectives. Sample was analyzed using UV-vis. spectrophotometry as well as HPLC (for experimentation with real extract). All the experiments have been accomplished thrice and the average data have been reported with standard error bars.

Table 2.4: Characteristics of tube and shell side of HFM and linear flow velocities

Pass	Volume, ($10^{-6} \times m^3$)	Cross section area A, ($10^{-4} \times m^2$)	Length (L), ($10^{-2} \times m$)	Flow velocity (v_f, v_s), ($10^{-2} \times m.s^{-1}$)
Tube side	21.3	1.07	20	0.06-0.62
Shell side	24.5	1.36	20	0.49

2.3.4.3 Testing of leakage

A provision for testing of leakage is provided prior to the transportation studies of the catechins in order to ensure the stability of the HF-SLM. Dilute sulphuric acid (0.1 M) is circulated through the lumen side at a linear velocity and deionized water is circulated through the shell side of the module at a constant velocity. The change of H^+ concentration in the water reservoir is measured by a pH meter. The change in pH of the shell side de-ionized water with time indicates the extent of water leakage [4]. A calibration curve for water leakage was constructed from the time course of $[H^+]$ obtained in the shell side during the test. The details are provided in Chapter-V.

2.4 Model calculation

The percentage extraction (%E) was calculated from the concentration of catechin before ($[CatOH]_{aq,i}$) and after ($[CatOH]_{aq}$) the extraction by the following equation:

$$\%E = \frac{[CatOH]_{aq,i} - [CatOH]_{aq}}{[CatOH]_{aq,i}} \times 100 \quad (2.1)$$

In order to find the best stripping agent among ethanol, *i*-propanol, NaOH, HNO_3, NaCl and pure water, multiple experiments were carried out whereby the extracted catechin was stripped by aqueous solution of individual stripping agent. The efficiency of recovery (% recovery) was estimated. The percentage recovery (%R) was calculated from the concentration of recovered catechin, $[CatOH]_{aq,r}$ in strip phase and the initial concentration of catechin in aqueous phase, $[CatOH]_{aq,i}$ by the following equation:

$$\%R = \frac{[CatOH]_{aq,r}}{[CatOH]_{aq,i}} \times 100 \quad (2.2)$$

The initial flux, J ($kg.m^{-2}.s^{-1}$) in steady state can be calculated according to the following equation:

$$J = \left(\frac{V}{A}\right)\left(\frac{d[CatOH]_{aq,r}}{dt}\right) \quad (2.3)$$

Where, *V* is the volume of the aqueous solutions, A is the effective exposed surface area of the membrane-strip interface, and $[CatOH]_r$ is the catechin concentration in the receiving phase ($kg.m^{-3}$) at elapsed time t(s).

Abbreviations

BLM	bulk liquid membrane
(-)ECG	(-)eppicatechingallate
EDX	electron dispersive x-ray
(-)EGC	(-)eppigallocatechin
(-)EGCG	(-)eppigallocatechingallate
FT-IR	fourier transformation-infrared
HPLC	high pressure liquid chromatoghraphy
LM	liquid membrane
PTFE	polytetrafluoroethylene
PVDF	polyvinylidenefluoride
PES	polyethersulphone
SEM	scanning electron microscope
SLM	supported liquid membrane
TBP	tributyl phosphate

UV	ultraviolet
XRD	x-ray diffraction

Nomenclature

A	interfacial surface area each side of the membrane phase
$[CatOH]_{aq}$	catechin concentration in aqueous phase
$[CatOH]_{aq,i}$	intial catechin concentration
J	flux across the strip side interface
$[CatOH]_{aq,r}$	catechin concentration in stripping phase
%E	percentage of extraction
% R	percentage of recovery
V	volume of the feed or stripping phase
V_p	volume fraction of the polymeric framework
ε	porosity of polymeric support membrane
τ	tortuosity of the polymeric support membrane

References

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[3] K.K. Bhatluri, M.S. Manna, P.K. Saha, A.K. Ghoshal, Separation of Cd(II) from its aqueous solution using environmentally benign vegetable oil as liquid membrane, Asia Pac. J. Chem. Eng., 8 (2013) 775-785.

[4] K. Takahashi, W. Goto, H. Takeuchi, Some observations on the stability of supported liquid membranes, J. Membr. Sci., 37 (1987) 19-31.

CHAPTER-III

Recovery of Catechin through BLM

CHAPTER-III

Recovery of Catechin through BLM

The results and discussion on the transportation of synthetic extract of catechin through bulk liquid membrane (BLM) prior to its recovery in aqueous ethanol were presented in this chapter. Influence of various parameters was studied for the optimization of catechin transport from its aqueous solutions. The experimental results were discussed elaborately in this chapter. Initially, two phase equilibrium studies were performed in order to select a suitable membrane phase for efficient transport of catechin. The effects of various parameters, such as pH, temperature and carrier concentration, on the equilibrium distribution of catechin were also studied. Based on the results of equilibrium study, the appropriate optimum operating conditions were detected. Further experimentations were carried out on the three phase transportation of catechin through BLM. The transportation performance were evaluated against various parameters such as stirring speed, temperature, carrier concentration, pH of feed phase, concentration of both feed and strip phases. A comparison of catechin transportation using two different solvents viz. conventional aliphatic n-decane and environmentally as well as physiologically benign vegetable oil (sunflower oil) was also highlighted in this chapter.

3.1 Theoretical background

The details about the molecular structure of catechins have been described in Section 1.2 of Chapter-I. The various catechins are generally present in the plant sources in different proportions as well as in different quantity in aggregate. The schematic representation of various catechins has been shown in Fig. 1.8 of Chapter-I. All the studies presented in this

chapter have been accomplished with synthetic extract of (+) catechin only. Various basic carriers *viz.* trioctylamine (TOA), *n-n*-dimethyloctylamine (DMOA), aliquat-336; two neutral carriers *viz*. tributyl phosphate (TBP), trioctyl phosphine oxide and the acidic carrier, di-2-ethylhexyl phosphoric acid (D2EHPA) were tried by mixing them in various vegetable oils *viz.* sunflower oil, soya bean oil, coconut oil and mustard oil in all possible combinations at various proportions for the extraction of catechin from its aqueous solution. Catechin could be extracted, in the present work, by the neutral extractant, TBP efficiently as discussed later part of this section. This has similarity to the report of Zidi *et al.* [1] for extracting phenol using TBP as carrier. The possible reaction mechanism could be the hydrogen bonding between the hydrogen of the hydroxyl group attached to the benzene ring in catechin or in phenol [1] and the double bonded oxygen in TBP, and subsequent complex formation (Fig. 3.1). The reaction takes place at the feed-membrane interface. The complex is more soluble in the membrane phase than pure catechin and diffuses through this phase due to concentration gradient and reaches the other end of the membrane phase *i.e*. membrane- strip interface. The catechin is stripped off by the stripping agent in the strip phase and the carrier is released at the interface. Released carrier diffuses back to the feed-membrane interface due to its concentration difference as shown in Fig. 3.2. The reaction of catechin with TBP can be described by the following reaction:

$$[CatOH]_{aq} + \mathrm{x}TBP_{org} \rightleftharpoons (CatOH.\mathrm{x}TBP)_{org} \tag{3.1}$$

and the equilibrium constant, K_{ex} by:

$$K_{ex} = \frac{[CatOH.\mathrm{x}TBP]_{org}}{[CatOH]_{aq}[TBP]_{org}} \tag{3.2}$$

where, $[CatOH]_{aq}$, $[\mathrm{TBP}]_{org}$ and $[CatOH.\mathrm{x}TBP]_{org}$ represent concentration of catechin in aqueous feed phase, concentration of TBP in organic membrane phase and the concentration

of the complex between catechin and TBP in organic membrane phase, respectively. Eqn. (3.2) can be rewritten to find the extraction reaction stoichiometry as follows:

$$CatOH]_{aq} = \frac{[CatOH.\mathrm{x}TBP]_{org}}{K_{ex}.[TBP]^{\mathrm{x}}_{org}} \tag{3.3}$$

$$\log[CatOH]_{aq} = \log\left\{\frac{[CatOH.\mathrm{x}TBP]_{org}}{K_{ex}.}\right\} - \mathrm{x}.\log[TBP]_{org} \tag{3.4}$$

The distribution coefficient (D_1) of catechin between organic phase (membrane phase) containing various concentration of carriers and aqueous phase was determined in two-phase equilibrium study. The experimental data are shown through Fig. 3.3. According to Eqn. (3.4) and Fig. 3.3, plot of aqueous phase equilibrium concentration, $[CatOH]_{aq}$versus carrier concentration, $[TBP]_{org}$ is linear with a slope of -1.8. So the stoichiometry can be approximated as 1:2 as x is nearly equal to 2. The FT-IR analysis results (covering pure catechin, pure TBP, and the combination of catechin + TBP) (Fig. 3.4) suggests that there has been no additional peak for the combination of catechin + TBP. The peak at O−H stretching (3200−3500 cm^{-1}) includes O−H hydrogen bonding also. So, this hydrogen bonding could be the only way of making the complex between catechin and TBP. The mass transfer of catechin across the membrane is described considering only diffusional parameters. The interfacial reaction is assumed to be very fast and hence the concentrations at the interfaces will be almost equal to the equilibrium concentrations. Fig. 3.2 presents a schematic of the transport of catechin through the LM. The mass transfer of catechin across the LM is described considering only diffusional parameters. The interfacial flux due to the chemical reaction has been neglected, as the chemical reaction is intrinsically very fast, and hence the concentrations at the interface will be almost equal to the equilibrium concentrations. The overall flux can be derived by Fick's first law of diffusion applying both to the feed phase

and LM if the composition of receiving solution is such that the extraction equilibrium is completely shifted to the left at the membrane-receiving interface.

At steady state, the diffusion flux through aqueous film and the LM, *viz.*, J_{aq} and J_{org} are represented as,

$$J_{aq} = \Delta_{aq}^{-1} \left([CatOH]_{tot} - [CatOH]_{i,tot} \right) \quad (3.5)$$

$$J_{org} = \Delta_{org}^{-1} \left([CatOH.2TBP]_{i,f} - [CatOH.2TBP]_{i,r} \right) \quad (3.6)$$

where, Δ_{aq} and Δ_{org} are diffusional resistances caused by the aqueous feed phase boundary layer and due to diffusion through the LM, respectively. $[CatOH]_{tot}$ and $[CatOH]_{i,\,tot}$ are the total catechin concentration in the feed phase and at the feed phase-membrane interface, respectively. $[CatOH.2TBP]_{i,f}$ and $[CatOH.2TBP]_{i,r}$ are the catechin-TBP complex concentrations at feed-membrane and strip-membrane interfaces, respectively. [CatOH.2TBP] releases CatOH quickly to the stripping phase and thus concentration of $[CatOH.2TBP]_{i,r}$ is negligible. Then the Eqn. (3.6) can be re-written as

$$J_{org} = \Delta_{org}^{-1} [CatOH.2TBP]_{i,f} \quad (3.7)$$

If the chemical reaction expressed by Eqn. (3.1) is assumed to be fast as compared to the diffusion rate, local equilibrium at the interface is considered to have reached instantaneously and concentrations at the interface are related through Eqn. (3.2). Thus, under steady state conditions the fluxes are equal (otherwise solute accumulation would occur), *i.e.* $J_{aq} = J_{org}$ and, in addition, are equal to overall flux *J*. The flux can be calculated The catechin concentration in the receiving phase is not constant but increases with time. The initial flux (*J*) can be calculated according to the Eqn. (2.3) described in the Section 2.4 of Chapter-II.

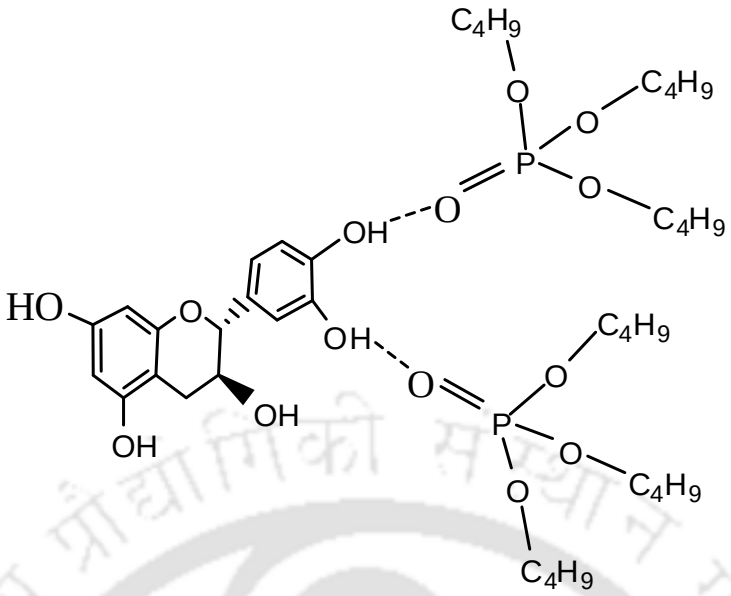

Figure 3.1: Model structure of catechin-2TBP complex

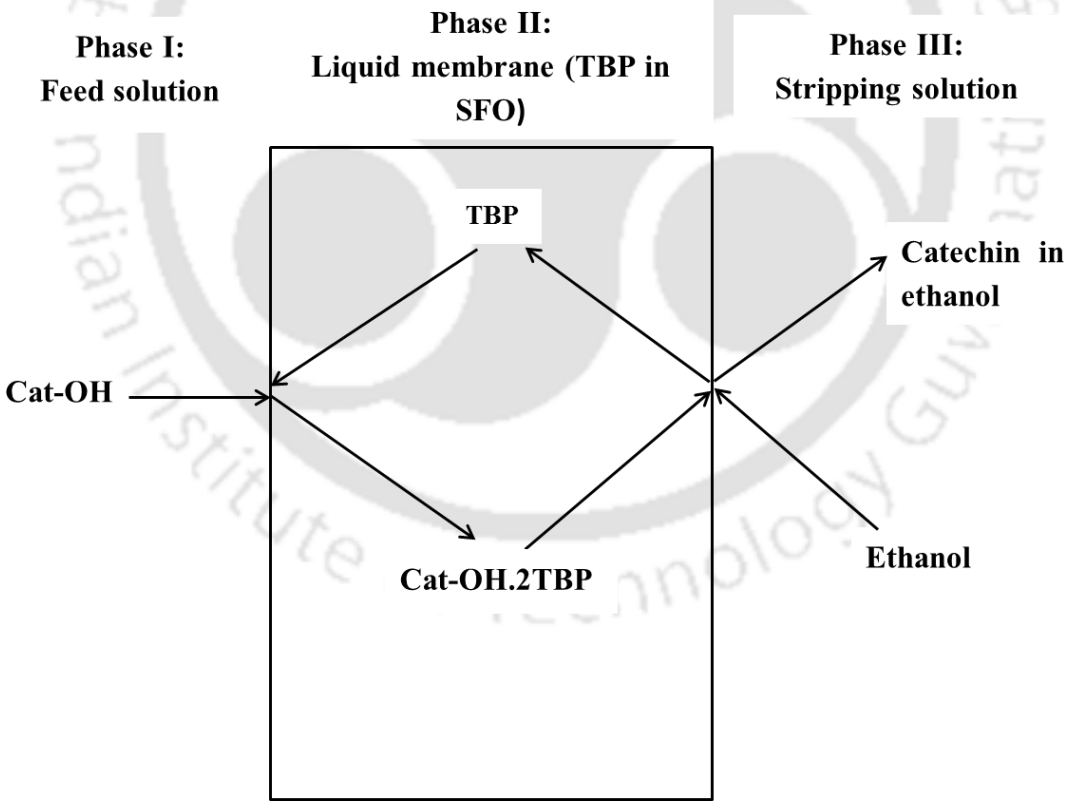

Figure 3.2: Schematic of the catechin transportation

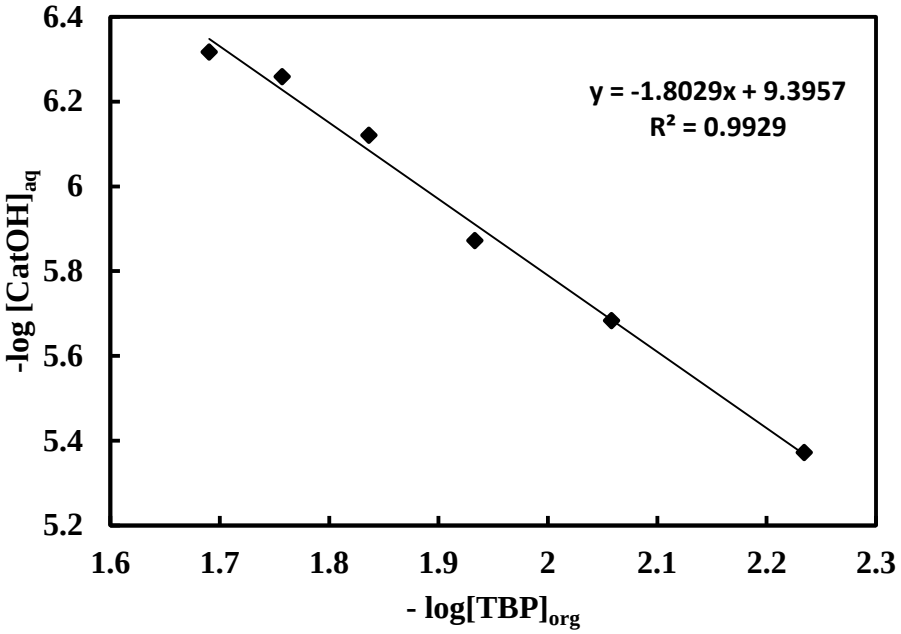

Figure 3.3: Stoichiometry of catechin-TBP complex

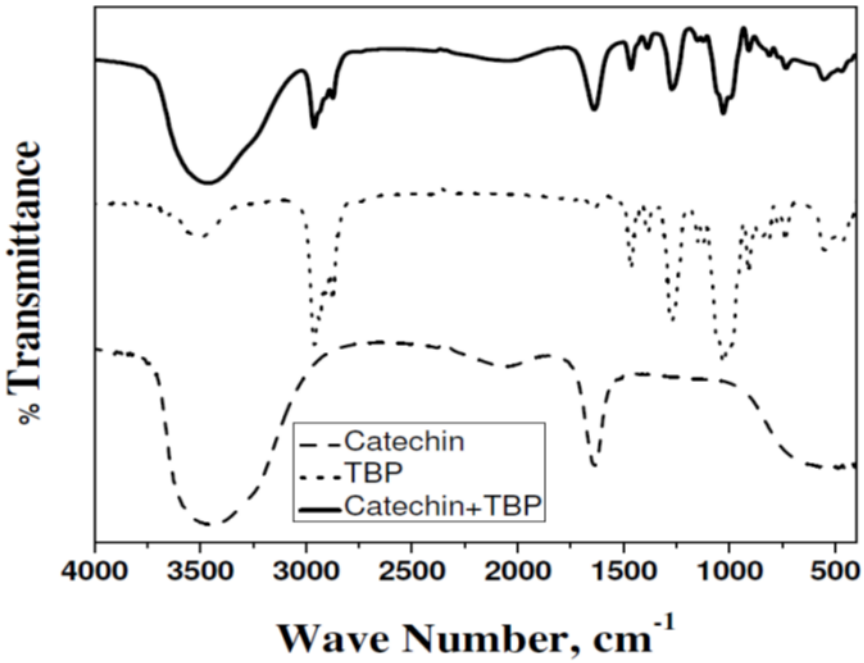

Figure 3.4: FTIR-analysis for determination of catechin-TBP complex

3.2 Results and discussion

3.2.1 Two phase equilibrium study

Equilibrium study was conducted as described in Section 2.3.1 of Chapter-II in order to select a suitable membrane. A high distribution coefficient (*a.k.a* separation factor) of the solute between the membrane phase and the aqueous feed phase necessarily means better transport of solute from feed phase to membrane phase. Effects of various physical parameters such as temperature, pH, carrier concentration in the membrane phase *etc*. on the equilibrium distribution of catechin have also been examined with the selected solvents. Distribution coefficient (D_1) of catechin was then calculated as the ratio of catechin in membrane phase to that in the aqueous phase at equilibrium. The value of distribution coefficient needs to be very high to ensure successful separation by LM based technique.

3.2.1.1 Solvent and carrier selection

Various combinations of solvents and carriers were tested for the extraction of catechin in two phase equilibrium studies under various physical and chemical conditions *viz.* temperature, stirring speed, carrier concentrations *etc.* Sunflower oil containing TBP as carrier has been found to be the best solvent-carrier combination. Catechin extraction of 78% was obtained in the membrane phase containing 0.8 M TBP as carrier with solvent sunflower oil followed by soya bean oil (75%), coconut oil (74%) and mustard oil (34%) (Fig.3.5). Two phase equilibrium studies were also conducted with conventional organic solvents containing same 0.8 M TBP as carrier. The extraction efficiency of various membrane phases in terms of distribution coefficient is presented in Table 3.1. According to the table, the presence of a carrier in membrane phase is the predominant factor for extraction of catechin. Various conventional organic solvents *viz. iso*-octane, *n*-heptane and *n*-decane and vegetable oils were tried as extractor in absence of any carrier agent. No extraction of catechin was observed in

the above cases due to very less solubility of catechin in vegetable oils as well as conventional solvents [2]. A carrier agent is very much needed for formation of solute-carrier complex which is soluble in the membrane phase that eventually facilitates active transport of solute *i.e.* carrier mediated transport. Vegetable oils are environmentally as well as physiologically benign solvents and they are inexpensive and easily available too. The liquid membrane comprising of TBP in SFO has the highest distribution coefficient (3.55). Because of the highest extracting capacity and the non-hazardous property, it was selected as the solvent of LM in further experimental work.

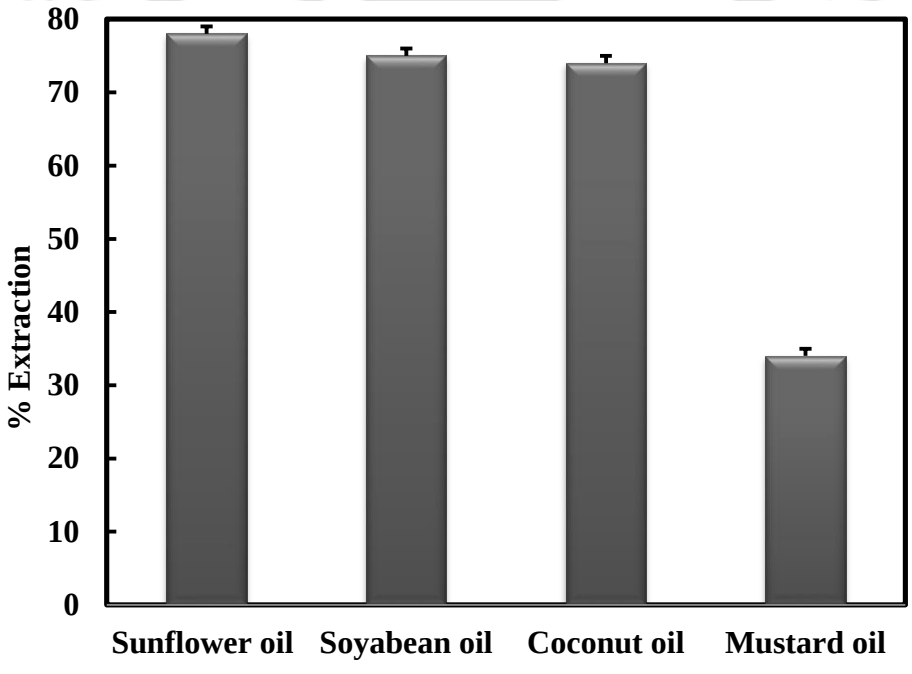

Figure 3.5: Selection of vegetable oil as solvent

Table 3.1: Distribution coefficient (D_1) of catechin using various solvents in two phase studies

Experimental conditions	Solvent used	Distribution coefficient (D_1)
Concentration of catechin in feed phase (C_{cat}):100 mg.L^{-1}, pH of feed phase: 5.0, carrier concentration (TBP): 0.8 M, Temperature: 25˚C, Stirring speed: 200 rpm, duration of stirring: 24 h	sunflower oil	3.55
	soyabean oil	3.0
	mustard oil	0.52
	coconut oil	2.84
	iso-octane	0.41
	n-decane	0.88
	n-heptane	0.39
	Any of the above solvents without carrier	0.0

3.2.1.2 Effect of pH

Feed phase pH has considerable effect on the extraction of any solute [3]. Feed phase pH was varied from highly acidic (pH=3.0) condition to highly alkaline (pH=13.0) one while two phase equilibrium studies were performed at 25ºC under stirred condition (200 rpm) for 24 h in shaking incubator. TBP (0.8 M) was used as carrier in SFO as this carrier-solvent combination was found to be the best. It was found that the maximum extraction occurred at pH of 5.85, and it was also observed that extraction does not vary much in acidic range of pH (Fig. 3.6). Catechin is chemically a polyphenol and a weak acid. So, it remains almost un-dissociated in acidic feed solution. Thus, neutral carrier such as TBP can extract catechin in molecular state from aqueous feed phase of acidic pH range. Similar observation has been reported by Zidi *et al*. [1] while extracting phenol from industrial wastewater of pH 3.0.

However, in alkaline range of pH, catechin in aqueous solution dissociates to its anionic state with increasing alkalinity leading to its reduced extraction rate by the neutral extractor TBP. Hence, in subsequent two phase equilibrium studies, pH of feed phase was maintained at moderate pH of 5.0.

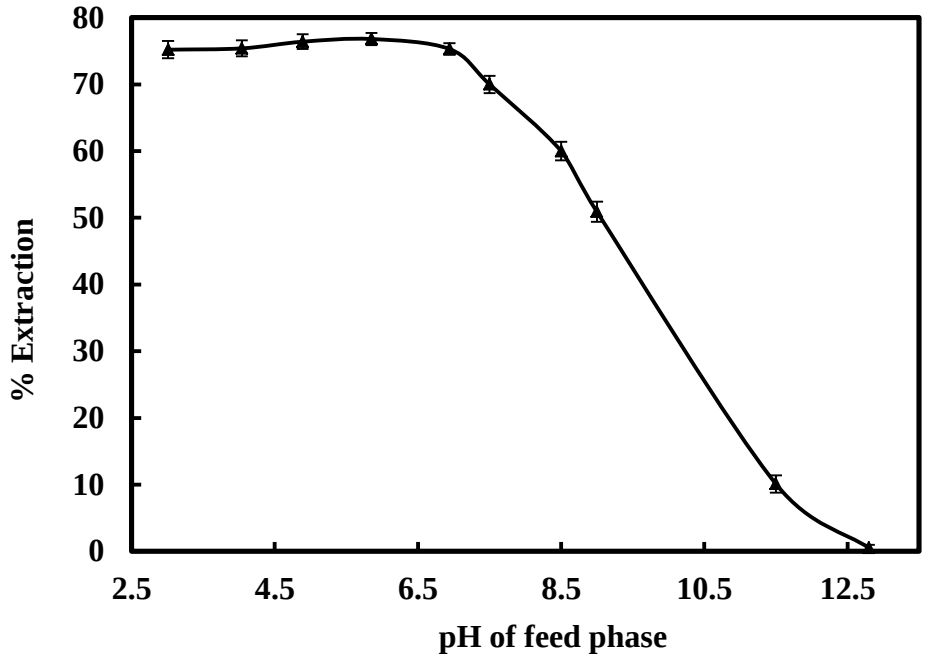

Figure 3.6: Effect of feed phase pH on catechin extraction in two phase study *(membrane phase = 0.8 M TBP in SFO, temperature = 25°C, stirring speed = 200 rpm, initial feed conc.= 100 mg.L^{-1}, run time = 24 h)*

3.2.1.3 Effect of carrier concentration

TBP concentration in membrane phase (SFO) was varied from 0.2-0.9 M while temperature and stirring speed were maintained at 25ºC and 200 rpm, respectively. The stirring speed of 200 rpm was selected by trial so as to ensure proper mixing between the aqueous and organic phases. It was observed that extraction increases rapidly as carrier concentration increases from 0.2-0.45 M and it increases slowly beyond carrier concentration of 0.45 M. Maximum

(79%) extraction of catechin occurs at TBP concentration of 0.9 M (Fig. 3.7), while it reaches almost a saturation point at TBP concentration of 0.8 M. When carrier concentration is low, the number of carrier molecules at feed-membrane interface is stoichiometrically less than catechin required for the complexation of the solute-carrier. Hence with the increase in concentration of carriers, initially extraction increases rapidly and then with a declining slope. However, beyond carrier concentration of 0.8 M, the membrane becomes almost saturated with carrier, hence, the extraction increases very slowly with increase of carrier concentration. Thus, the optimum working range of carrier concentration for the purpose of catechin extraction is 0.45-0.80 M. Increase of carrier concentration has two opposite effects, *viz*. viscosity and benignity. Viscosity of the liquid membrane reduces with increasing carrier concentration as carrier itself is low viscous compared to solvent (SFO) whereas benignity of the organic phase is compromised with increasing concentration of carrier. Lower viscosity of the organic phase provides more flux at the cost of benignity of the liquid membrane. Hence, 0.8 M carrier was used in the membrane phase for the sake of the physiological benignity of the extracted and recovered bioactive catechin. For further studies, an organic phase of 0.8 M TBP in SFO has been chosen as organic phase.

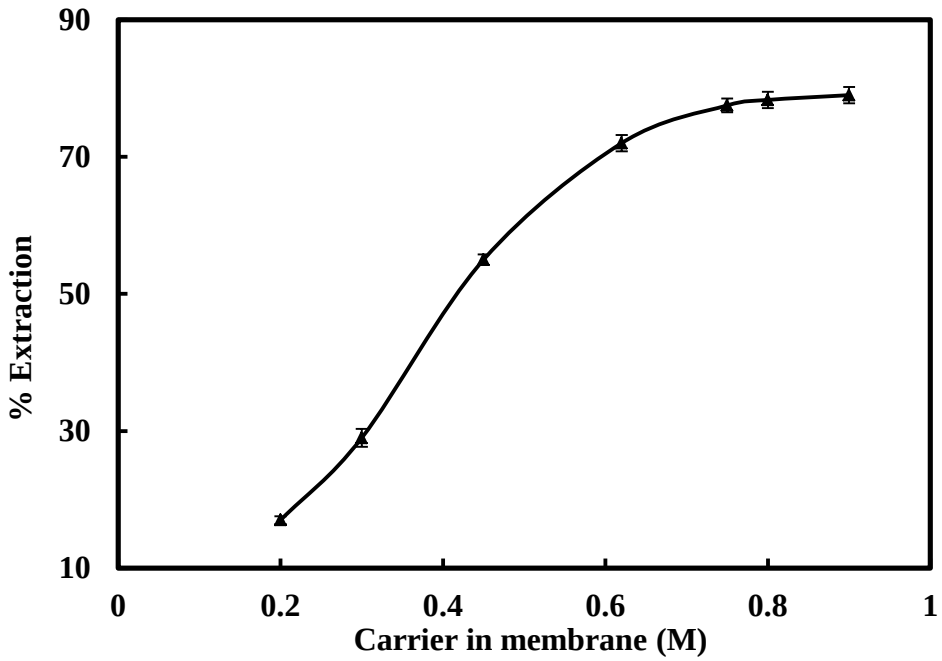

Figure 3.7: Effect of carrier concentration in liquid membrane (TBP in SFO) on extraction efficiency in two phase study *(temperature= 25 ºC, stirring speed = 200 rpm, initial feed conc.=100 mg.L^{-1}, feed phase pH=5.0, run time = 24 h)*

3.2.1.4 Effect of extraction time

The kinetics of the process such as rate of inter-phase diffusion and interfacial reactions involved in the extraction are important to understand the viability of this separation process in continuous mode of operation in industrial scenario. Two phase study was performed for 24 h with an operational set up of 0.8 M carrier (TBP) in SFO at 25ºC, pH 5.0 and 200 rpm. Samples were collected periodically. It has been observed that 50.2% and 70% extraction were over by 1 and 5 h, respectively whereas maximum extraction, 78.5%, was reached after 24 h (Fig. 3.8). Hence, it can be concluded that most of the catechin is extracted quickly as the equilibrium between two phases approaches. Nevertheless, each of the remaining two phase studies were conducted for 24 h duration with the aim of highest possible extraction.

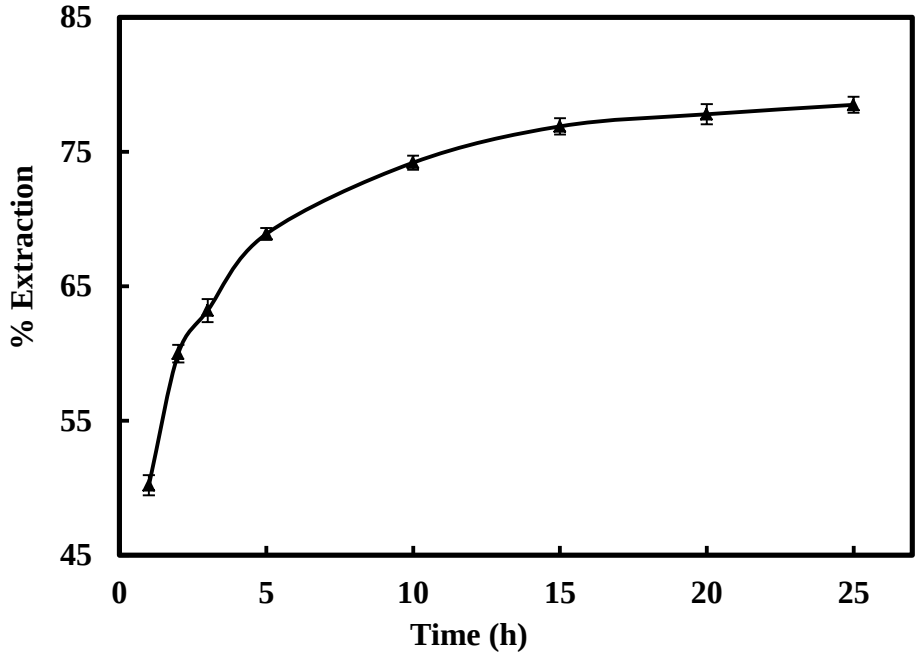

Figure 3.8: Effect of extraction time in two phase study *(membrane phase = 0.8 M TBP in SFO, temperature= 25ºC, stirring speed = 200 rpm, initial feed conc.= 100 mg.L^{-1},fesd phase pH = 5.0, run time = 24 h)*

3.2.1.5 Effect of temperature

Bioactive compounds are very temperature sensitive. However, catechin is stable up to a reasonably high temperature of 60^{o}C [4]. The effect of the temperature, on the extraction of catechin, was examined in the range 15-50ºC as the temperature can affect the solubility, viscosity, diffusivity of the membrane resulting in the change of rate of solute transport. The experimental results are shown in Fig. 3.9. Viscosity of oil decreases marginally with the increase in oil temperature (Table 3.2), hence, the solubility of TBP-catechin complex increases marginally. Thus, the catechin extraction also increases marginally from 76% at 15ºC to 78.1% at 25 ºC. However, at higher temperature (beyond 25ºC), extraction of catechin decreases first slowly and then rapidly due to the instability of the TBP-catechin complex at higher temperatures. It is, however, confirmed by UV-vis spectral analysis of

aqueous phase that carrier loss (transfer of TBP from LM to aqueous phase) does not take place at higher temperatures. Maximum catechin extraction (78.1%) was obtained at 25ºC. Hence, remaining two phase experiments have been performed at 25ºC and all three phase studies were conducted in room temperature which was in the range of 15-25ºC.

Table 3.2: Variation of viscosity of sunflower oil (SFO) with temperature

Temperature(˚C):	15	20	25	33	40	50
Viscosity (mPa.s):	47	42	38	34	30	28

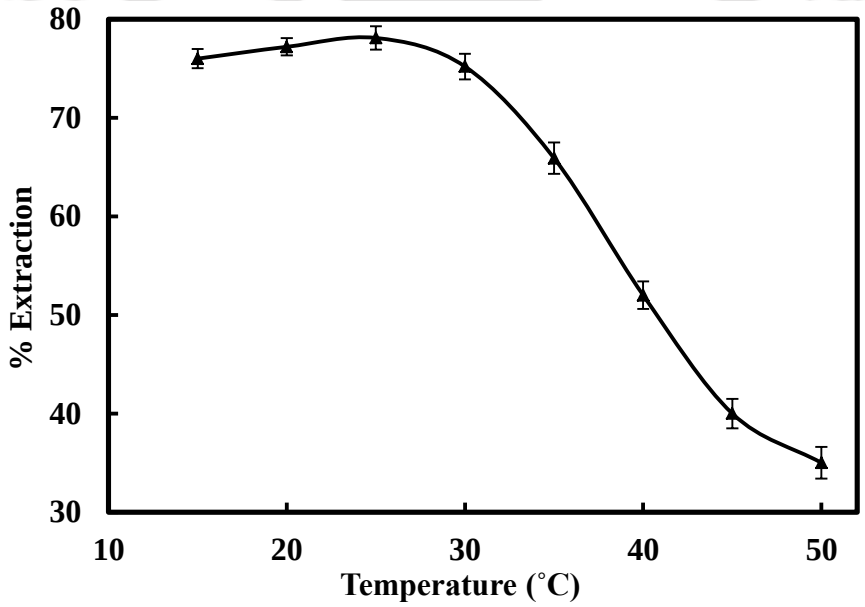

Figure 3.9: Effect of temperature on extraction in two phase study *(stirring speed =200 rpm, pH of feed phase = 5.0, initial feed concentration = 100 mg.L^{-1}, membrane phase = 0.8 M TBP in SFO, run time = 24 h)*

3.2.1.6 Effect of initial feed concentration

The influence of the initial feed concentration on catechin extraction was studied in the concentration range of 25-400 mg.L^{-1} with operating condition of 0.8 M TBP in SFO at 25

ºC, pH of 5.0, stirring speed of 200 rpm. It was observed that the distribution coefficient was almost constant in the feed concentration up to 150 $mg.L^{-1}$ and beyond that distribution coefficient decreases with initial feed concentration. Marginally increasing distribution coefficient in the lower feed concentration could be due to the fact that carrier concentration, 0.80 M, is in excess stoichiometrically and the excess carrier imparts resistance to solute diffusion. On the other hand, for very high solute concentration, the percentage extraction is low due to either more crowding of solute, carrier, and/or solute−carrier complex at the aqueous−organic interface leading to the steric hindrance to solute extraction or limited supply of carrier to take catechin (low TBP:catechin ratio) at the feed−membrane interface. Thus, it can be concluded that for 0.8 M carrier concentration in the membrane phase, the favorable initial feed concentration is in the range of 100−150 $mg.L^{-1}$.

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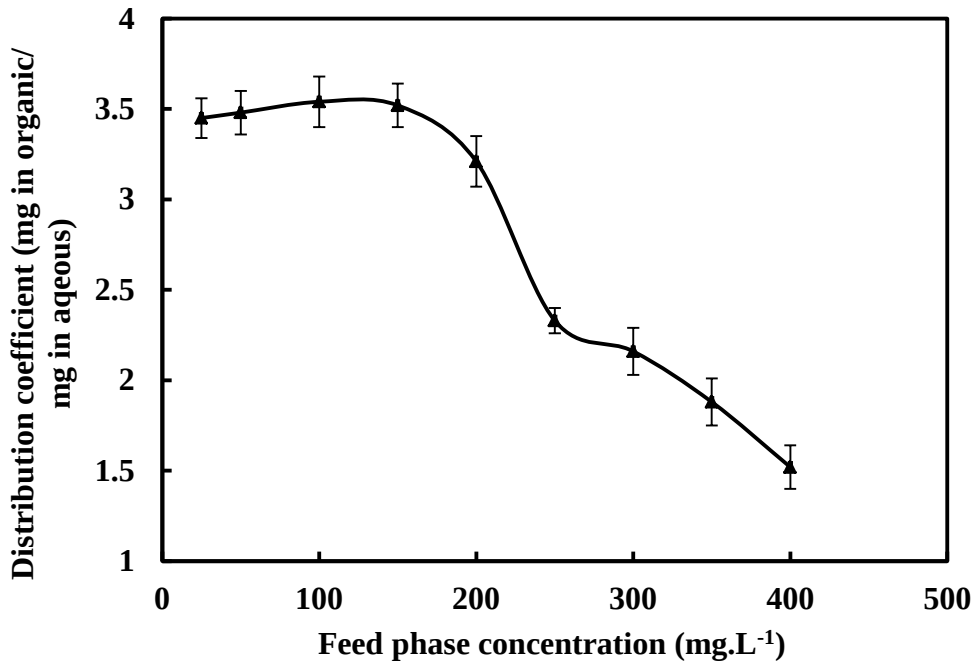


Figure 3.10: Effect of initial feed concentration on the extraction in two phase study

(temperature =25ºC, stirring speed = 200 rpm, pH of feed phase = 5.0, membrane phase = 0.8 M TBP in SFO,run time = 24)

3.2.2 Three phase studies

The three phase studies were carried out in a BLM set up as described in Section 2.3.2. The effects of various process variables, such as stirring speed, carrier concentration and feed and strip phase concentration on the transport of catechin, were studied. Continuous stirring ensured the aqueous solutions to be well mixed and the bulk concentration to be uniform throughout, at the same time care was taken so that interfaces of membrane phase with each of the aqueous phases were not disturbed. The pH of feed phase was maintained at 4.0 to ensure the catechin in its molecular state. The effects of process variables on the transport of catechin were evaluated by measuring the concentration of catechin in both feed and strip phase after 24 h for calculation of extraction and recovery, respectively. The change in the fluxes J_F (flux of feed phase) and J_S (flux of strip phase) were calculated at one hour interval as follows, where t_2-t_1 = 1 hour.

$$J_F \text{ or } J_S = \frac{V\left([C_{cat}]_{t_2} - [C_{cat}]_{t_1}\right)}{S(t_2 - t_1)} \tag{3.8}$$

3.2.2.1 The choice of stripping agent

Stripping of the solute from extract phase (membrane phase) is conducted by a stripping agent present in the stripping phase. Hence, the choice of a suitable stripping agent and its concentration in the stripping phase is most important for the recovery of target solute. Various stripping agents *viz.* ethanol, *iso*-propanol, sodium hydroxide (NaOH), sodium chloride (NaCl) in their aqueous solutions, were tried for the recovery of catechin in two phase equilibrium study. Catechin was stripped/re-extracted from membrane phase by various stripping agents and the results in terms of distribution coefficients have been shown in Table 3.3. Ethanol and *iso*-propanol have nearly the same recovery efficiency. No catechin recovery was found by sodium hydroxide, whereas sodium chloride extracts less amount of

catechin. Ethanol was selected as the stripping agent due to its low cost and easy availability. Moreover, it is extensively used in medical practices.

Table 3.3: Selection of stripping agent: Distribution coefficient (D_2) of catechin between membrane phase (containing 100 mg.L^{-1}catechin) and various stripping phases

Stripping agent:	Ethanol (0.2 M)	*Iso*-propanol (0.2 M)	NaCl (0.2 M)	NaOH (0.2 M)
Distribution Coefficient (D_2):	0.86	0.79	0.64	0.0

3.2.2.2 Effect of pH

Based on the result of two phase equilibrium study, the effects of pH on extraction and recovery of catechin in three phase studies were examined in the pH range of 2.0-8.0 and it was observed that both extraction and recovery were favorable in the pH range of 4.0-7.0 (Fig. 3.11). The maximum extraction and recovery were obtained at feed phase pH of 4.0 instead of 5.85 which was observed for two phase equilibrium study (Section 3.2.1.2). This minute shift of the favorable pH can be explained by the fact that one extra phase *i.e.* strip phase is involved in three phase studies which may affect the working pH and also with the fact that variation of % extraction was very less in the pH range 4.0-7.0 in two phase studies. Hence, moderate acidic pH (pH=4.0) of feed phase was maintained to ensure the catechin in its molecular state in all subsequent three phase studies.

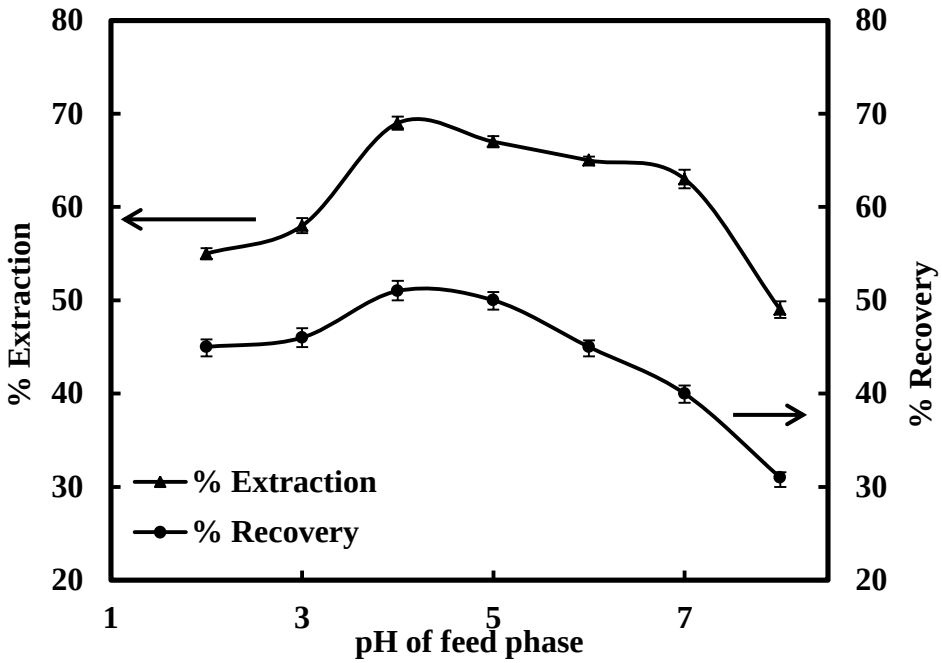


Figure 3.11: Effect of feed phase pH in three phase study *(V_F = 50 mL, V_S = 50 mL, V_M =30 mL, membrane phase =0.8 M TBP in SFO, stirring speed = 400 rpm, initial feed concentration =100 mg.L^{-1}, run time = 24 h)*

3.2.2.3 Effect of carrier concentration

The effects of carrier concentrations on the simultaneous extraction and recovery of catechin were studied with 100 mg.L^{-1} aqueous catechin solution and 0.2M ethanol as feed and stripping phases, respectively. Results are shown in Fig. 3.12. Extraction of catechin attained a maximum value of 70.2% using 1.2 M TBP as carrier in SFO. Any higher value of carrier concentration does not yield any betterment of extraction. On the other hand, recovery of catechin in the stripping phase attains a maximum value of 44% using 1.2 M TBP as well. Though both the extraction and recovery were maximum at 1.2 M carrier concentration, they are nearly same at carrier concentration of 0.8 M. The optimum concentration in three phase study is dependent on the feed-membrane interfacial surface area and the diffusivity of the solute-carrier complex in the membrane phase. The carrier is freed from its complex after

releasing the solute at the membrane-strip interface and diffuses back to the feed-membrane interface (Fig. 3.2). Thus, higher concentration beyond the optimum value doesn't yield any better extraction; rather the recovery is decreased marginally due its resistance on transport of the complex through the membrane. This adverse effect on the catechin recovery occurs due to the presence of extra carrier that creates extra resistance at the membrane-aqueous interface against solute transportation. Hence 0.8 M carrier concentration was maintained for all the subsequent three phase experiments.

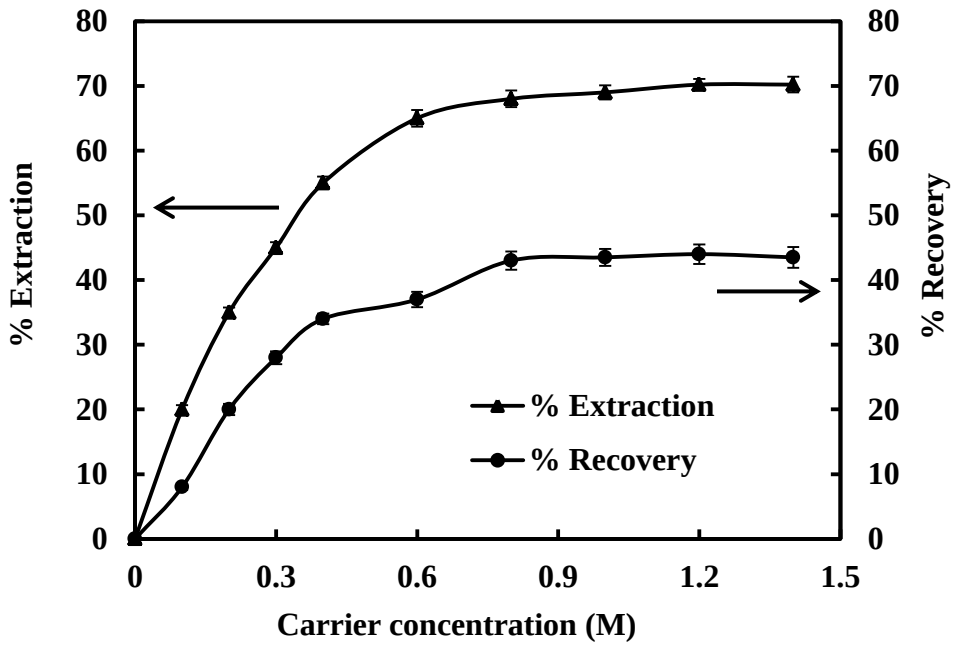


Figure 3.12: Effect of carrier concentration in three phase study *(V_F =50 mL, V_S =50 mL, V_M =30 mL, stripping phase =0.2 (M) ethanol, stirring speed=400 rpm, feed phase pH=4.0, run time = 24 h)*

3.2.2.4 Effect of stripping phase concentration

The effects of stripping phase concentrations were studied in the same BLM set up with pH 4.0 of feed phase and 0.8 M carrier in membrane phase. It was observed that 0.2 M ethanol in

stripping phase is optimum for both extraction (70%) and recovery (44 %) of catechin (Fig. 3.13). Both the extraction and recovery are enhanced with increase of the ethanol concentration up to 0.2 M. However, beyond this concentration both of them start declining. This adverse effect occurs due to crowding of excess ethanol in the stripping phase which inhibits the catechin transportation from membrane-strip interface to the bulk strip phase.

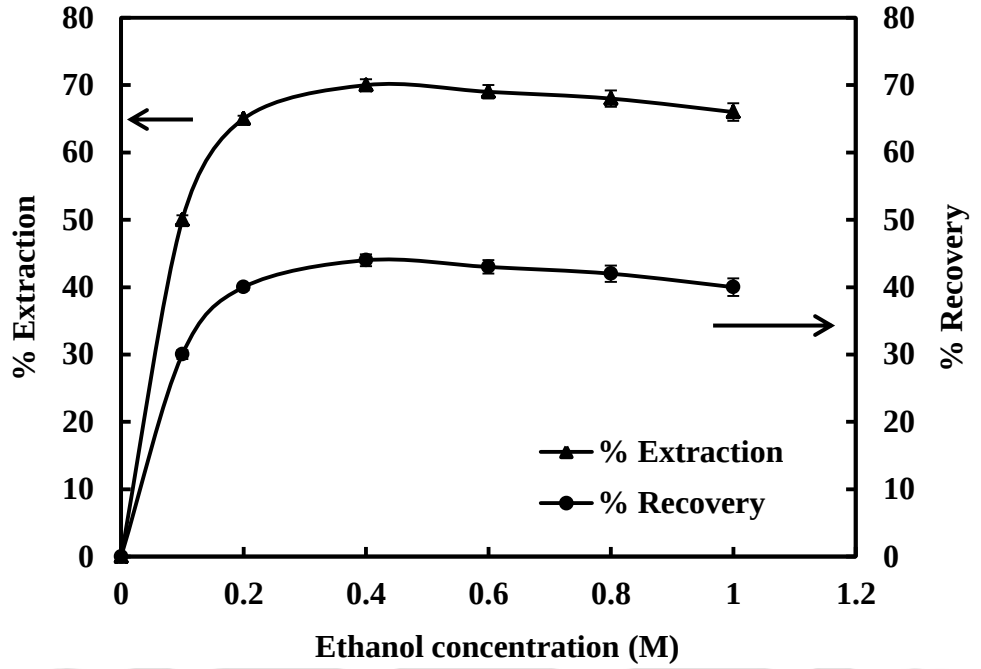
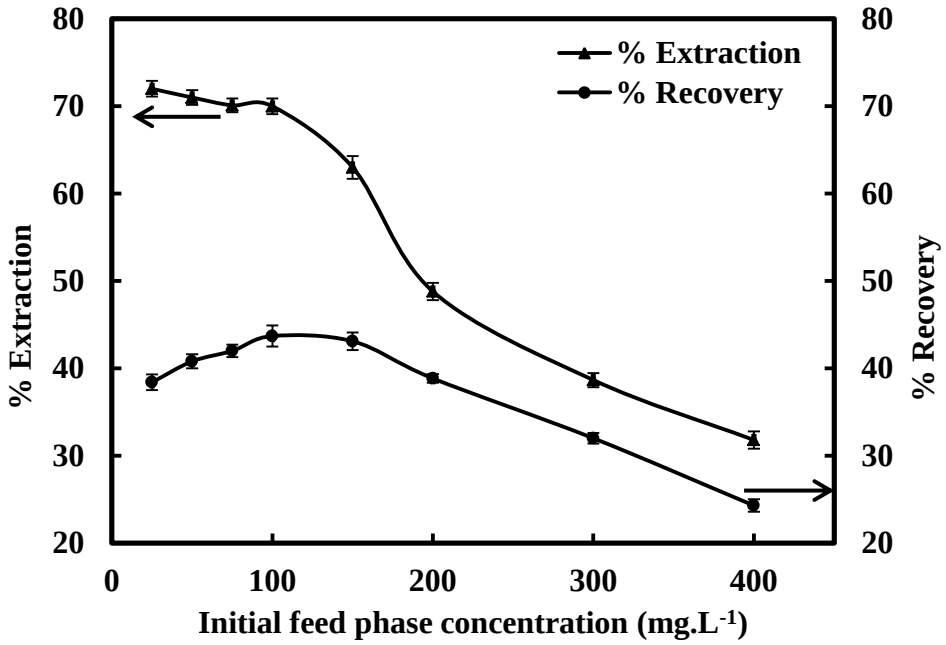


Figure 3.13: Effect of stripping phase concentration in three phase study *(V_F =50 mL, V_S =50 mL, V_M =30 mL, membrane phase =0.8 TBP in SFO, stirring speed=400 rpm, feed phase pH=4.0, run time = 24 h)*

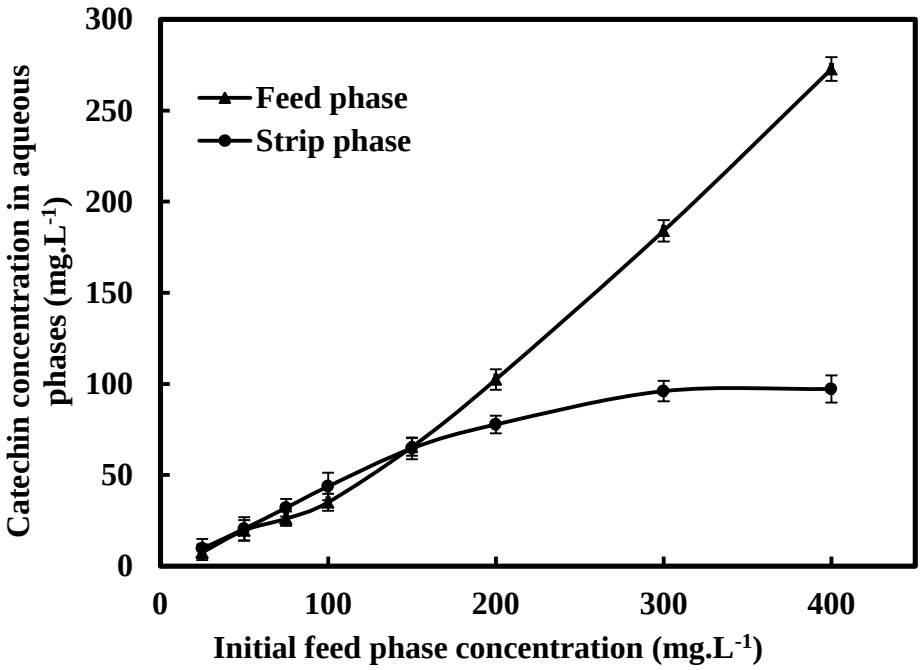
3.2.2.5 Effect of initial catechin concentration

The influence of initial catechin concentration on the transport of catechin in three phase experimentation was studied in the range of 25-400 mg.L^{-1}. Figs. 3.14 (a) and (b) show the effect of initial catechin concentration on extraction and recovery of catechin and absolute catechin concentration in both feed and strip phases, respectively. It was observed from Fig.

3.14 (a) that with increase in catechin concentration, extraction decreases very slowly from 25 to 100 mg.$L^{-1}$of initial catechin concentrations and beyond this concentration extraction decreases rapidly due to concentration polarization of the solute in the feed-membrane interface with respect to the carrier concentration. However, the recovery increases very slowly from 25-150 mg.L^{-1} and beyond that it decreases very slowly as it depends upon extraction. Though the percentages of extraction and recovery decrease beyond certain initial feed concentration (150 mg.L^{-1}), the absolute concentration in feed phase increases rapidly with the initial feed concentration and the stripping phase increases slowly up to initial feed concentration of 300 mg.L^{-1} and beyond that it becomes saturated. Hence, the optimum initial feed concentration for the favorable extraction and recovery was obtained in the range of 100-150 mg.L^{-1} for carrier concentration of 0.8 M in the membrane phase and in all subsequent BLM studies initial feed concentration of 100 mg.L^{-1} catechin was maintained.



(a)



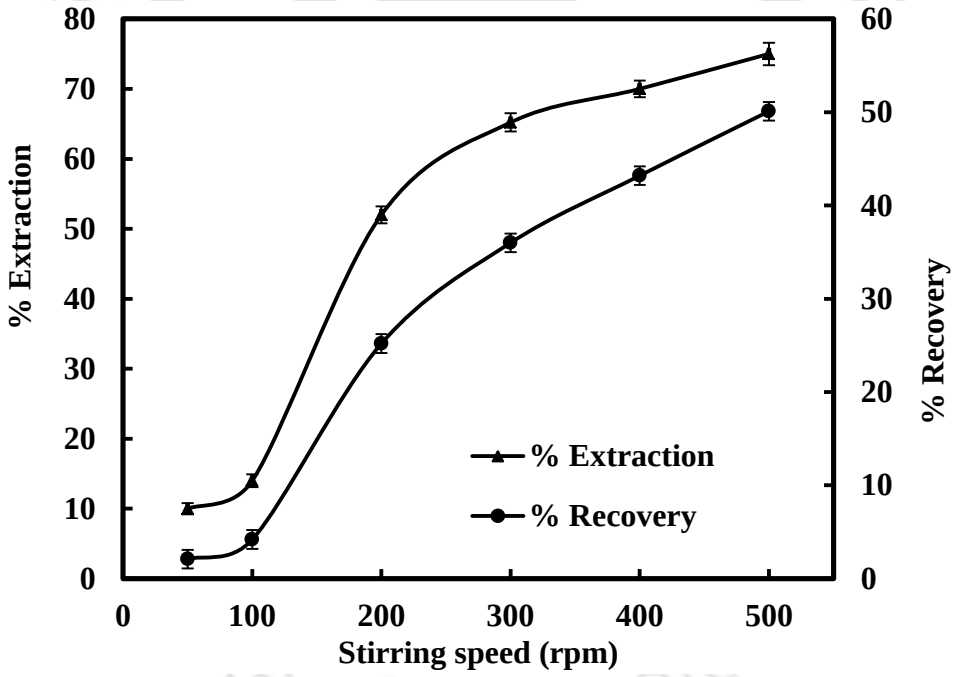
(b)

Figure 3.14: Effect of initial feed concentration in three phase study: (a) % extraction and % recovery (b) catechin concentration in aqueous phases *(V_F =50 mL, V_S =50 mL, V_M =30 mL, feed phase pH =4.0, rpm =400, strip conc. =0.2M, run time = 24 h)*

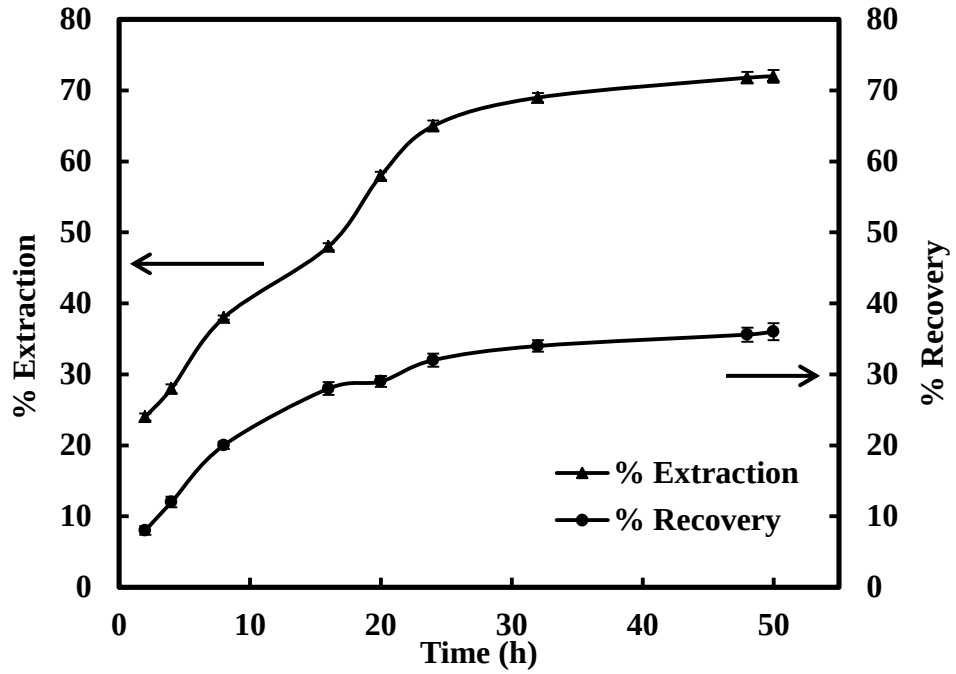
3.2.2.6 Effect of stirring speed

Effects of hydrodynamic conditions on the transport of catechin were studied at various stirring conditions of the phases involved. To identify the control regime in extraction and stripping, experiments were carried out by stirring either one of the phases or both the phases as and when required. The results have been shown in Fig. 3.15 (a-d). It was observed that both extraction and recovery increase with rate of stirring and at 500 rpm, % extraction and % recovery were 75% and 55.1 % of initial concentration of feed phase, respectively (Fig. 3.15a). But at 500 rpm the problem of emulsion formation was high due to minor mixing of both the aqueous phases with the membrane phase. Hence, stirring speed of 400 rpm was maintained in the aqueous phases in all the subsequent three phase experiments. Time profiles of % extraction and % recovery of catechin were recorded in the figures (Fig. 3.15b-

d) for various stirring conditions. It is observed that after an experimental run of 50 h duration, the extraction and recovery achieved 72 % and 36 %, respectively when only feed phase was in stirred conditions (Fig. 3.15b) and that were 68% and 34% when only strip phase was stirred (Fig. 3.15c). However, the extraction and recovery were 80 % and 43.5 % respectively when both the aqueous phases were stirred (Fig. 3.15d) and that were only 20 % and 5 %, respectively when none of the phases were stirred (data not shown). The above results postulate the effectiveness of stirring in both the aqueous phases of a three phase liquid membrane based separation process.



(a)



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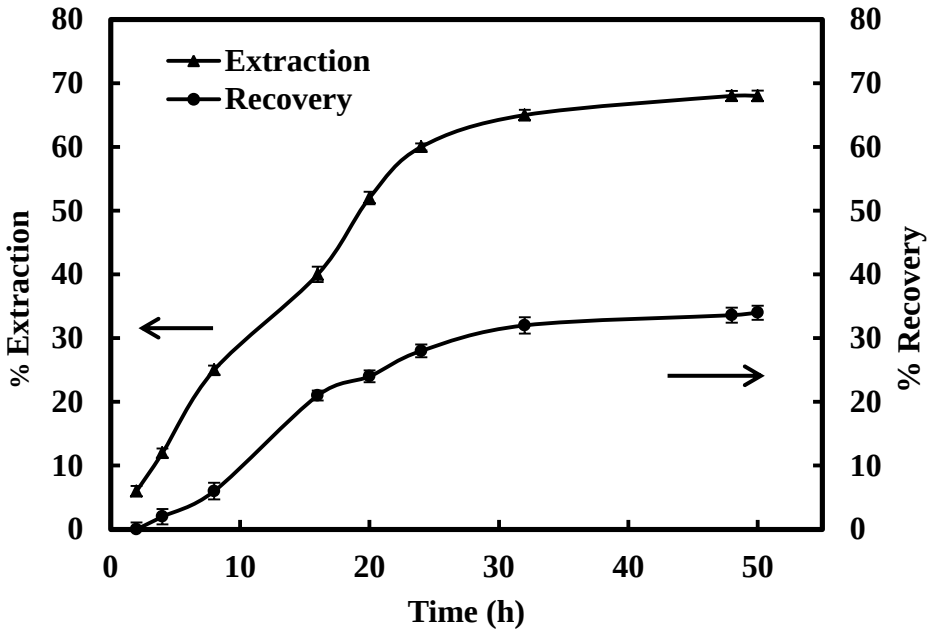
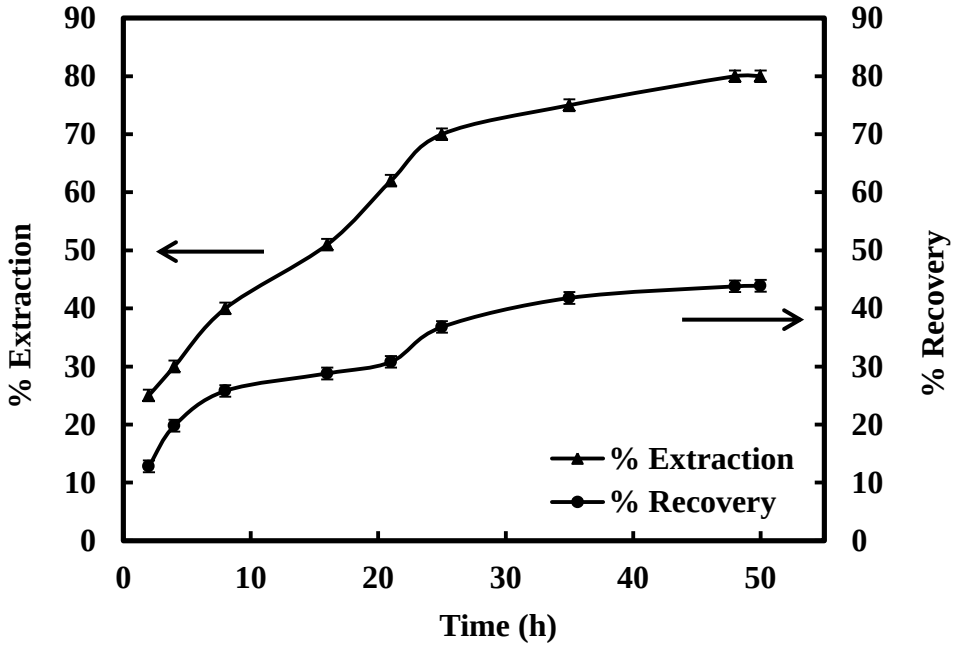


(c)



(d)

Figure 3.15: Effect of stirring in three phase (a) effect of variation of stirring speed while stirring time is 24 h (b) only feed phase stirred at 400 rpm (c) only strip phase stirred at 400 rpm (d) both feed and the strip phases stirred at 400 rpm: *(initial feed concentration =100 mg.L^{-1}, feed phase pH =4.0, strip conc. =0.2 M, V_F =50 mL, V_S =50 mL, V_M =30 mL)*

3.2.2.7 Kinetics of the process

Solute fluxes across the interfaces are important estimates of the process kinetics which, in turn, provide the idea about the separation feasibility. Catechin fluxes across the feed and strip side interfaces were determined with the help of Eqn. (3.8) of Section 3.2.2. Calculations are done at the optimum process conditions, namely initial feed concentration = 100 mg.L^{-1}, feed phase pH = 4.0, stirring speed = 400 rpm, and strip phase concentration = 0.2 M. A maximum flux of 5.7×10^{-8} kg.m^{-2}s^{-1}was estimated across the feed side interface at 2 h and that across the strip side interface was 4.9×10^{-8} kg.m^{-2}.s^{-1}at 5 h. The above result can

be explained in terms of driving force to mass (catechin) transfer through the membrane phase. The complex formation occurs instantaneously as assumed, in the feed-side interface. Flux of catechin (complex with TBP) through the feed side interface is very high up to 2 h due to high concentration gradient of complex between two interfaces. But as the diffusion is the slower step,catechin takes time to reach the strip-side interface and the maximum flux through strip-side interface occurs at 5 h (Fig.3.16). A sharp decline is observed in the flux of extraction and recovery up to 15 h, beyond which fluxes decrease rather slowly. The transportation of catechin ceases beyond this time and catechin initially present in the feed phase remains distributed among three phases as per its equilibrium concentration.

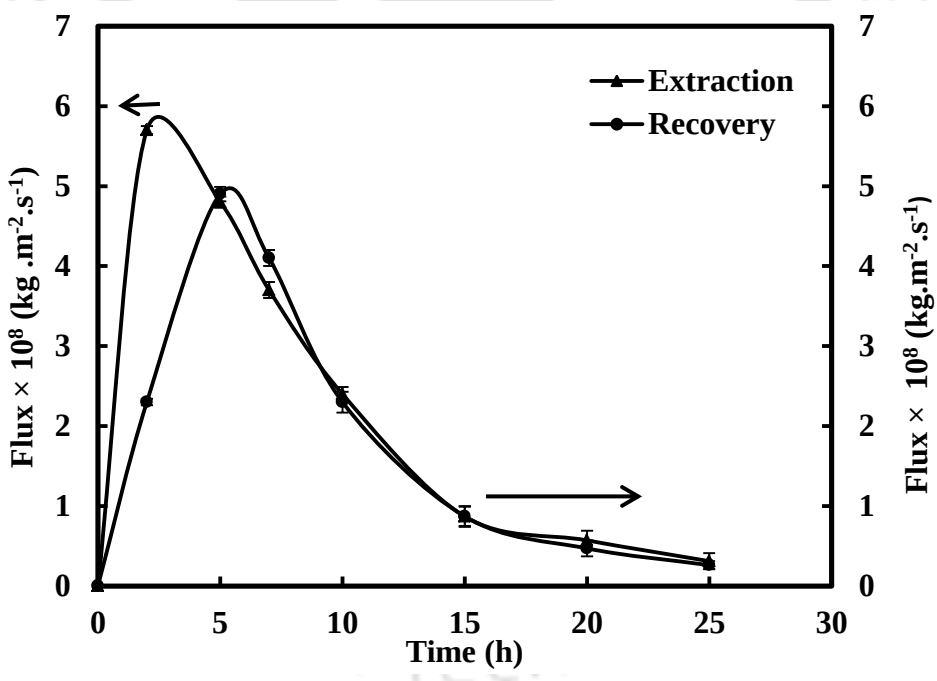


Figure 3.16: Flux of catechin through feed and strip side interface in three phase study ***(V_F =50 mL, V_S =50 mL, V_M =30 mL, initial feed concentration = 100 mg.L^{-1}, feed phase pH =4.0, stirring speed =400 rpm, strip phase concentration=0.2M)***

3.2.2.8 Fed batch system

The three-phase BLM operation was carried out in a fed batch manner in order to test whether the same liquid membrane and the stripping solution are able to transport and recover more amount of catechin repeatedly. A successful test would be a fair indication of the liquid membrane process for its feasibility to be used in continuous mode of operation. After every 24 h of operation, aqueous catechin was added to the feed phase to top it up to 100 $mg.L^{-1}$ again and it was done by the mass balance for catechin with the help of remaining concentration of exhausted feed phase and that of fresh catechin solution. At the end of three such cycles, catechin concentration of the stripping phase reached 95 $mg.L^{-1}$ (Fig. 3.17). Though the concentration of the solute transported to the stripping phase is limited by its distribution coefficient (D_2) between the membrane and stripping phases, it can be concentrated in strip phase by several times of that remaining in feed phase before that limitation arises.

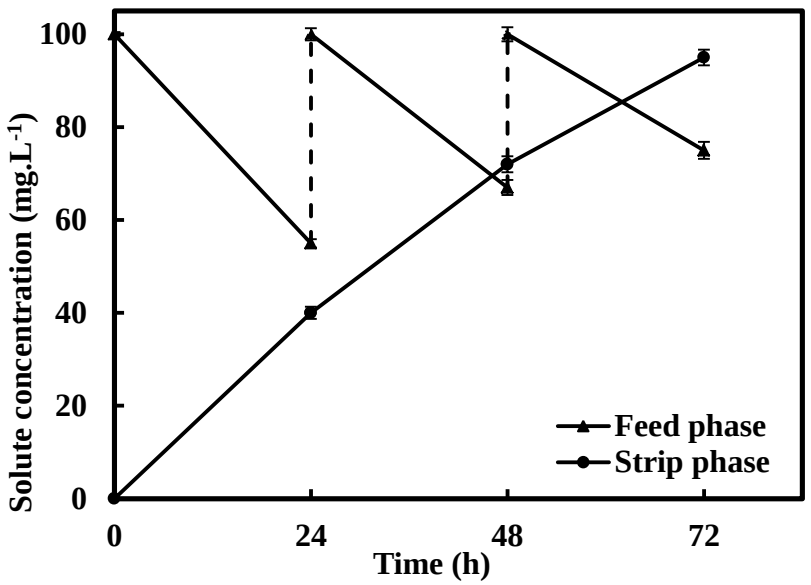


Figure 3.17: Fed batch system *(V_F =50 mL, V_S =50 mL, V_M =30 mL, feed phase pH = 4.0, stirring speed =400 rpm, strip phase concentration=0.2M)*

3.2.2.9 Comparison between different types of solvents

Two different types of solvents (diluents) were studied for comparison of their merits and demerits as the diluents of membrane phase. The extraction capacity of environmentally benign sunflower oil was found nearly four times greater than that of conventional solvent, *n*-decane in terms of distribution coefficients (Table 3.1). But one of the important steps in liquid membrane technique is the mass (target solute) transfer by diffusion in the membrane phase. Hence, the rate of solute diffusion in the membrane phase is deciding factor for the transportation or recovery process to be viable commercially. The rate of the diffusion can be measured as the flux of the solute at the strip side interface of the liquid membrane system. The fluxes of catechin in liquid membrane of two types of solvents were determined with all other components of liquid membrane and physico-chemical parameters intact. The physico-chemical parameters were already optimized for the liquid membrane with SFO as the solvent. The values of fluxes with time were determined with those optimized parameters. The maximum flux of catechin in liquid membrane with solvent *n*-decane was 11.2 $x10^{-8}$ $kg.m^{-2}.s^{-1}$ as compared to 4.9 $x10^{-8}$ $kg.m^{-2}.s^{-1}$ for that with SFO. The maximum flux across the stripping side interface was obtained at 2 h in case of *n*-decane as the solvent whereas maximum flux was obtained at 5 h in case of SFO as solvent. The results have been reported in Fig. 3.18. So, it can be concluded that though the extraction capacity of SFO is nearly four times compared to that of *n*-decane, the catechin flux in the liquid membrane with SFO is two times less compared to that with *n*-decane.

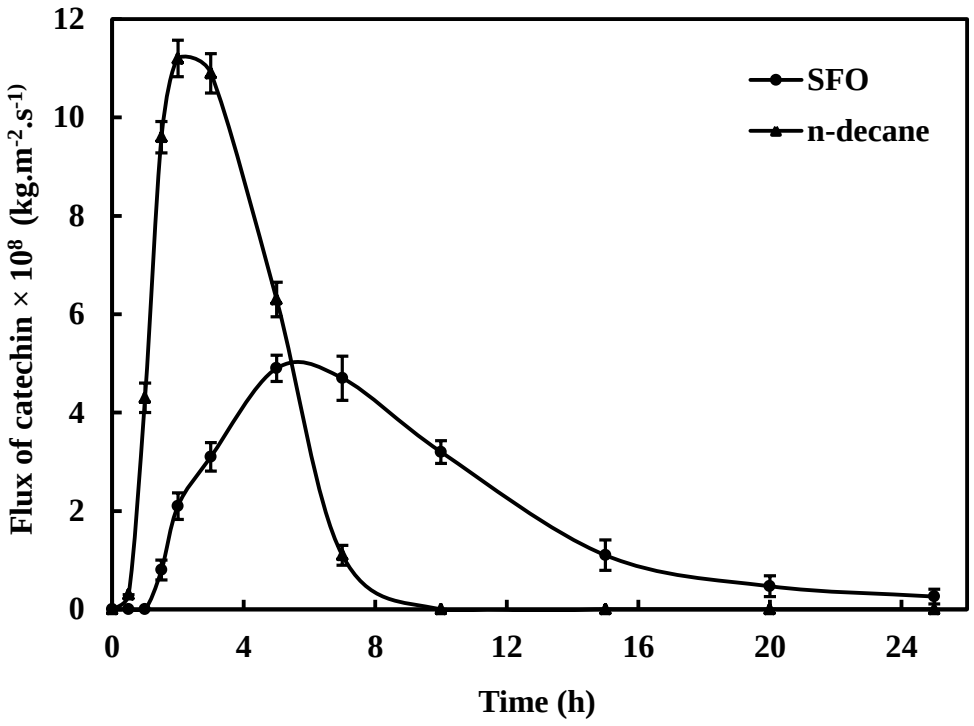


Figure 3.18: Comparison of fluxes with two different types of solvents (*(V_F =50 mL, V_S =50 mL, V_M =30 mL, feed phase pH = 4.0, stirring speed =400 rpm, TBP concentration in both the membrane phases =0.8 M, strip phase concentration=0.2M)*

3.3 Summary of the recovery of catechin through BLM

- LM based separation technique has been found to be an efficient means to extract bioactive component *viz.* catechin and the same LM may be employed to recover catechin from tea leaves extracts. The above fact has been proved through both two phase as well as three phase studies
- TBP and SFO have been found to be the best carrier-solvent combination for the above purpose when environmentally benign solvent was employed.
- The two phase studies demonstrate that the optimum extraction of catechin can be obtained at feed phase pH of 5.0, temperature of 25˚C, carrier concentration in the

membrane phase of 0.8 M and initial feed concentration of 100 $mg.L^{-1}$ in extraction time of 24 hours

- Ethanol has been proved to be an excellent stripping agent for recovery of catechin from membrane phase
- The optimum conditions for three phase studies have been found to be pH of 4.0, carrier concentration in the membrane phase of 0.8 M, ethanol concentration of 0.2 M in the stripping phase, stirring speed of 400 rpm and initial feed concentration of 100 $mg.L^{-1}$
- An operation of the system in a "Fed-batch" manner has ensured the possibility of complete separation and pre-concentration of catechin with a relatively small amount of stripping agent
- The feed phase must be maintained in acidic pH range to ensure the catechin in its molecular *i.e.* unionized state to be extracted by a neutral carrier
- Flux of the catechin in high viscous (38.0 mPa.s) SFO is less. It can be replaced by low viscous conventional solvent for higher flux in expense of purity of the recovered catechin
- The use of physiologically benign vegetable oil(SFO) as liquid membrane for the recovery of bioactive substances should be taken into consideration, as an important application, by pharmaceutical industries
- Extraction of 70% and recovery of 44% were achieved in the BLM transportation process from initial catechin concentration of 100 $mg.L^{-1}$ in the feed phase
- Twenty six percent of catechin remained in the membrane phase
- Nearly same concentration of catechin as in the feed phase was recovered in the strip phase by 3 runs in "Fed-batch" process.

Abbreviation

BLM	bulk liquid membrane
DMOA	dimethyl octyl amine
D2EHPA	di-2-ethyl hexyl phosphoric acid
FT-IR	fourier transform infra-red
SFO	sunflower oil
TOA	trioctyl amine

Nomenclature

$[C_{cat}]_{t_1}$	catechin concentration at time t_1
$[C_{cat}]_{t_2}$	catechin concentration at time t_2
$[CatOH]_{aq}$	catechin concentration in feed phase
$[CatOH]_{r}$	catechin concentration in receiving phase
$[CatOH]_{tot}$	total catechin concentration in feed
$[CatOH]_{i,tot}$	total catechin concentration in feed – membrane interface
$[CatOH.TBP]_{i,f}$	catechin-TBP complex concentrations at feed-membrane interface
$[CatOH.TBP]_{i,r}$	catechin-TBP complex concentrations at strip-membrane interface
$[CatOH.TBP]_{org}$	catechin-TBP complex in organic membrane phase
D_1	distribution coefficient between membrane phase and feed phase
D_2	distribution coefficient between stripping phase and membrane phase
J	flux of catechin transport
J_F	flux of catechin transport at feed sideinterface
J_S	flux of catechin transport at strip side interface

J_{aq}	diffusion flux through aqueous film
J_{org}	diffusion flux through the membrane
Δ_{aq}	diffusional resistances caused by the aqueous feed phase boundary layer
Δ_{org}	diffusional resistances caused due to diffusion through the membrane
K_{ex}	equilibrium constant
V	volume of aqueous solution
A	effective exposed surface area of the feed-membrane and membrane-strip interface
$[TBP]_{org}$	concentration of tributyl phosphate in organic membrane phase

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CHAPTER-IV

Recovery of Catechins through FS-SLM

CHAPTER-IV

Recovery of Catechins through FS-SLM

The results and discussion on the transportation of catechins through flat sheet supported liquid membrane (FS-SLM) prior to their recovery in ethanol were presented in this chapter. TBP in n-decane was used as the LM phase. Various physico-chemical parameters were studied and optimized for transportation of catechin primarily from aqueous solution of synthetic extract of catechin. All the optimized parameters were employed for the case study i.e. recovery of various catechins in aggregate from real extract of green tea leaves. Preparation of real extract from green tea leaves and the identification and quantification of various catechins were also described here. Issues regarding transportation of catechins from real extract that were different from the transportation of synthetic extract of catechin were discussed elaborately in this chapter.

4.1 Results and discussion

4.1.1 Selection of solvent-carrier-stripping agent combination

Extraction and stripping performances of the four different organic phases are reported in terms of distribution coefficients (D_1 *and* D_2) in Table 4.1. From the values of D_1 and the quantity of catechin recovered in stripping phase, it can be concluded that performances of *n*-decane and *n*-dodecane are more or less similar for extraction and stripping. The solubility in aqueous phase (0.05 $mg.L^{-1}$ at 25˚C) and the toxicity (Oral rat LD_{50} > 5000 $mg.kg^{-1}$) of *n*-decane and *n*-dodecane are comparable. Since *n*-decane is readily available and quite inexpensive compared to *n*-dodecane, TBP in *n*-decane was selected as ML for catechin transportation. Among various stripping agents used (Table 4.2), ethanol and *i*-propanol

showed the best and comparable performance. Since ethanol is more inexpensive and less toxic than *i*-propanol, aqueous ethanol solution (0.4 *M*) has been chosen as stripping agent for all the remaining experiments. Thus, the combination TBP in *n*-decane and aqueous ethanol solution (0.4 *M*) was chosen as solvent-carrier-stripping agent combination for the FS-SLM studies.

Table 4.1: Extraction of catechin from its aqueous solution (100 mg.L^{-1}) using various organic phases and stripping of those organic phases using 0.4 *M* ethanol

Organic phase	Catechin extracted in organic phase (in mg.L^{-1})	Catechin recovered in stripping phase (in mg.L^{-1})	Distribution coefficient (D_1) between organic/source phases	Distribution coefficient (D_2) between strip/organic phases
	x	y	x/(100-x)	y/(x-y)
n-decane (without carrier)	0	0	0	0
n-decane (with 1.2 M TBP)	46.4	26	0.87	1.27
n-dodecane (with 1.2 M TBP)	47	25.8	0.89	1.22
cyclohexane (with 1.2 M TBP)	84.2	4.9	5.33	0.06
i-octane (with 1.2 M TBP)	66.3	11	1.97	0.20

Table 4.2: Selection of stripping agent

Organic (LM) phase	Stripping phase	D_2 = (Catechin in strip phase/catechin in organic phase)
1.2 *M* TBP in *n*-decane containing 100 mg.L^{-1} catechin	0.4 *M* ethanol	1.27
	0.4 *M* *i*-propanol	1.22
	0.4 *M* NaCl	0.54
	0.4 *M* NaOH	0.00
	0.4 *M* HNO_3	0.00
	Deionized water	0.40

4.1.2 Selection of polymeric support

Polymeric supports of various chemical compositions and physical properties were tested under the same experimental conditions and their performances have been reported in the Fig. 4.1. Best recovery was found with PVDF membrane followed by PTFE, PES and Nylon, 6. Zidi *et al.* [1] reported that chemical composition of the supports are also important parameters along with its physical parameters *viz.* porosity (ε), thickness (L) and tortuosity (τ). The value of the group (ετ/L) is lower for PVDF membrane compared to that of PTFE membrane support which should lead to the lower flux (Table 2.2 of Section 2.3.3.2). But the effect of chemical compositions is more on the flux and it surpasses the adverse effect of physical parameters. The effect of chemical composition of the support on the performance (flux) lies in the fact that the wettability of various support materials by the ML and the compatibility of the support materials with ML are different. Yang *et al.* [2] reported that the lipophobic membrane is better in comparison with lipophilic ones in terms of flux as loss of ML with time is more in case of lipophilic membrane. Visual observation showed that the

PVDF membrane was most lipophobic and hence, most transparent among the tried materials [2]. In our experiment, in a run time of 24 h, we have achieved best recovery (49%) of catechin through PVDF support followed by PTFE, PES and Nylon, 6. So in this work, we have chosen PVDF support for subsequent experimentations.

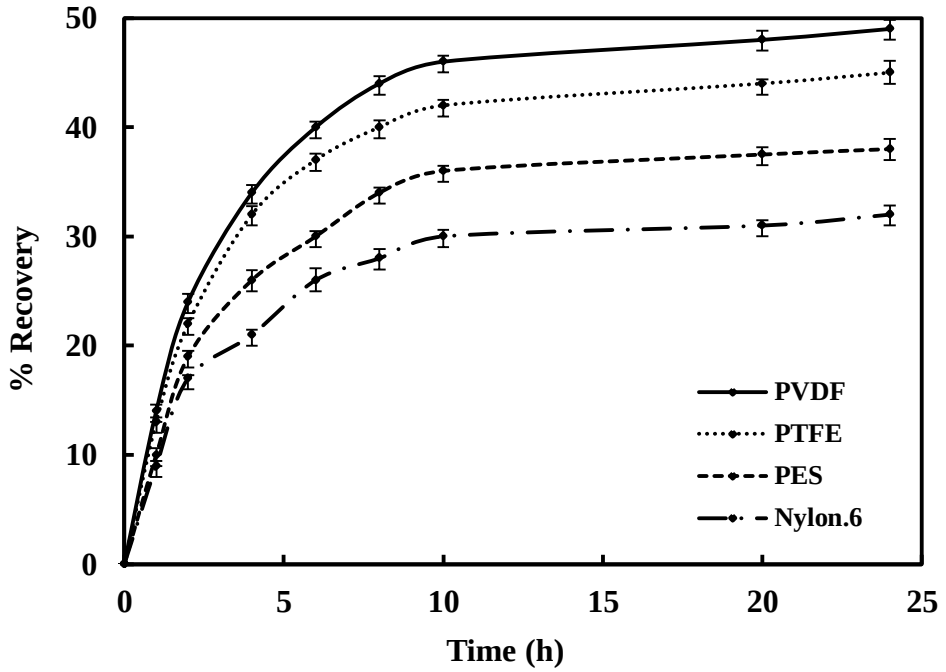

Figure 4.1: Selection of polymeric support for the LM *(catechin conc. = 100 mg. L^{-1}, membrane phase=1.2 M TBP in n-decane, strip phase=0.4 M ethanol, stirring speed = 200 rpm, temperature = 25˚C, run time 24 h)*

4.1.3 Optimization of parameters for efficient transport of catechin

4.1.3.1 Role of ethanol concentration in stripping phase

Experiments were conducted with variation in ethanol concentration in stripping phase in the FS-SLM set up (with feed phase of pH = 4, initial catechin concentration of 100 $mg.L^{-1}$ and 1.2 *M* carrier in membrane phase). It was observed that 0.4 *M* ethanol in stripping phase yields optimum performance for both extraction (53%) and recovery (49%) of catechin (Fig.

4.2). Extraction and recovery increased with increase in ethanol concentration up to 0.4 *M*. However, decline of performance beyond this concentration occurs due to crowding of excess ethanol in the stripping phase which inhibits the catechin transportation from membrane-strip interface to the bulk strip phase. Similar adverse effect was observed (though very less) at very high concentration ethanol in strip phase in BLM studies [3]. Moreover, ethanol being relatively non-polar compared to water, ML solubility in more concentrated ethanol (stripping phase) adversely affects the membrane stability inside the pores.

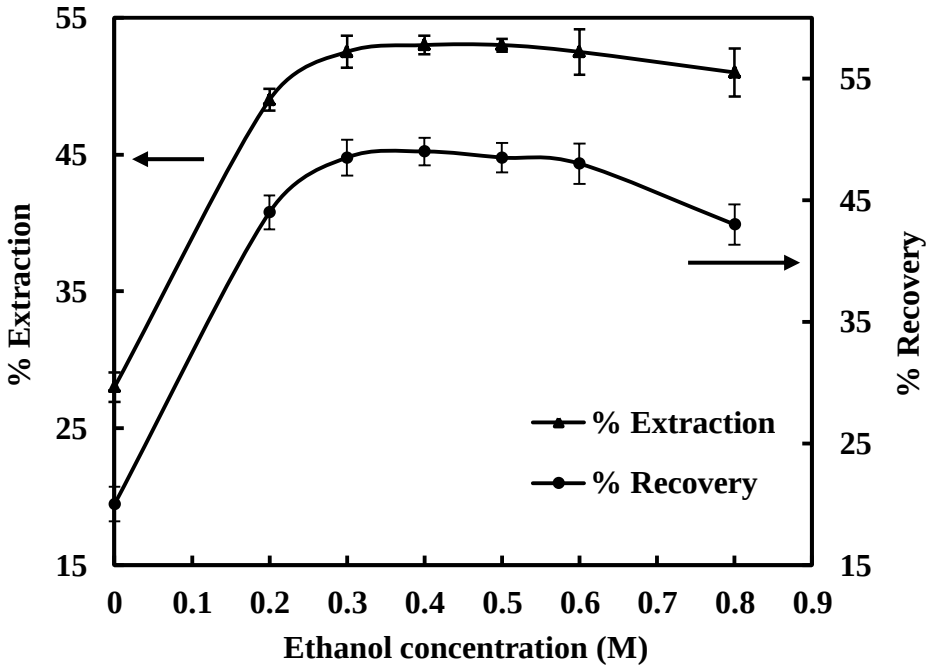

Figure 4.2: Role of ethanol concentration in stripping phase *(catechin phase pH=4.0, feed conc. =100 mg.L^{-1}, membrane phase=1.2 M TBP in n-decane, stirring speed = 200 rpm, temperature = 25˚C, run time = 24)*

4.1.3.2 Role of TBP concentration in membrane phase

The effect of carrier concentration on the simultaneous extraction and recovery of catechin were studied with 100 mg.L^{-1} aqueous catechin solution and 0.4 *M* ethanol as feed and

stripping agents, respectively. Results are shown in Fig. 4.3. Extraction of catechin attained a maximum value of 53% using 1.2 *M* TBP as carrier in *n*-decane and the recovery (49%) is closely linear with the extraction percentage as the distribution coefficients of catechin in organic phase to aqueous feed phase and aqueous strip phase to organic phase are very close (Table 4.1 and Table 4.2). Further increase in carrier concentration in ML decreases the catechin permeation due to higher viscosity of carrier (3.8 mPa.s) compared to *n*-decane (0.92 mPa.s) resulting in the higher viscosity of ML that consequently reduces the recovery of catechin. Hence, the optimum concentration of TBP is dependent on the diffusivity of the catechin-TBP complex in the membrane phase. Apart from the diffusivity, the adverse effect on the catechin transportation at high carrier concentration occurs also due to the extra resistance created by the excess carrier molecules at the membrane-aqueous interface. Zidi *et al.* [1] and Shukla *et al.* [4] had similar observations with optimum carrier (TBP) concentration as 20% (0.73*M*) in kerosene and 30% (1.2 *M*) in *n*-dodecane for transport of phenol and uranyl ions, respectively. The difference in the amount of optimum carrier can be explained from the fact that phenol was transported through complex formation with one molecule of TBP (as (PhOH$_*$TBP)) whereas uranyl ion formed the complex with 2 molecules of TBP (as $UO_2(NO_3)_{2*}$2TBP). The requirement of TBP is thus more with uranyl. Beyond a certain concentration the flux decreased in both cases due to presence of extra carrier in ML leading to the lower diffusivity of complex (due to higher viscosity) and higher resistance to mass transfer. Hence 1.2 *M* carrier concentration was maintained for all the subsequent three phase experiments.

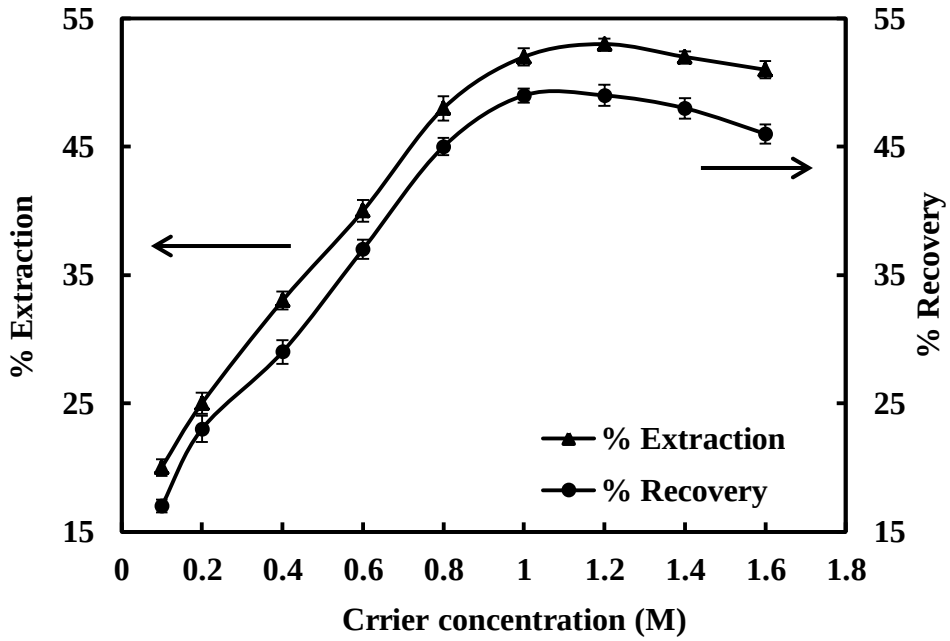

Figure 4.3: Role of carrier concentration *(catechin phase pH=4.0, feed conc.= 100 mg.L^{-1}, strip phase=0.4 M ethanol, stirring speed = 200 rpm, temperature = 25˚C, run time = 24 h)*

4.1.3.3 Role of feed phase pH

Catechin being slightly acidic in nature has the pKa value of 8.6 [5]. So, in acidic aqueous medium it remains un-ionized and makes the complex with the neutral carrier TBP in membrane phase at the feed side interface. Levin *et al.* [6] also reported that the catechin is stable in acidic medium only . The effects of feed phase pH on extraction and recovery of catechin in three phase studies were examined in the pH range of 2.0-10.0. It was observed that both extraction and recovery were favorable in the pH range of 2.0-6.0 with marginally higher value at pH 4.0 (Fig. 4.4). Slightly lower value of extraction and recovery in lower pH (2.0-4.0) is due to the excess H^+ ion which competes with the catechin to make complex with the carrier in ML. However, catechin is ionized in alkaline medium quantitatively and is not extracted by the neutral carrier TBP. So, both the extraction and recovery are reduced with

the increasing alkalinity of feed phase. Hence, all the subsequent studies were performed for feed phase pH of 4.0.

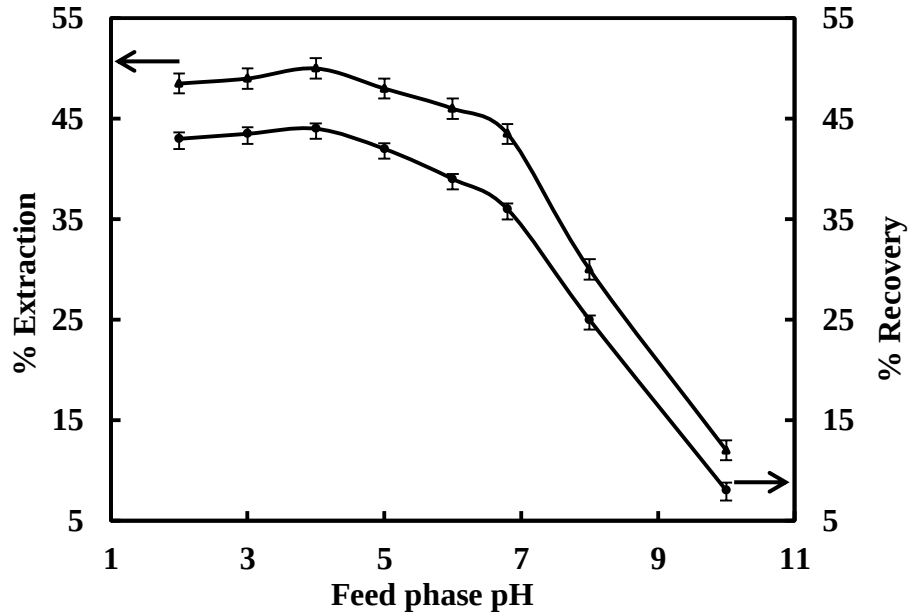

Figure 4.4: Role of feed phase pH *(catechin conc. = 100 mg L^{-1}, membrane phase=1.2 M TBP in n-decane, strip phase=0.4 M ethanol, stirring speed = 200 rpm, temperature = 25° C, run time = 24 h)*

4.1.3.4 Role of initial catechin concentration in the feed phase

The efficiency of the transport process was examined by the measurement of the catechin flux with varied initial concentration in the feed phase. TBP (1.2 M) in *n*-decane and 0.4 M ethanol were employed as the ML and the stripping phase, respectively. Both the aqueous phases were 130 mL each. The steady flux increases with the increasing catechin concentration up to 100 $mg.L^{-1}$ and the rate of increase of flux with respect to catechin concentration increases. This indicates that the catechin-TBP complexation has not reached its saturation condition until catechin concentration up to 100 $mg.L^{-1}$ [Fig. 4.5]. In other

words, some amount of carrier is still un-utilized up to that catechin concentration. Beyond catechin concentration of 100 mg.L^{-1} the flux increases, however with declining slope which indicates that the said saturation condition is reached for the higher catechin concentration and the flux is increasing as driving force for the transportation *i.e.* concentration difference increases with the increasing initial catechin concentration. Similar results were observed by Chakrabarty *et al.* [7, 8] for the separation of lignosulfonate (LS) as well as simultaneous separation of mercury and LS by trioctylamine (TOA) in dichloroethane. LM system requires more carrier and /or interfacial area for higher feed concentrations. So, for the present FS-SLM transportation system, the initial catechin concentration of 100 mg.L^{-1} was found as the optimum and maintained in subsequent experiments for the maximum efficiency (% transportation) of the transportation. The flux can be higher for the higher initial catechin concentration but with reduced efficiency of the transportation as the interfacial area remains fixed in all cases

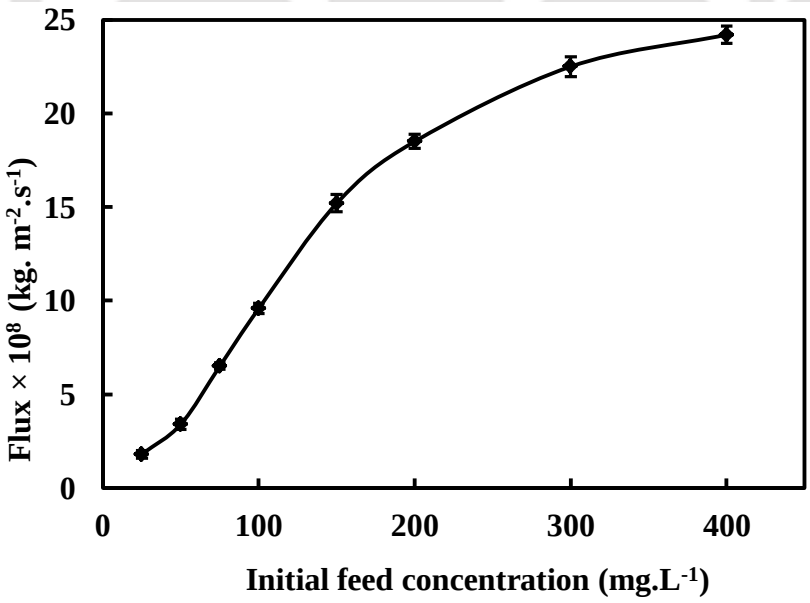

Figure 4.5: Role of initial catechin concentration in the feed phase *(feed phase pH, membrane phase=1.2 M TBP in n-decane, strip phase=0.4 M ethanol, stirring speed = 200 rpm, temperature = 25˚C, run time = 24 h)*

4.1.4 Fed batch system

Although batch study of FS-SLM process yields favourble results, a continuous mode of operation is more relevant in the context of commercial application. Nonetheless, a fed batch mode of operation is midway between a batch mode and a continuous mode, whereby feed phase is periodically topped up with fresh raw feed of high catechin concentration while the strip phase is left uninterfered. The strip phase continues to accumulate the catechin until it gets fully saturated with it. The successful operation of fed batch process provided useful information about the feasibility of continuous mode of operation and its design. Catechin transportation study was performed in FS-SLM in a fed batch fashion in order to examine the improvement of the catechin enrichment factor in the stripping phase and also to investigate whether any betterment is achieved while using same LM and the strip phase. After each 24 h, catechin concentration in feed phase was topped up to 100 $mg.L^{-1}$. Electrolyte (NaCl) was added in required amount in the respective aqueous phases. It was revealed from the experimental results (Fig. 4.6) that the concentration of strip phase increased over the runs, however the enrichment factor, which is defined as the ratio of final concentration of catechin in strip phase to that in feed phase, remained nearly same (1.032, 1.02 and 1.01 after 1^{st}, 2^{nd} and 3^{rd} run, respectively). The enrichment factor decreases over the runs primarily due to two issues. Firstly certain quantity of liquid membrane oozes out of the pores of support material and causes instability of FS-SLM. The second issue is that the enrichment factor is bounded by the distribution coefficient of catechin (D_2=1.27) between stripping phase (0.4 *M* ethanol) and organic phase (1.2 M TBP in *n*-decane) and the ethanol concentration in stripping phase was not increasing with subsequent runs. After three runs catechin concentration in the strip phase was found to be 88 $mg.L^{-1}$, which is fairly close to the initial catechin concentration (100 $mg.L^{-1}$) in the feed phase. Hence, an FS-SLM can be run in a fed

batch fashion to extract and recover catechin efficiently yet using rather less quantity of stripping agent.

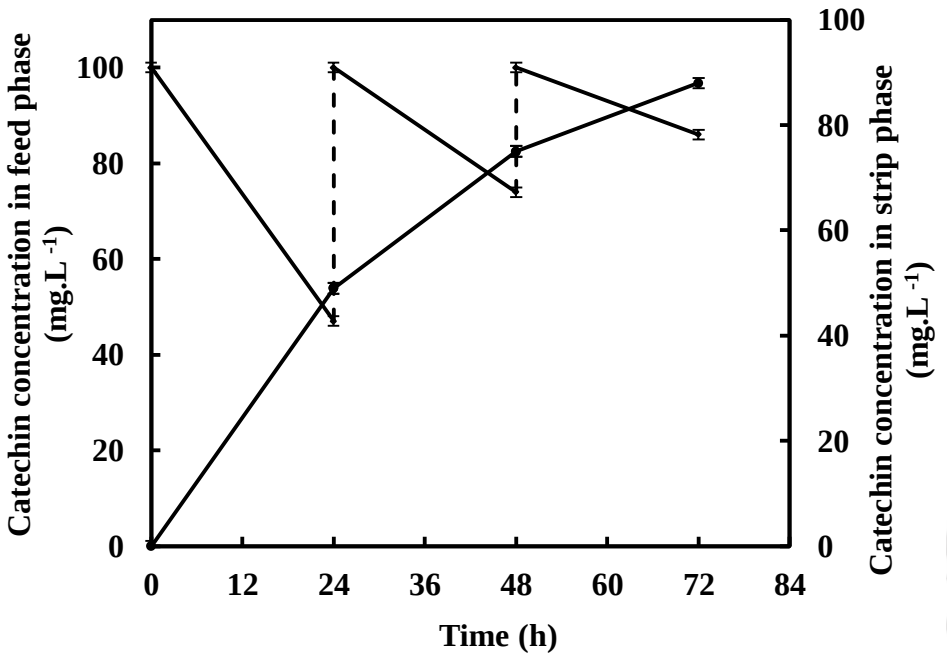

Figure 4.6: Fed batch system in SLM *(catechin conc. =100 mg.L^{-1}, membrane phase=1.2 M TBP in n-decane, strip phase=0.4 M ethanol, stirring speed = 200 rpm, temperature = 25˚ C, run time = 24 h)*

4.1.5 Case study: application of FS-SLM for recovery of various catechin from "real extract" of tea leaves

The efficacy of the FS-SLM process and its optimized operating conditions can be substantiated when it is useful in recovering catechin and its derivatives from real extract of tea leaves rather than its synthesized counterpart. The procedure for extracting aqueous solution of catechin compounds from raw tea leaves was described in Section 2.1.2 and the technique for their recovery/purification using FS-SLM was same as that of the synthesized

extract of catechin. The optimum operating conditions, as evaluated in Section 4.2.3 were incorporated in the experiments. The results are presented in the following subsections.

4.1.5.1 Identification and quantification of catechin in real feed, strip and hot water extract of green tea leaves

Identification of each catechin derivative was confirmed by comparison of retention time and chromatography with the authentic standards and quantification (as described in Section 2.2.1) was accomplished with calibration equations derived from each of the standards depicted in Table 2.1 & Fig. 2.2. Based on the chromatographic analysis mentioned in Section 2.2.1, tea leaves (Assam, India) contain 207 mg catechin compounds per gram (g) of green tea leaves (dry basis). The water content of tea leaves was found as 80.27%. The major catechin derivatives present in the tea leaves are (+) catechin (65.6%) and (-)EGC (33.8%) followed by very less amount of epicatechin gallates, (-)EGCG (0.64 %) and (-)ECG (0.04 %). The composition of Assam tea leaves were compared with the literature value in Table 4.3. The minor change in the compositions may be due to the seasonal change in growth of tea leaves. Zhu *et al*. [9] studied the degradation of eleven brands of canned and bottled green tea drinks and reported that the green tea had total catechin compounds ranging from 80 mg to 150 mg per gram of dry tea leaves . Jin *et al.* [10] also studied the separation of various catechin compounds from different teas by successive solvent extraction and reported the total catechin content in indian black tea as 14.99 mg per gram of dry leaves. Nevertheless, the total catechin content in Assam tea leaves is 1.38−2.59 times more than eleven brands of canned and bottled green tea drinks reported by Zhu *et al*. [9] and neary 14 times more than Indian black tea reported by Jin *et al.* [10]. The feed was prepared by extracting 1g of ground green tea leaves (Assam, India) in 130 mL deionized water at 60˚C for 10 h under continuous stirring at 600 rpm. The extract was initially filtered by 5.0 µm

pore size filter paper (Advantec, Japan). The catechins concentration in the feed was confirmed to be 269.7 $mg.L^{-1}$ by HPLC method.

Table 4.3: Quantification of some catechins in green tea leaves of Assam

Catechin compounds	This study		Literature value	
	Amount ($mg.g^{-1}$)	%	Amount ($mg.g^{-1}$)	%
(-) EGC	69.97	33.8	34.2	15
(+) Catechin	135.79	65.6	104.4	45
(-) EGCG	1.32	0.64	69.3	30
(-) ECG	0.08	0.04	23.1	10
Total	207	100	231	100

4.1.5.2 Transport of catechins from green tea leaves extract

The effectiveness of developed separation process was examined with some modifiactions for the recovery of various catechins from water extracts of Assam (India) green tea leaves. Polytetrafluoroethylene (PTFE) and polyvinylidene fluoride (PVDF) membranes were employed in these experiments as PTFE and PVDF were found as two better membrane supports (based on performace in transportation) among the support membranes examined in synthetic catechin transport. Feed phase of 269.3 $mg.L^{-1}$ and pH of 4.0 was used in the experiments in which 36% of the catechins initially present in feed phase was transported to the stripping phase of 0.4 M ethanol in 24 h using PTFE support. The stirring rate of both aqueous phases were maintained at 200 rpm throughout the transportation process of 24 h. The experiment was performed in ambient temperatue of (25±2) ˚C. The composition of the recovered catechins in the strip phase were found to be 59.5 % (+)catechin, 39% of (-)EGC and 1.5 % (-)ECG. The change in the strip composition reflects that more the number of (–OH) groups in the individual catechin, more is the % transportation of the compound. The

above result can be explained by the fact that ECG has the highest number (7 no.) of (-OH) group followed by (-)EGC (6 no.) and (+) catechin (5 no.) [10]. Solute- carrier interaction by hydrogen bonding is facilitated by more number of (-OH) group [3]. The performance comparison of two membranes has been illustrated as the fluxes of catechins with time in Fig. 4.7. The maximum fluxes were estimated as 19.17×10^{-8} kg.m^{-2}.s^{-1} and 18.0×10^{-8} kg.m^{-2}.s^{-1} for PTFE and PVDF, respectively. They are comparable with each other as well as with the flux (20.6×10^{-8} kg.m^{-2}.s^{-1}) of pure catechin for the same catechin concentration of 269.3 mg.L^{-1} (Fig. 4.5). Nevertheless, with advancement of time the flux declines more rapidly for PVDF membrane compared to PTFE membrane. The PTFE membrane was found less prone to be stuck with the colour substances, tannins and others impurities in the membrane pore surfaces and the problem of membrane clogging was found less compared to PVDF. Hence, the PTFE membrane was found to be better support material for the case study of recovery of catechin compounds from tea leaves extract using FS-SLM technique.

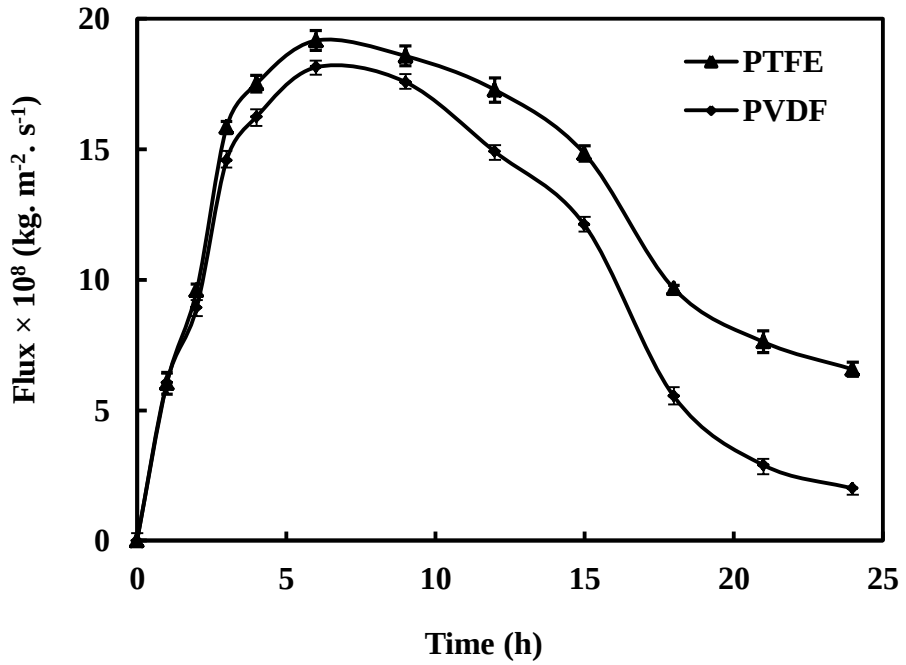

Figure 4.7: Total flux of catechins in tea leaves extract through FS-SLM *(catechin conc. = 269.3 mg L^{-1}, membrane phase=1.2 M TBP in n-decane, strip phase=0.4 M ethanol, stirring speed = 200 rpm, temperature = 25˚C, run time = 24 h)*

4.2 Summary of the FS-SLM based catechins transportation

- FS-SLM based separation technique has been found to be an efficient means for purification of bioactive components, *viz.* catechins, from tea leaves extract.
- TBP in *n*-decane immobilized into micro-pores of polymeric PVDF and PTFE membranes have been found to be suitable carrier-solvent-support combinations for the above purpose. PVDF membrane support was found best for synthetic catechin whereas PTFE membrane was found best for recovery of various catechins in aggregate from real extract of green tea leaves.
- Ethanol has been proved to be an excellent stripping agent for recovery of catechin from membrane phase.

- The optimum conditions for three phase studies have been found to be feed solution pH of 4.0, TBP concentration in the membrane phase of 1.2 *M*, ethanol concentration of 0.4 M in the stripping phase, stirring speed of 200 rpm and initial catechin concentration of 100 $mg.L^{-1}$
- An operation of the system in a fed batch fashion has ensured the possibility of 88% separation of the catechin in the feed with a relatively small amount of stripping agent.
- The feed phase must be maintained in acidic pH range to ensure the catechin in its molecular (*i.e.* un-ionized) state to be extracted by a neutral carrier in *n*-decane.
- The extracted catechin was recovered by a stripping agent in its pure *i.e.* molecular form. Hence, no extra technological step is required for purification of recovered catechin.
- The developed technique is quite applicable for the recovery of catechins from real extract of tea leaves without serious problems.
- The only problem associated with this technique was the adsorption of the colored substances, tannins to the pore surface of the support material.
- The solution to this problem is a new challenge that can be explored in future.

Abbreviations

BLM	bulk liquid membrane
FS-SLM	flat sheet supported liquid membrane
LM	liquid membrane
ML	membrane liquid
PES	polyethersulphone
PTFE	polytetrafluoroethylene

PVDF	polyvinylidenefluoride
TBP	tributyl phosphate

Nomenclature

D_1	distribution coefficient between membrane phase and feed phase
D_2	distribution coefficient between strip phase and membrane phase
L	thickness of membrane (μm)
ε	porosity
τ	tortuosity

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CHAPTER-V

Recovery of Catechins through HF-SLM

CHAPTER-V

Recovery of Catechins through HF-SLM

Catechins were recovered from tea leaves in medicinal grade ethanol through hollow fiber supported liquid membrane (HF-SLM) module. In the first phase, synthetic solution of single catechin compound was used for experimentation to optimize operating conditions. The tests with real extract of green tea leaves followed in the second phase with the similar operating conditions as optimized in the first phase. Tributyl phosphate (or TBP) as a carrier component dissolved in solvent n-decane was used as membrane liquid (ML). Ethanol was found to be ideal strippant of catechins. The flow rates of feed/strip streams and differential pressures across the membrane surfaces were optimized for achieving a stable SLM. The established models for the solute transportation in FS-SLM, as found in various literatures, were revisited to explain the experimental results. Preliminary suggestions for the once-through transportation of catechins were also provided for possible application in a pilot-plant. The studies were conducted to eliminate the problem of membrane fouling in the recovery of catechins from real extract of green tea leaves. A membrane cleaning protocol was found out experimentally.

5.1 Theoretical background

5.1.1 Extraction equilibrium

A complexation reaction between un-ionized catechin and tributyl phosphate (a neutral extractant) dissolved in *n*-decane (aliphatic solvent) occurs through hydrogen bonding at the feed side interface. The catechin-TBP complex thereby diffuses through the liquid membrane (LM) to reach the strip side interface and to the bulk of strip side thereon. The catechin-TBP

complexation reaction and the equilibrium constant (K_{ex}) have been demonstrated in the Section 3.1 of Chapter-III.

The distribution coefficient (D) of catechin is written as:

$$D = \frac{[CatOH.2TBP]_{org}}{[CatOH]_{aq}} \tag{5.1}$$

So the equilibrium constant, K_{ex} can be expressed with distribution coefficient as following:

$$K_{ex} = \frac{D}{[TBP]^2} \tag{5.2}$$

The performance of TBP as carrier and the ethanol as stripping agent have been reported elsewhere [1, 2].

5.1.2 Measurement of permeability coefficients

The distribution coefficient of catechin between the membrane phase and the stripping phase is much lower than that between the feed phase and the membrane phase. Hence, the final equation for permeability in the circulation mode could be obtained as suggested by Danesi *et al.* [3] and Aamrani *et al.* [4]:

$$\ln\left(\frac{C_f}{C_0}\right) = -\frac{AP}{V_f}.\left(\frac{\phi}{\phi+1}\right)t \tag{5.3}$$

$$\phi = \frac{Q_f}{PL\,\epsilon\pi r_i} \quad , \quad \phi > 1 \tag{5.4}$$

where P is the permeability coefficient modified for hollow fiber membrane (HFM) module on the basis of various assumptions made for it. C_f is catechin concentration in feed stream at elapsed time t and C_0 is initial concentration of feed. A and V_f are the effective surface area of membrane and volume of the feed stream respectively. Q_f is the flow rate ($m^3.s^{-1}$) of feed stream passing through the lumen of the hollow fibers. Slope of the plot of Eqn. (5.3) is

designed as S_p and the catechin permeability (P) through the membrane is calculated as follows [5]:

$$P = \frac{S_p r_i v_f}{\frac{2\pi r_i^2 L N v_f}{V_f} - S_p L \epsilon} \tag{5.5}$$

where, N, L, r_i, ϵ, v_f are number of fibers in the module, length of the fiber, internal radius of hollow fiber membrane, porosity of hollow fiber and flow velocity of the feed stream, respectively.

5.1.3 Mass transfer modeling

The design of the HF-SLM module for the transportation of catechin centers on a robust mass transfer modeling of the process. The transport of catechin encounters three mass transfer resistances in series [5]. First resistance is encountered due to the feed stream flowing in the lumen of hollow fibers. The second resistance is due to the diffusion of catechin-TBP complex through the membrane phase and the third resistance is experienced at strip side boundary layer of the stripping solution. The resultant permeability coefficient is written in terms of individual resistances as following [6]:

$$\frac{1}{P} = \frac{1}{k_i} + \frac{r_i}{r_{lm}}\frac{1}{P_m} + \frac{r_i}{r_o}\frac{1}{k_s} \tag{5.6}$$

where r_{lm} is the log mean radius of hollow fibers, k_i and k_s are mass transfer coefficients in lumen and shell side respectively, r_i and r_o are internal and external radii of the hollow fiber, P_m is solute-carrier complex permeability in the membrane phase [5]. P_m is further related to distribution coefficient of catechin, D and mass transfer coefficient in membrane phase (k_m) as Eqn. (5.7) [6]:

$$P_m = D.k_m = K_{ex}.[TBP]^2 k_m \tag{5.7}$$

On the basis of the assumption that the stripping reaction is very fast, the effect of the strip side resistance is negligible and the overall permeability can be written from Eqns. (5.6) and (5.7) as:

$$\frac{1}{P} = \frac{1}{k_i} + \frac{r_i}{r_{lm}} \frac{1}{K_{ex} \cdot [TBP]^2 k_m} \tag{5.8}$$

The effective diffusivity of solute-carrier complex is calculated from the following equation [6] :

$$D_{eff} = k_m \delta \tau \tag{5.9}$$

where, δ and τ are the thickness and tortuosity of the fibers of HFM module.

5.2 Results and discussion

5.2.1 Study of the membrane stability through leakage curve

The most contributory reason of the instability of liquid membrane is the imbalance of various forces applied on it. The resultant force can be measured in terms of the differential pressure across the surface of liquid membrane. If the liquid membrane is forced out of the pores, permeation of both feed and strip solutions occurs across the membrane and a rate of leakage curve is produced by measuring pH of shell side stream as described before (Section 2.3.4.3). Sudden change of pH indicates a serious leakage in the membrane and mixing of the streams. The rate of leakage (R_L) at a particular velocity of stream in the lumen side (or differential pressure across the membrane) is plotted (Fig. 5.1) against the time of operation. The time t_b (maximum life time of SLM), after which the membrane raptures (*i.e*. sudden change of pH is detected), can be detected from the sudden change in slope of the plot. The membrane stability up to the time t_b is confirmed by the fact of fixed volume of both streams in the circulation vessels. The maximum life time of LM was found to be 500 minutes when

the velocity of stream (v_f) at the lumen side is 0.49 cm.s^{-1}. Hence, subsequent experiments have been performed at $v_f = 0.49$ cm.s^{-1}. The detailed results have been presented in Table 5.1 and Fig. 5.1.

Table 5.1: Various operational parameters for stable membrane

Sl. No.	1	2	3	4	5	6
$R_L \times 10^8$ (m^3/m^2.s) at t_b	0.1	0.1	0.3	0.5	0.25	0.1
t_b (min.)	300	350	400	420	500	150
Flow rate $\times 10^6$ (m^3/s)*	4	8	16	24	32	40
Linear velocity (v_f) $\times 10^2$ (m.s^{-1}) *	0.06	0.12	0.25	0.37	0.49	0.62
$\Delta H \times 10^2$ $(m\ H_2O)^{\#}$	-5	-3	-1	-0.5	0.5	1

* Lumen side, $^{\#}\Delta H = H_s - H_f$, H_s=differential height of strip side, H_f = differential height of feed side

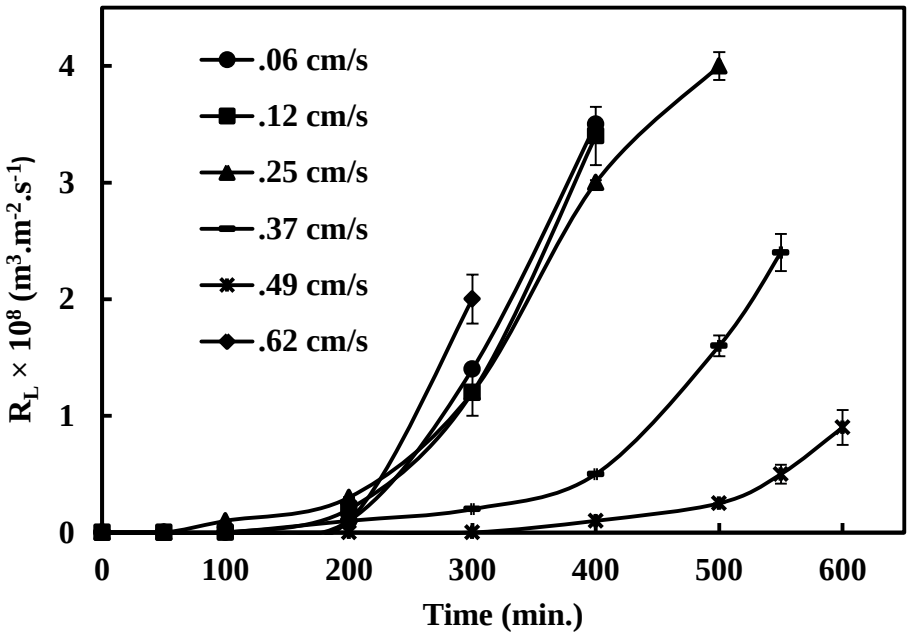

Figure 5.1: Effect of lumen side stream velocity on water leakage across fiber thickness from feed side to strip side *(shell side linear velocity = 0.49 × 10^{-2} m.s^{-1})*

5.2.2 Influence of flow rate on permeation

Flow rates of feed and strip streams are important parameters of permeation of solute through liquid membrane. The flow rate should be such that it satisfies the required condition of developed flow in both shell side and the lumen of hollow fibers of the module. At the same time, it should not force the liquid membrane out of the pores of the fibers. Moreover, the Reynolds number should be such that flow streams are in laminar zone. In this study, flow rate of feed stream was varied in the range of (4×10^{-6} to 40×10^{-6} m^3. $min.^{-1}$) in the lumen side while flow rate of strip solution was maintained constant at 40×10^{-6} $m^3.min.^{-1}$ in the shell side. The thickness of aqueous boundary layer that causes the maximum resistance to mass transfer decreases with increase in velocity of the stream. Hence, the permeability of catechin increases with the increase in flow rate of the feed in the lumen up to 32×10^{-6} $m^3.min.^{-1}$. At higher flow rate, permeability becomes independent of velocity as the convective resistance becomes negligible with respect to diffusional resistance to solute transport inside the membrane (Fig. 5.2). Permeability of catechin for various flow rates has been evaluated with the Eqn. (5.3) & (5.5). The optimum permeability of catechin was found to be 9.69×10^{-6} $m.s^{-1}$ when initial concentration of feed was 0.001 M and flow rates of feed and strip streams were 32×10^{-6} and 40×10^{-6} $m^3.min^{-1}$, respectively. The values of Reynolds numbers for the optimum permeation were calculated as 1.07 and 99.08 for flow rates in feed and strip sides respectively which are quite in laminar flow range.

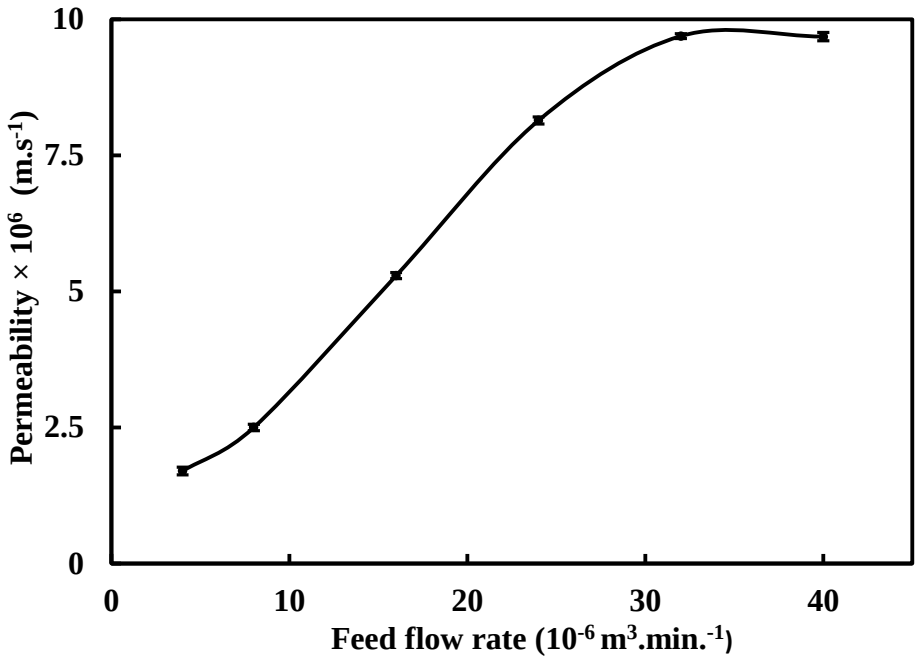

Figure 5.2: Catechin permeability with change of feed flow rate in the hollow fiber lumen *(feed concentration = 0.001 M, feed flow rate = 32 × 10^{-6} $m^3.min^{-1}$, ethanol concentration of 0.4 M, ethanol flow rate = 40 × 10^{-6} $m^3.min^{-1}$)*

5.2.3 Change of transportation of catechin with time

The experiments were conducted in a more stable conditions of the liquid membrane system *i.e.* flow rates of feed and strip streams at 32 ×10^{-6} and 40 × 10^{-6} m^3.min.$^{-1}$, respectively. The initial concentrations of catechin in the feed stream and that of ethanol in the strip stream were at 0.001 M and 0.4 M, respectively. The results have been plotted in Fig. 5.3. The rate of extraction is very high during the first 2 minutes of operation due to higher difference in concentrations of catechin in the feed and the membrane phases. The rate gradually decreases and attains a maximum value of 60% extraction after 70 minutes. On the other hand, rate of recovery of the catechin was found negligible during the first 5 minutes as the catechin takes time to diffuse through the membrane phase. Thereafter, the rate was found increasing and attained the maximum recovery of 57% after 70 minutes of operation. The flux of catechin

through the strip side interface was plotted with time in Fig. 5.4. The maximum flux was observed to be 18.33 × 10^{-8} kg.m^{-2}.s^{-1} at 20th minute of operation. The results are comparable to that obtained with the experimentation with FS-SLM at identical process conditions [2].

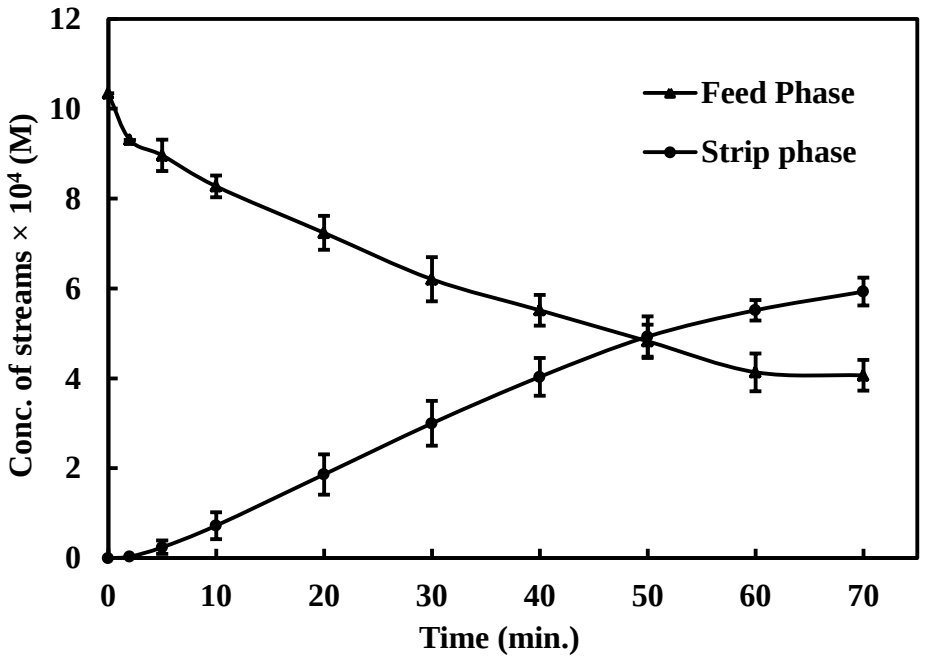

Figure 5.3: Change of concentration with time *(feed concentration = 0.001 M, feed flow rate = 32 × 10^{-6} m^3. $min.^{-1}$, ethanol concentration of 0.4 M, ethanol flow rate = 40 × 10^{-6} m^3. $min.^{-1}$)*

5.2.4 Influence of catechin concentration on permeation

Effect of initial concentration of catechin on its flux was examined for three different concentrations *viz.* 0.0005M (case A), 0.001M (case B) and 0.002 M (case C) through identical interfacial area of transportation. The flux was computed from slope analysis technique [6]. The maximum flux in all three cases was reached at 20th minute of operation. The values of flux are found to be 10.55 × 10^{-8} kg.m^{-2} s^{-1}, 18.33 × 10^{-8} kg.m^{-2} s^{-1} and 26.66 × 10^{-8} kg.m^{-2}.s^{-1} for cases A, B and C, respectively (Fig. 5.4). The higher feed concentration

yields higher flux due to higher concentration gradient between the aqueous streams across the liquid membrane. The flux through the membrane phase was calculated with following relationship [7]:

$$J = A\left(\frac{T}{\mu}\right)[TBP]_{org}^{2}[CatOH] \tag{5.10}$$

Where J, T, μ are flux of catechin in the membrane phase, operating temperature of the process and the viscosity of the membrane phase respectively. All the parameters except concentration of catechin in Eqn. (5.10) were same for all cases. Theoretically, as per Eqn. (5.10), the flux should increase linearly with increase in concentration of feed solution. However, experimental results yield lower values of flux than the theoretical ones. This result can be explained in the following two ways: (i) the amount of carrier in the LM may be less than required at higher concentration of catechin (ii) there might be crowding of the solute-carrier complex in the membrane phase at higher feed concentration that creates extra resistance to their transportation through the membrane phase. For the higher initial concentration of catechin (Case C ≡ 0.002 M) the flux remained at the maximum value for a longer duration (from 10^{th} min. to 50^{th} min.) as catechin in the feed phase was available to maintain that maximum steady flux for longer time.

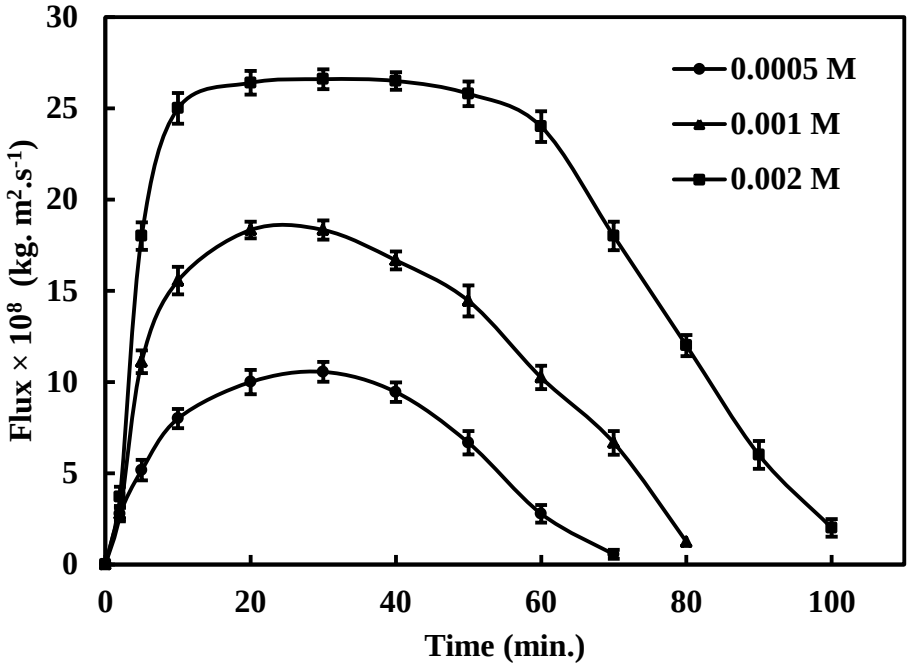

Figure 5.4: Change of catechin flux with initial catechin concentration *(linear velocity in the lumen = 0.49 × 10^{-2} m.s^{-1}, strip phase concentration = 0.4 M)*

5.2.5 Evaluation of mass transfer coefficient and diffusion coefficient

The mass transfer coefficient (k_m) and the effective diffusion coefficient (D_{eff}) of catechin-carrier complex in the membrane phase are predicted by plotting the permeability values against carrier concentrations in the membrane (refer Fig. 5.5 and Eqn. (5.8)). The permeability for each carrier concentration in membrane phase was calculated employing Eqn. (5.3) and Eqn. (5.5). It is assumed that all the carriers (TBP) present in the membrane phase are bound to the catechin and no free carrier is left in the membrane. The effective diffusion coefficient (D_{eff}) of the catechin-carrier complex in the membrane phase is calculated using Eqn. (5.9). The calculated values k_m and D_{eff} were found to be 16.3 × 10^{-7} m.s^{-1} and) 3.69 × 10^{-10} m^2.s^{-1}, respectively. The value of the regression coefficient (R^2) was found as 0.97. Both the parameters were found comparable with the literature values

reported by Rathore *et al*. [6] for diffusion of plutonium nitrate-TBP complex through similar kind of liquid membrane comprising of 1.2 M TBP in *n*-dodecane.

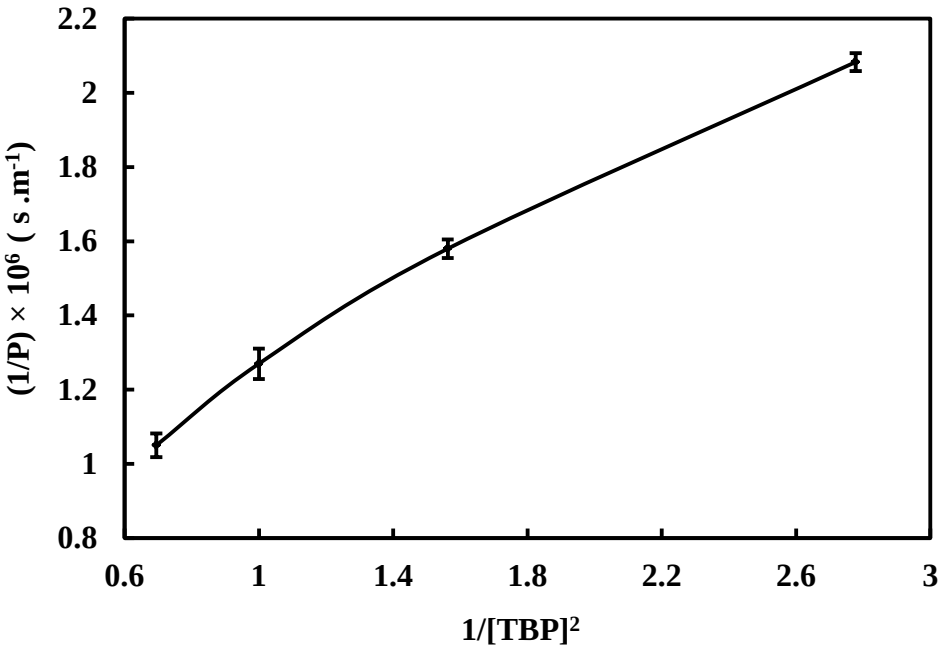

Figure 5.5: Plot of 1/P vs.1/[TBP]^2 *(linear velocity of feed flow in the lumen = 0.49 × 10^{-2} m s^{-1}, strip phase concentration = 0.4 M)*

5.2.6 Recovery of catechins from “real extract" of green tea leaves: A case study

The experimentation of transport of four catechins extracted from green tea leaves was carried out in the same set up and with similar optimal conditions that was observed in the experimentation with synthetic catechin. The results were found to be slightly different due to the effect of impurities *viz.* colored substances, tannins *etc*. The initial concentration of feed in aggregate of all four catechin compounds was 0.001 M. The extraction was found very high in the initial few minutes of operation while the recovery was less. The maximum extraction was found to be 55% as opposed to 60% in case of synthetic solution of catechin whereas the recovery was 51.3% with real extract as opposed to 57% with synthetic solution

of catechin. The results are depicted in Fig. 5.6. It is further revealed that the progression of extraction and recovery are identical with that of synthetic solution of single catechin compound, however the maximum extent of extraction and recovery are found to be 5% and 5.7 % lesser for extraction and recovery respectively, primarily due to the interference effect of impurities in the real extract. The impurities would crowd in the feed side interface and/or clog the membrane pores.

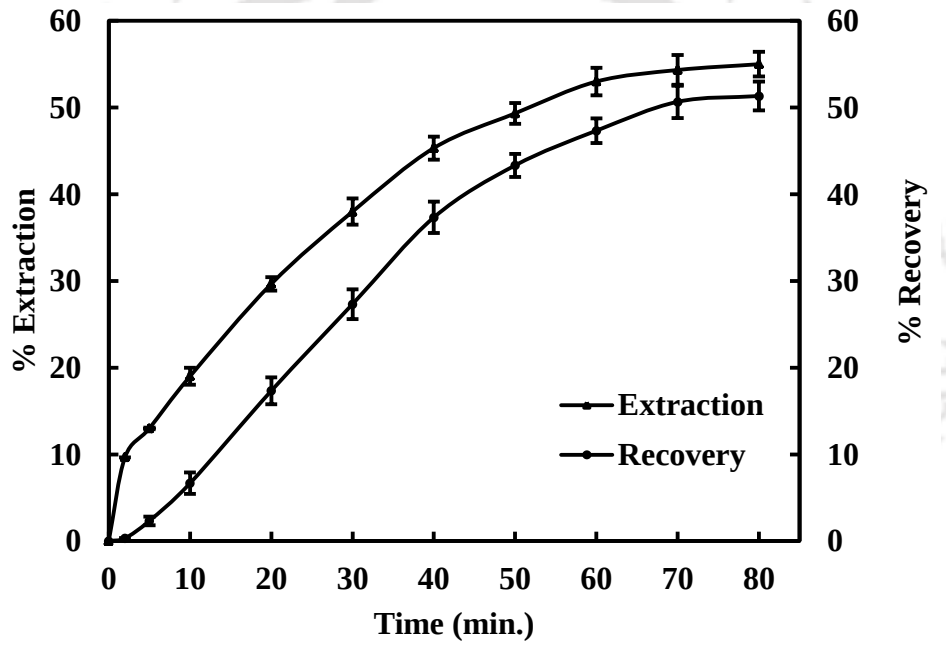

Figure 5.6: Effect of interfering compounds on catechins transportation with time from real extract *(initial catechin concentration=0.001 M, strip concentration=0.4 M, feed flow rate= 32 ×10^{-6} m^3.min.$^{-1}$, run time = 80 min.)*

5.2.7 Cleaning and the reusability of the HFM

One of the limitations of the SLM is the fouling of the micro-pores of the support in addition to the instability. The problem of fouling is maximum when various catechins are transported from real extract in HFM module. The real extract contains several impurities such as

tannins, caffeine, theophylline, theobromine *etc*. They are not candidates for "selective separation" but clog the surface of pore mouth. The fouling leads to reduction in flux. In this work, physical and chemical methods for cleaning have been demonstrated separately. Cleaning agents and their specifications were suggested as outcome of the results. The cleaning procedure was investigated with moderate positive findings.

A filtration protocol against the fouling of membrane is evaluated comparing with the flux (J) of clean water. Extent of fouling after the use of membrane as well as the cleaning efficiency after each run has been determined. The lumen side outlet was locked and the de-ionized water was allowed to pass through from lumen side to shell side at a particular hydraulic pressure (30×10^{-2} m of H_2O) through the pores. Any changes in flux will determine the extent of fouling or cleaning efficiency. Flux (J_0) through the fouled membrane after a 70 minutes run is found to be reduced to 70% of the initial flux (through fresh membrane). Two steps cleaning were executed. Water backwashing is performed for 30 minutes to reach a clean water flux of (J_1), followed by chemical washing by 10 $mg.L^{-1}$ sodium hypochlorite (NaOCl) solution for 60 minutes to reach a clean water flux of (J_2). Sodium hypochlorite dissociates in water solution to hypochlorite ion (-OCl) and proton (H^+) and the extent of dissociation depends on the pH of the solution. The reactivity and the oxidizing power of sodium hypochlorite depend on the available chlorine in the solution but, as a solution it is much easier to handle sodium hypochlorite for cleaning than handling chlorine. The hydroxyl groups of the macro-molecule tannins are oxidized by strong (–OCl) to form various salts and water. The salts are highly soluble in aqueous solution and thereby pores get cleaned. The same procedure for cleaning is performed after each run of transportation through the same HFM module. The flux values are used to generate a fouling profile pursuant to this filtration protocol and quantified using Unified Membrane Fouling Indices ($UMFIs$). The principles of

$UMFIs$ have been reported in details elsewhere [8]. $UMFIs$ are the measures of rates of membrane fouling observed within a certain time frame of interest and calculated using the following equation:

$$\frac{J_{if}}{J_{ff}} = (1 + UMFI \times V) \tag{5.11}$$

where J_{if} and J_{ff} are the values of fluxes before and after fouling (transportation experiment) and V is the volume of circulated feed for washing. Based on the principle of $UMFIs$, Unified Membrane Cleaning Indices ($UMCIs$) was also defined and calculated from following equation:

$$\frac{J_{ic}}{J_{fc}} = (1 + UMCI \times V) \tag{5.12}$$

where J_{ic} and J_{fc} are the values of fluxes before and after cleaning. The cleaning efficiency has been calculated in terms of flux of water after the cleaning (Table 5.2) and that has been re-checked in terms of recovery efficiency for individual run (Table 5.2). The maximum recovery of catechins in subsequent runs decreases despite cleaning of the membrane after each run. It reduces to 44.3% at 3rd run from 51.3% in the first run. The fluxes in these three runs of transportation have been studied with progression of time and the results are plotted in Fig. 5.7. With increasing number of runs it takes more time to attain maximum recovery of catechins.

Table 5.2: Efficiency of cleaning: hydraulic head (ΔH) = 30 cm of water, permeate volume (V) in 70 min. = 0.1143 m^3/m^2, surface area = 0.06 m^2, J = 27.22 $kg/m^2.s$

Membrane	$\frac{J_0}{J} \times 100$ (%)	$\frac{J_1}{J} \times 100$ (%)	$\frac{J_2}{J} \times 100$ (%)	V ($m^3.m^{-2}$)	*UMFI* ($m^2.m^{-3}$)	*UMCI* ($m^2.m^{-3}$)	% Recovery
Fresh membrane (1st run)	70	75	95	0.1143	12.12	3.124	51.3
After 1st cleaning (2nd run)	65	85	90	0.096	8.09	4.006	46.3
After 2nd cleaning (3rd run)	63	83	87	0.085	7.90	4.48	44.3

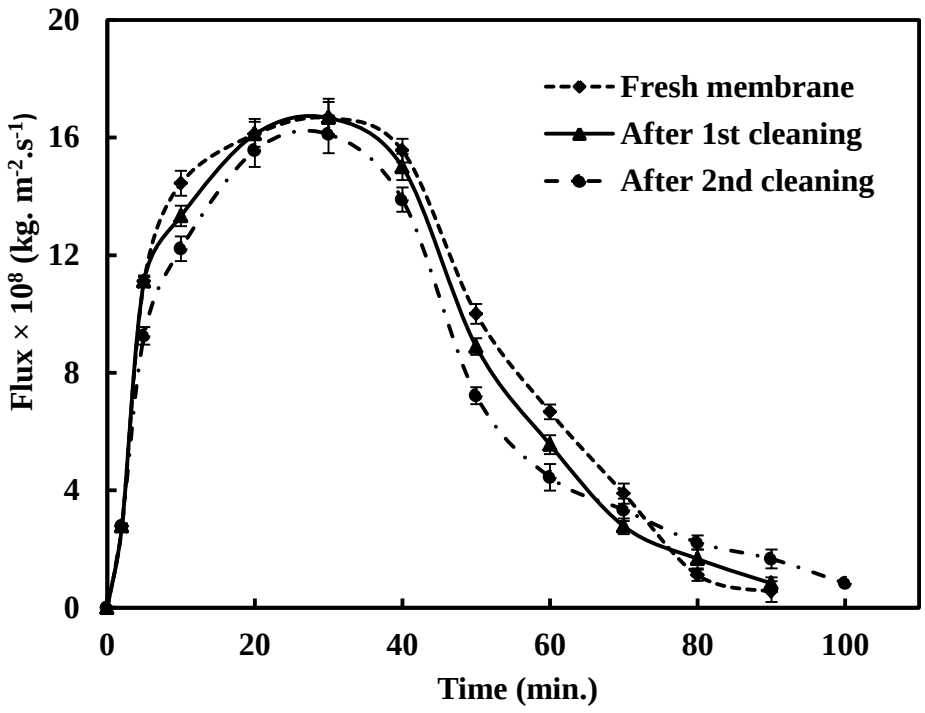

Figure 5.7: Comparison of catechins flux with time with successive cleaning of hollow fiber membrane *(initial catechin concentration=0.001 M, strip concentration=0.4 M, feed flow rate= 32 × 10^{-6} $m^3.min.^{-1}$)*

5.2.8 Combination of membrane modules for once-through transportation

The transportation process for the recovery of various catechins from tea leaves is better to be once-through process for its commercial application. A process of separation could be continuous if it is once-through and no circulation is needed. Then large amount of feed (catechins) can be treated with minimum operational cost. A simple mathematical calculation, assuming the linearity of the characteristics behavior of the multiple modules in series, parallel or in series-parallel, may yield the approximate outcome of the pilot-scale operation prior to its commercial application. For a single module of 20 $\times 10^{-2}$ m length, the maximum recovery of 51.3% was achieved in 60 minutes with a feed/lumen side linear velocity of 0.49 $\times$ 10^{-2} m.s^{-1}. Hence number of recycles the lumen-side stream has experienced is

$$\frac{(60 \times 60)}{\left(\frac{20}{0.49}\right)} = 88.2 \cong 90$$

In other words, the number of such modules in series and/or parallel which will provide the same target recovery in once-through mode of operation is 90. The fiber length has a limitation due to lack of mechanical stability for longer fibers and it can be utmost twice the present module *i.e.* 40 $\times$ 10^{-2} m. Hence, the number of modules required will be halved (≈ 45) and they will contain a total of $45 \times 2800 = 126000$ fibres. Usually commercial HFMs contain higher number of fibres. If we contain these fibers in four such modules, then a single module will contain $126000/4 = 31500$ fibers which is very common in available commercial HFM module [9]. Four such modules in series or parallel combination will perform the same duty of catechins recovery in once-through mode. The recovery of catechins in the strip phase by this LM technique is accomplished in a single technological step (simultaneous extraction and stripping). In addition, the once-through transportation of

catechins probably will provide favorable technique over solvent extraction for the recovery of catechins.

5.3 Summary of the recovery of catechins through HFM module

- Catechin and its derivatives from tea leaves extract has been recovered in HF-SLM module
- TBP in *n*-decane immobilized into the micro-pores of polyethersulfone (PES) membrane have been found to be suitable carrier-solvent-support combination for the above purpose
- The flow characteristics have been optimized to obtain the stable liquid membrane in the pores of the fibers using synthetic catechin
- Flow rates and other various physical parameters have been optimized to achieve the maximum efficiency of the transportation
- The established model equations available in the liquid membrane techniques have been employed for the study of the experimental results
- The mass transfer parameters such as solute diffusivity in the membrane phase (De_{ff}), mass transfer coefficient in the membrane (k_m), permeability (P) and the flux (J) through strip side interface *etc*. have been evaluated
- The developed technique is quite applicable for the recovery of catechin compounds from hot water (60˚C) extracts of tea leaves with minor problem of membrane fouling due to adsorption of the colored substances, tannins *etc.* to the pore surface of the support material.
- A membrane cleaning protocol has been experimentally established and reported on the basis of measured fluxes of catechins

- The skills and methodologies developed in this research can be utilized in a wider context such as purification of other bioactive compounds.
- A simple mathematical calculation has been provided for the recovery of catechins using HF-SLM in once-through mode.

Abbreviation

(-)ECG	epicatechin gallate
(-)EGC	epigallocatechin
(-)EGCG	epigallocatechin gallate
HFM	hollow fiber membrane
HF-SLM	hollow fiber supported liquid membrane
LM	liquid membrane
TBP	tributyl phosphate
UMCI	unified membrane cleaning index
UMFI	unified membrane fouling index

Nomenclature

A	effective area of catechin transportation
C_f	final concentration of catechin
C_0	initial concentration of catechin
D	distribution coefficient
D_{eff}	effective diffusion coefficient
K_{ex}	equilibrium constant

J	flux through virgin membrane
J_i	initial flux before fouling
J_f	final flux after fouling
J_M	flux through membrane phase
J_0	flux through fouled membrane (before cleaning)
J_1	flux after physical cleaning
J_2	flux after chemical cleaning
k_i	mass transfer coefficient in feed side
k_m	mass transfer coefficient in the membrane phase
k_s	mass transfer coefficient in strip side
L	length of the hollow fiber
N	number of the hollow fiber
P	permeability
P_m	permeability through membrane phase
Q_f	flow rate of the feed
R_L	rate of leakage
r_i	inner radius of fiber
r_o	outer radius of fiber
r_{lm}	log mean radius of fiber
T	absolute temperature
t_b	time of liquid membrane breaking

V_f	volume of the feed
v_f	velocity of the feed
ΔH	hydraulic pressure differential
γ	interfacial tension
δ	thickness of the fiber
τ	tortuosity of the fiber
ε	porosity of the fiber

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CHAPTER-VI

Recovery and Enrichment of Catechins through Iron-complexation using FS-SLM

CHAPTER-VI

Recovery and Enrichment of Catechins through Iron-complexation using FS-SLM

The idea of metal complexation of catechins integrated with FS-SLM technology for their recovery as an enriched bioactive product was introduced. Primarily synthetic catechin was employed for transportation prior to the its recovery as precipitated complex. Seven metal ions(Al^{3+},Cu^{2+}, Ni^{2+}, Mg^{2+}, Mn^{2+}, Zn^{+2} and Fe^{3+}) were examined as complexing/precipitating agents for catechin in a two-phase stripping set-up and Fe^{3+} was selected on the basis of its best capacity for catechin complexation and precipitation. Owing to the property of precipitation of Fe-catechin complex inside stripping section, the pre-concentration and recovery of the target solute became very easy. As a consequence of complex precipitation, equilibrium of stripping reaction shifted towards the right which augmented the continuous operation of extraction and recovery of catechins manifold. Parameters influencing the recovery of the catechins as complex were optimized. The optimized FS-SLM parameters were applied for the case study with catechins from real extract of green tea leaves. Various analytical methods for the characterization of iron-catechin complex were also presented.

6.1 Experimental procedure

The experimental procedure was followed as described in Section 2.3.3 of Chapter-II. The feed phase (130 mL) was aqueous solution of catechin (0.001 M or 300 $mg.L^{-1}$) at pH 5.5. The strip phase (130 mL) was aqueous solution of metal salts of various concentrations. Both aqueous phases were stirred at 200 rpm using mechanical stirrers (Make: Remi; Model: RGQ 121/D) in order to reduce concentration polarizations at the interfaces. The temperature was

maintained at 25 ± 2˚C. A required amount of electrolyte (NaCl) was dissolved in the aqueous feed phase in order to increase interfacial tension and thereby to reduce loss of ML from the pores of the solid membrane. This enhances the stability of LM in the pores of solid support. The use of electrolyte (NaCl) in the aqueous phases has been reported in detail in Section 7.2.3 of Chapter-VII [1]. All the transportation experiments were conducted with carrier (TBP) concentration of 1.2 M and this concentration was optimized in Chapter-IV [1, 2]. Vacuum-dried precipitate of metal-catechin complex was weighed and recovery was estimated from the stoichiometry of iron catechin complexation. Feed and strip phase samples (about 3 mL) were periodically taken out and concentration of catechin content in those samples were determined using UV-vis. spectrophotometer. The schematic of permeation cell, the model equation for flux of catechin were described in Chapter-II. The calculations for the extent of extraction and recovery have been reported later in result and discussion section of this chapter. All experiments were carried out in triplicate and the results were shown with standard error bars.

6.2 Results and Discussion

6.2.1 Chemistry of the Fe-catechin complexation

Catechin, a secondary metabolite of tea plants, is a polyphenol. It has of two benzene rings (called the ring-*A* and ring-*B*) and a dihydropyran heterocycle (the *C*-ring) with a hydroxyl group on carbon-3 in its structure as shown in Fig. 1.8 of Chapter-I. It has been extracted in the present work by the neutral extractant, TBP, whose chemical structure has been described in Section 3.1 of Chapter-III and reported elsewhere [1]. The possible reaction mechanism and the stoichiometry of extraction have also been discussed there and reported elsewhere [3]. As per the chemical coordination mechanisms of catechin, the electron donating catechol

3', 4' dihydroxyl groups (at ring−B, catechol moiety) make the coordinate bond with the ferric ion (Fe^{+3}) to form the complex. The coordination of ferric ion occurs with the oxygen atoms of two catechol groups (−OH) in ring−B. These two hydroxyl groups are favorable as they are in *ortho*-position and bond length is minimum. The other two hydroxyl groups (−OH) in ring−A will not form complex as they are in *meta*-position. The phenoxyl group (−OH) attached to the ring−C does not form the coordination bond with metal ion as per the coordination chemistry. The complexation site between catechin and the ferric ion is shown in Fig. 6.1. The stoichiometry between metal (ferric nitrate) and catechin in complex is determined employing Job Method [4, 5]. The detailed procedure and the results have been discussed in the following section. Complexation occurrs in the strip side interface and the complex diffuses into the bulk strip solution prior to precipitation of so formed complex beyond certain concentration (solubility product) of it. It is assumed that the mass transfer of catechin across the membrane occurs only through diffusion mechanism. The interfacial flux due to the chemical reaction has been neglected, as the chemical reaction is intrinsically very fast, and hence the concentrations at the interfaces will almost be equal to the equilibrium concentrations [3, 6]. The overall flux (J_{org}) can be derived by Fick's first law of diffusion applied to the membrane phase (Eqn. 6.1).

$$J_{org} = \Delta_{org}^{-1} \left([CatOH.TBP]_{i,f} - [CatOH.TBP]_{i,r} \right) \quad (6.1)$$

where $[CatOH.TBP]_{i,f}$ and $[CatOH.TBP]_{i,r}$ are the concentrations of catechin-TBP complex at feed-membrane and strip-membrane interfaces, respectively, and Δ_{org} is the resistance to the diffusion of complex in the membrane phase.

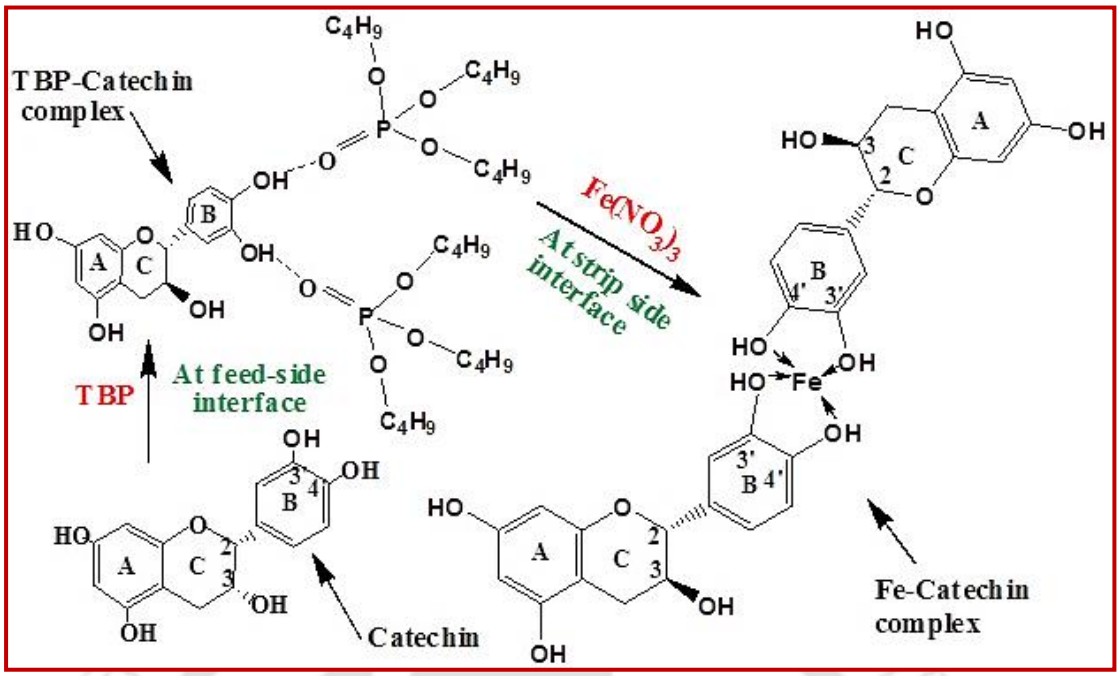


Figure 6.1: Schematic of Fe-catechin complex formation

6.2.2 Stripping phase selection

Aqueous solutions of seven metal ions (Al^{3+}, Cu^{2+}, Ni^{2+}, Mg^{2+}, Mn^{2+}, Zn^{+2} and Fe^{3+}) were tested for their efficacy as stripping solutions as well as complexing agents for catechin. Two phase equilibrium studies were conducted for complexation and precipitation. Seven equimolar (0.001M) solutions of 10 mL each, each consisting of catechin and one metal ion, were prepared. Each of them was poured in separate 250 mL Erlenmeyer flasks and stirred for 24 hours. The resulting solutions were centrifuged and the retentates were analyzed spectrophotometrically to measure the concentration of unreacted catechin as well as to obtain the spectral change of the solutions due to formation of metal-catechin complex. The results have been shown in the Table 6.1 which indicates the Fe^{3+} as the second best complexing (92%) and stripping agent after Cu^{+2}. Brownish black precipitate was found in the solution mixture in case of iron salt (ferric). Ferric ion (Fe^{+3}) has been selected as complexing agent over the Cu^{+2} due to its comparable better non-toxic nature and biological

compatibility. In previous works, in case of catechin recovery in aqueous ethanol solution in Chapter-IV [1, 3], it was difficult to achieve extraction and recovery more than 53% and 49%, respectively *i.e* 'uphill transport' was not obtained adequately. In this work, extraction and recovery of catechin were augmented manifold due to formation and precipitation of metal-catechin complex and subsequent shifting of equilibrium of stripping reaction to the right. Hence, ferric nitrate was selected as stripping as well as complexing agent.

Table 6.1: Selection of best metal ions to be included in the stripping phase for metal complexation of catechin (with initial concentration of catechin 0.001M in feed phase)

Metal ions	Standard reduction potential, E˚(V)[20]	Unreacted catechin ($\times 10^4$ M)	Metal-catechin complex ($\times 10^4$ M)	%Complexation
Al^{3+}/Al^0	-1.66	4.2	2.9	58
Cu^{2+}/Cu^0	0.34	0.5	4.75	95
Ni^{2+}/Ni^0	-0.25	1.4	4.3	86
Mg^{2+}/Mg^0	-2.37	6.1	1.95	39
Mn^{2+}/Mn^0	-2.18	4.2	2.9	58
Zn^{+2}/Zn^0	-0.76	2.03	3.98	79.7
Fe^{3+}/Fe^0	-0.04	0.8	4.6	92

6.2.3 Stoichiometry of complexation reaction

The stoichiometry between metal (ferric nitrate) and catechin was determined by employing Job Method. Two stock solutions, one of catechin (0.001 M and 5.5 pH) and the other of ferric nitrate (0.001 M and 1.55 pH) were prepared for this study. Seven 2 mL mixtures of

these stock solutions in varied molar ratio were collected in as many 100 mL Erlenmeyer flasks and stirred at a speed of 200 rpm for 24 h. This allowed complexation reaction between catechin and ferric nitrate and a chemical equilibrium was believed to be attained in this period. The samples were then centrifuged at 5000 rpm and filtered through 0.2 µm pore size cellulose acetate filter prior to analysis by HPLC to measure unreacted catechin remaining in the reaction mixture. The calibration equation for measurement of catechin has been provided in Section 2.2.1 of Chapter-II and can be found elsewhere [1]. The amount of formed complex was supposed to be equivalent amount of reacted catechin in the mixture. Reacted catechin concentration in the mixture versus catechin mole fraction was plotted and the graph indicates that the maximum complexation occurred at 0.67 mole fraction (X_{max}) of catechin. Thus,

$$n = \frac{X_{\max}}{(1 - X_{\max})} \tag{6.2}$$

$\mathrm{X} = \text{mole fraction of catechin}$

$n = \text{no. of mole catechin bonded with one metal}$

$X_{\max} = \text{mole fraction of catechin at maximum complexation}$

Putting value of $X_{\max}$ (0.67) in Eqn. (6.2), one obtains $n = 2$. Hence, the stoichiometry of complexation indicates 2:1 molar ratio of catechin to Fe *i.e.* two molecules of catechin is attached to one Fe atom. McDonald *et al.* also observed the same stoichiometry in catechin-metal ion (Cu^{+2}) complex precipitation in their study on optimal conditions and origin of precipitation of metal ions by plant polyphenols [7]. The equilibrium data for stoichiometry of complexation has been plotted in Fig. 6.2.

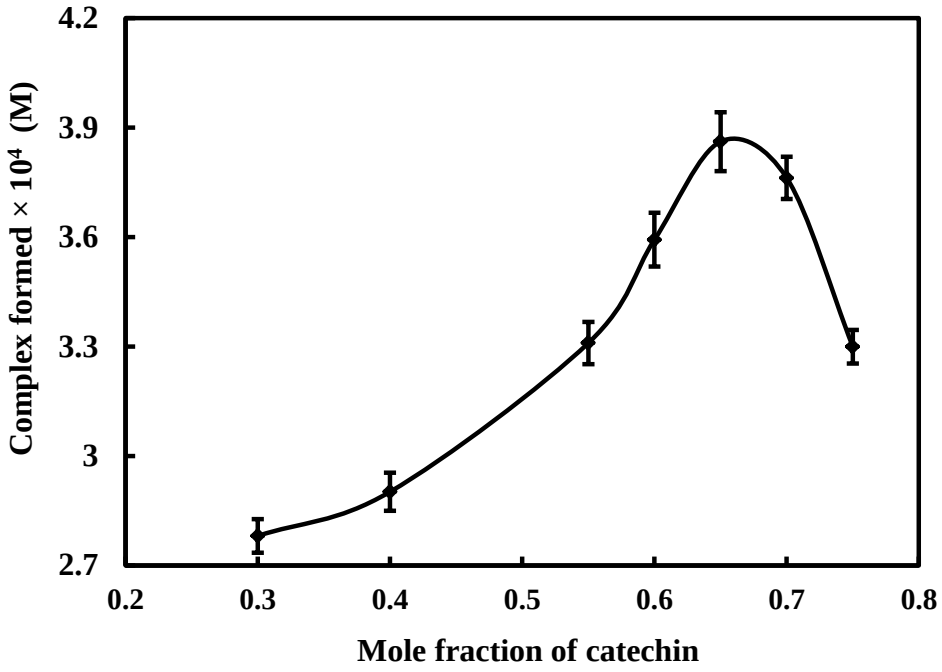


Figure 6.2: Reaction stoichiometry of complexation

6.2.4 Identification of metal-catechin complex

Metal-catechin complex was analyzed by UV-vis. spectra and the formation of complex was confirmed by XRD, FT-IR, SEM and EDX analyses. The UV-vis. spectral analyses of the retentate are shown in Figure 6.3. The absorption spectra of retentate containing complexes with Ni^{++} and Cu^{++} were maximum in between 386-391 nm, whereas the same for Al^{+++} indicates no trace of production of any complex at all. On the other hand, the absorption spectra of retentates involving Fe-catechin complex produced an interesting result. Two separate retentates were studied in this case, one produced in two phase equilibrium study and the other recovered from the stripping phase after a three phase liquid membrane transport study. Both the spectra are nearly same. Hence, lower concentration of catechin in the retentate of the stripping phase indicates the occurrence of complexation reaction, whereas the absence of any UV-vis. spectra of Fe-catechin complex in the retentate necessarily indicates conglomeration and precipitation of that complex during centrifuge.

Bark *et al*. reported similar results by UV-vis. spectral analyses of the catechin complexes with various metals [8].

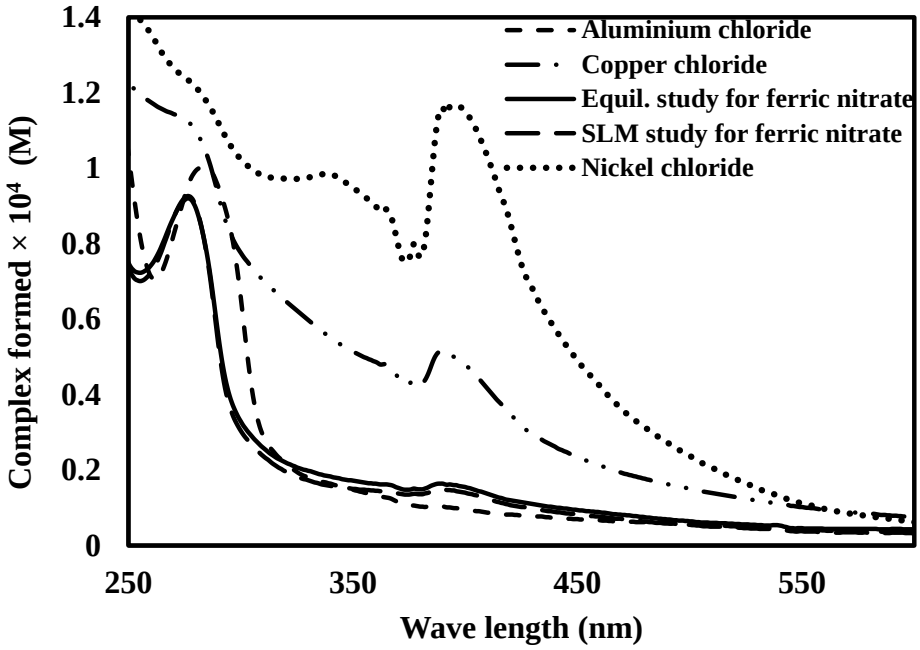


Figure 6.3: UV-vis Spectra of complex (un-precipitated) formed in strip solution ($Fe(NO_3)_3$) and in two phase equilibrium studies

The X-ray diffraction analysis of the precipitated compound with respect to that of pure catechin confirms the formation of ion-complex. Catechin being a crystalline compound has the maximum XRD peak at 2θ = 15˚ along with other major peak at 2θ = 24.84˚. On the other hand, metal-complex has non-crystalline characteristics that shows no peak in XRD analysis at 2θ in the range of 3-40˚ (Fig. 6.4). Chen *et al*. also reported the non-crystalline nature of iron-catechin complex through XRD analyses [9].

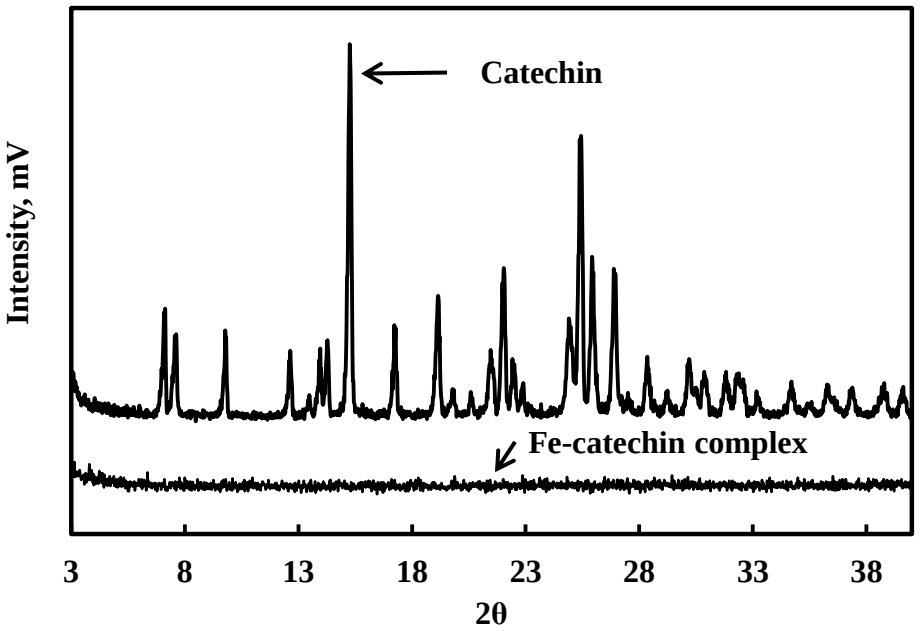


Figure 6.4: XRD analysis of complex and pure catechin

The formation of metal-catechin complex was confirmed by FT-IR spectrum analyses too. The wave numbers of FT-IR spectra of pure catechin at 970, 1030, 1143, 1282 ,1471, 1519 and 1612 cm^{-1} were assigned to C-H alkenes, -C-O alcohols, -OH aromatic, C-O alcohols, C-H alkanes, C = C aromatic ring and C = C alkenes respectively [9]. Stretching of O-H was found at 3210 cm^{-1}. The absorption bands at 1030, 1143, 1471 cm^{-1} were absent in the iron-catechin complex. The other wave numbers at 1519 and 1612 were also shifted to 1504 and 1606 cm^{-1} respectively. A new spectrum was found for Fe-O stretching at 1382 cm^{-1} and the intensity of O-H spectrum at 3210 cm^{-1} became reduced and shifted to 3170 cm^{-1} which might be due to coordination bonding of iron (Fe) with 'O' atom of –OH group (Fig. 6.5) [10].

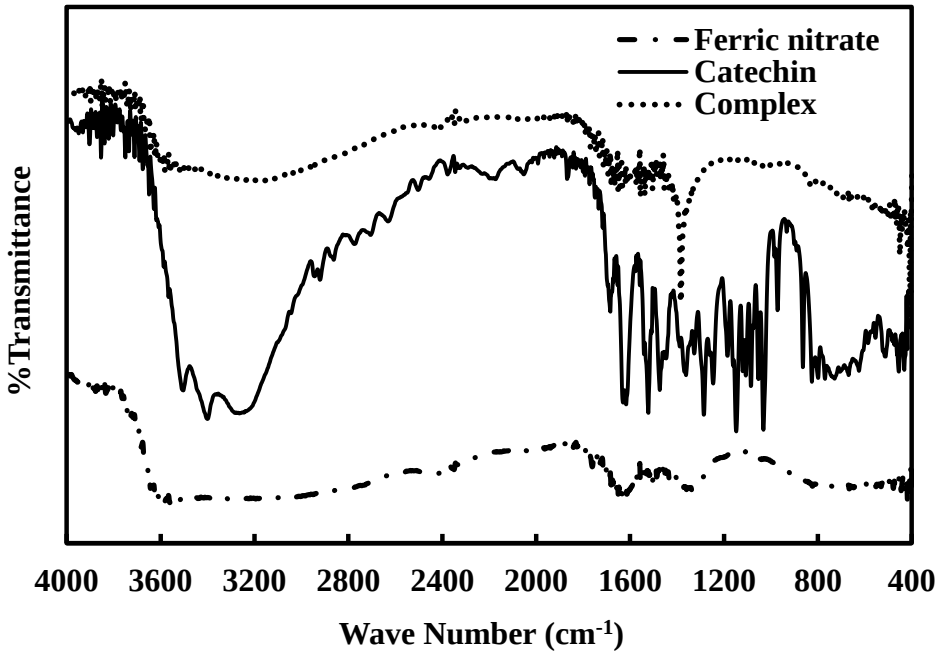
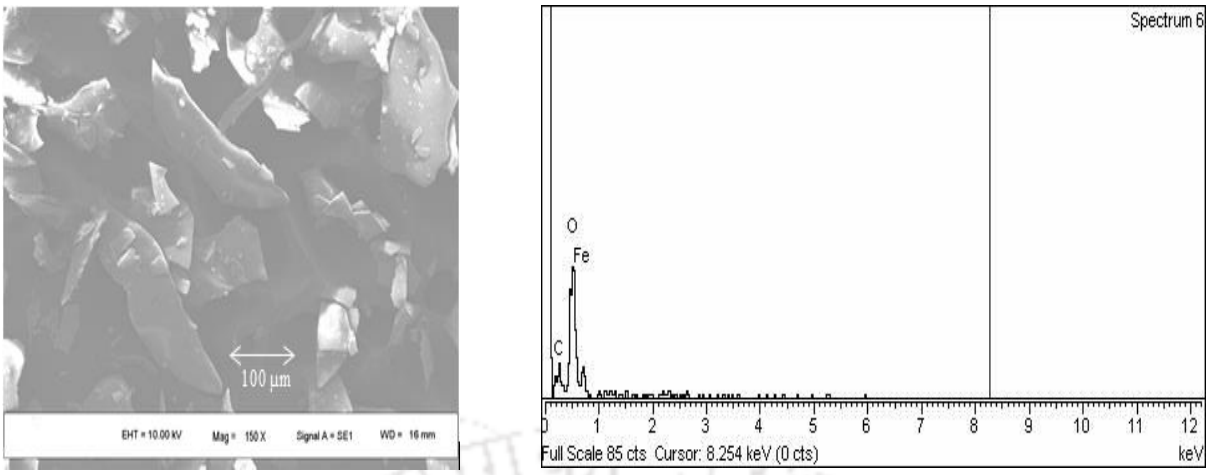


Figure 6.5: Comparison of FT-IR spectra of metal-catechin complex and pure catechin with $Fe(NO_3)_3$.

The vacuum-dried iron-catechin complex was characterized by scanning electron microscopy (SEM) combined with energy dispersive X-ray (EDX) analysis in order to characterize the morphology of surface of the complex and to examine global chemical composition of the complex, respectively. The average particle size of complex was 100 µm (Fig. 6.6 (a)). The particles were in irregular arrangements indicating the amorphous nature of the complex in accordance with the results of XRD analyses. The presence of iron (Fe) in the complex was detected in EDX spectrum (Fig. 6.6 (b)).



(a) **(b)**

Figure 6.6: (a) Vacuum dried Fe-catechin complex powder as observed under Scanning Electron Microscope (b) EDX spectra of vacuum-dried Fe-catechin complex

6.2.5 Significance of concentration of stripping solution

The precipitation of metal-catechin complex starts at its value of solubility product in the stripping solution. On the other hand, stoichiometry between metal and catechin in their complex depends upon the molar ratio of the reactants (2:1::catechin:metal) as obtained by the study of complexation reaction stoichiometry through Job plot. The ferric ion concentration in the stripping solution is important for precipitation and/or recovery due to above two reasons. In order to find the most favorable concentration of the stripping solution, it was varied from 0.0002M to 0.003 M, while the initial concentration of catechin was fixed at 0.001M. The pH of stripping solution was 1.55 at the beginning of the complexation. Both extraction and recovery increased with the increase in concentration of strip solution up to 0.001M. On further increase in concentration of strip solution slight decline in extraction and recovery was observed. This is due to the excess Fe^{+3} and nitrate ions in the stripping solution that possibly create extra resistance to diffusion of complex in the bulk of strip phase which eventually reduces the quantity of precipitation. The maximum values of extraction and

recovery were 83% and 78.4%, respectively at 0.001 M stripping solution (Fig. 6.7). Recovery includes the catechin contained both in precipitate as well as in the solution. A quantitative analysis, as shown in Table 6.2 indicates that 72% of the transported catechin precipitated in the strip side chamber and 6.4% remained in the strip solution.

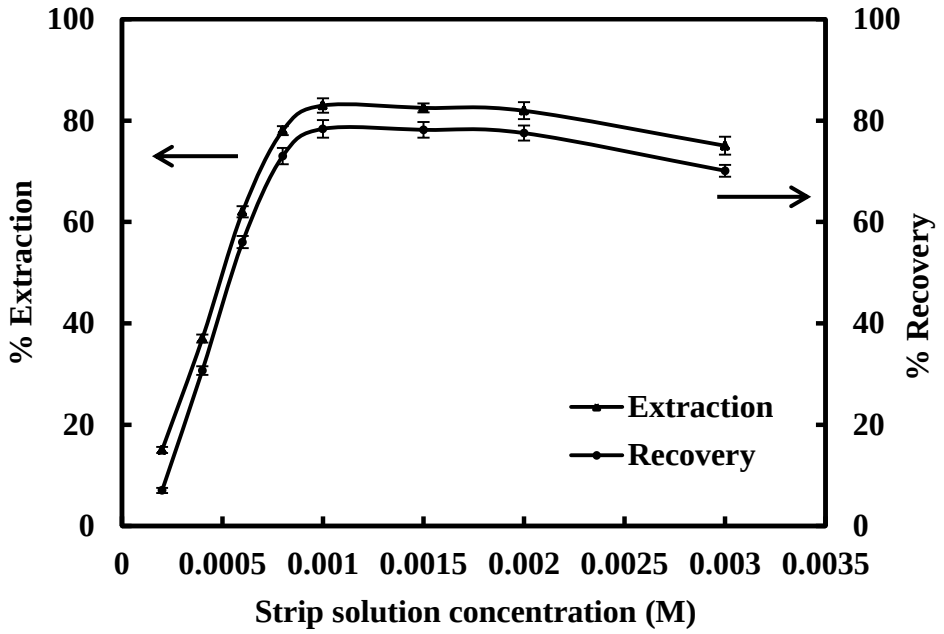


Figure 6.7: Role of concentration of metal ion (Fe^{+3}) in the strip solution *(Feed phase concentration = 0.001M, feed phase pH = 5.5, stripping solution pH = 1.55 M, stirring speed = 200 rpm, transportation time = 24 h)*

Table 6.2: Calculation of catechin content recovered through precipitation

Component	Molar/ atomic weight	One equivalent mole of precipitate (g)	Precipitate (g)
Dried precipitate	-	-	0.0309
Molar ratio of catechin to iron (Fe) in the complex = 2: 1			
Catechin	290.14	$2 \times 290.26 = 580.52$	$\frac{0.0309 \times 580.52}{580.52 + 58.845} = 0.02806$
Fe	58.57	$1 \times 58.845 = 58.845$	$\frac{0.0309 \times 58.845}{580.52 + 58.845} = 0.00284$
Catechin recovered as precipitate = 0.02806 g 130 mL of 300 ppm feed solution contains $(300 \times 130)/(1000 \times 1000) = 0.039$ catechin Catechin recovered as precipitate = $(0.02806 \times 100)/0.039 \sim 72$ % of catechin			

6.2.6 Role of pH in metal complexation of catechin

The pH is an important parameter for extraction of catechin. Source phase of catechin (feed phase) is maintained at pH of 5.5 because aqueous solution of catechin has pH of 5.5 and it is stable in the acidic range of pH. Further, the effect of pH of stripping solution on the transportation of catechin was studied in the range of 1.2 to 2.96 as the complexation reaction at the strip-side interface is pH dependent. As expected, with decrease in pH of stripping solution transportation of catechin increased. The extraction increased from 68.1% to 72%, whereas the recovery increased from 62.1% to 65.6% for the pH reduction from 2.96 to 1.92. The results have been shown in Fig. 6.8. On further decrease of pH, a sharp increase in the

transportation was observed as the complexation reaction, hence, the precipitation of complex was favored in this range of pH. Equilibrium of complexation reaction shifted to the right leading to the more catechin transportation from feed solution to strip side interface for further complexation and precipitation. Hence, overall transportation increased owing to the maximum available driving force (concentration gradient of complex across the membrane phase) to transportation. On further lowering of pH below 1.8, marginal changes were observed in both the extraction and recovery, with a maximum transportation at pH of 1.55.

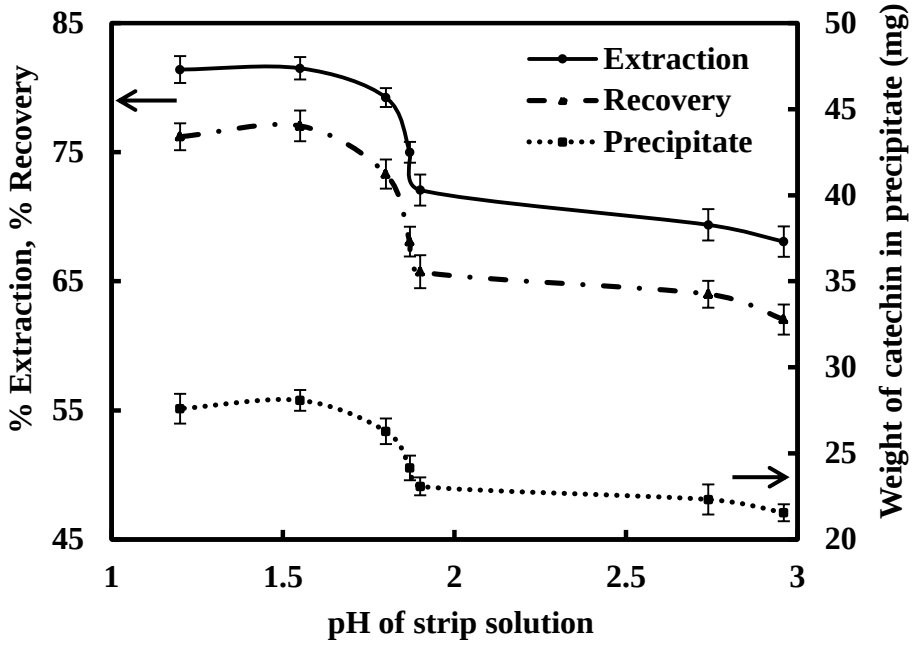


Figure 6.8: Role of pH of stripping solution *(Feed phase concentration = 0.001 M, feed phase pH = 5.5, stripping solution concentration = 0.001 M, stirring speed = 200 rpm, transportation time = 24 h)*

6.2.7 Significance of initial concentration of feed

The effect of initial concentration of catechin in the feed solution had to be investigated with varied concentration of strip solution. Because the molar ratio of concentrations of feed and

strip solution is more contributory to the rate of transportation of catechin rather than the concentration of catechin in the feed solution only. Based on a concentration of feed at 0.001 M, three different concentrations of strip phase were chosen *viz*. 0.002 M (Case I: twice the feed conc.), 0.001 M (Case II: same as the feed conc.) and 0.0005 M (Case III: half of the feed conc.). A series of experiments were carried out with varying initial concentrations of feed phase while keeping the concentrations of strip phase fixed at either of the above three cases. Each experiment was run for 24 hours. The overall transportation was calculated on the basis of both the precipitated and un-precipitated complex in the stripping solution. For Case II, transportation of catechin decreased from 88% to 78% rather slowly with increase of concentration of feed from 0.000172 M to 0.001 M. Further increase of concentration of feed resulted in rapid decline in percentage transportation (Fig. 6.9). It may be argued that for feed concentration below 0.001 M, the interfacial area of the membrane (11.3×10^{-4} m^2) is the only limiting factor that had influence on transportation of solute and this factor does not change even with increase in feed concentration. However, for the feed concentration above 0.001 M, the concentration of strip, which is rather insufficient for facilitating higher percentage transportation of solute, also contributed to lowering its percentage value. Cases I and III demonstrate similar trends where the 'breaking points' of concentration of feed were 0.0012 M and 0.0007 M for cases I and III, respectively. Hence, for the existing SLM set up with its available interfacial area for transportation and stripping solution concentration of 0.001 M, the initial concentration of feed was fixed at 0.001 M in all other experiments.

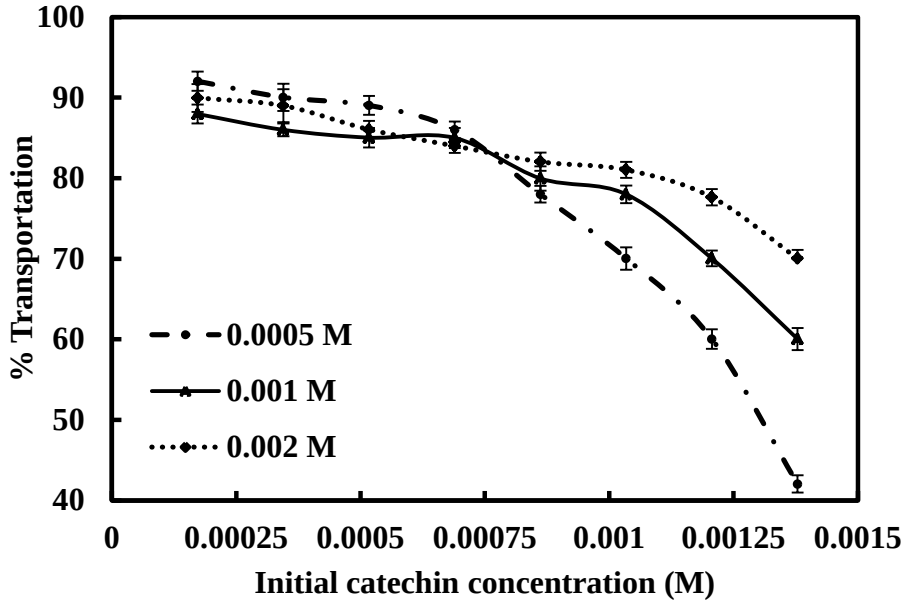


Figure 6.9: Role of initial concentration of catechin *(Feed phase pH = 5.5, stripping solution pH = 1.55)*

6.2.8 Kinetic behavior of the transportation

Kinetics of the transportation has been studied in order to investigate the rate controlling step of the whole process. The process of formation of solute-carrier complex is assumed to be instantaneous, so is the formation of complex between catechin and Fe^{+3}. Hence, diffusion of the solute-carrier complex from feed-membrane interface to the membrane-strip interface is the rate limiting process. According to our previous study of catechin recovery through FS-SLM in Chapter-IV, PVDF support membrane performed better for the case of synthetic catechin among various other polymeric supports like polytetrafluoroethylene (PTFE), Nylon 6, and Polyethersulphone (PES) [1]. So, experiments were carried out using a support made of PVDF. Flux of transportation of solute across the membrane surface (area 11.3×10^{-4} m^2) was measured and the result has been reported in the Fig. 6.10. The flux increased very fast up to 2 h due to presence of maximum number of carrier molecules at the interface. Marginal

increase of flux occurred from 2 to 5 h and the steady flux was obtained at ~34.5 × 10^{-8} kg.m^{-2}.s^{-1} and sustained for another 8 h. Flux started decreasing after 13 h due to the decreasing concentration of solute. In our previous study of transportation of catechin with ethanol as the stripping agent, the maximum flux was obtained at 21.0 × 10^{-8} kg.m^{-2}.s^{-1} with same interfacial area (same type and specification of support material) of transport [1]. In the present case, the maximum flux is nearly 1.8 times higher than that in previous case. This is due to the complexation reaction and subsequent precipitation that resulted in higher driving force (concentration gradient) for transportation in the stripping phase.

6.2.9 Case study with real extract

The efficacy of simultaneous transportation and complexation of catechin and the optimum operating conditions of that process can be substantiated when it is applicable with or without some modifications in recovering catechins from real extract of tea leaves rather than its synthetic counterpart. The procedure for extracting aqueous solution of catechins from raw tea leaves has been described in experimental procedures. The real extracts of tea leaves were undergone the same treatment procedure for transportation and complexation of catechins at the optimum operating conditions.

6.2.9.1 Transportation and complexation of catechin extracted from green tea leaves

The real extract of green tea leaves was undergone the same experimentation using FS-SLM technique described so far. The concentration of feed was 0.001 M and its pH was 5.5. All other operating conditions were maintained at their optimum values (*e.g.* stirring speed of 200 rpm and transportation time 24 h) as found with synthetic solution of pure catechin. Polytetrafluoroethylene (PTFE) and polyvinylidene fluoride (PVDF) membranes were the two most effective support materials for recovery of catechins from real extract as obtained in Chapter-IV and reported elsewhere [1]. The comparison of performance of two supports has

been illustrated in Fig. 6.10 where individual fluxes for various catechins are combinded and reported as "aggregate flux" of all catechins. The maximum fluxes were estimated as 32.0 $\times 10^{-8}$ kg.m^{-2}.s^{-1} and 32.5 $\times$ 10^{-8} kg.m^{-2}.s^{-1} for PTFE and PVDF supports, respectively at 8 h. They are comparable with each other, albeit 6.15% less than the flux (34.5 $\times$ 10^{-8} kg.m^{-2}.s^{-1}) observed in case of synthetic solution of pure catechin. The recovery of catechins from real extract was calculated to be 70% as opposed to 77% for synthetic solution of catechin. With time the flux declines more rapidly for PVDF in comparison with that of PTFE and the same trend was observed in case of recovery of real extract by ethanol (Section 4.1.5.2 of Chapter-IV) [1]. The PTFE membrane was less prone to be stuck with colour substances, tannins and other impurities in its pores and the problem of clogging was found less compared to that of PVDF. Hence, the PTFE membrane support was found to be a better choice as a support material for the case study of transportation, complexation and recovery of Fe-catechin compounds from extract of tea leaves using FS-SLM technique.

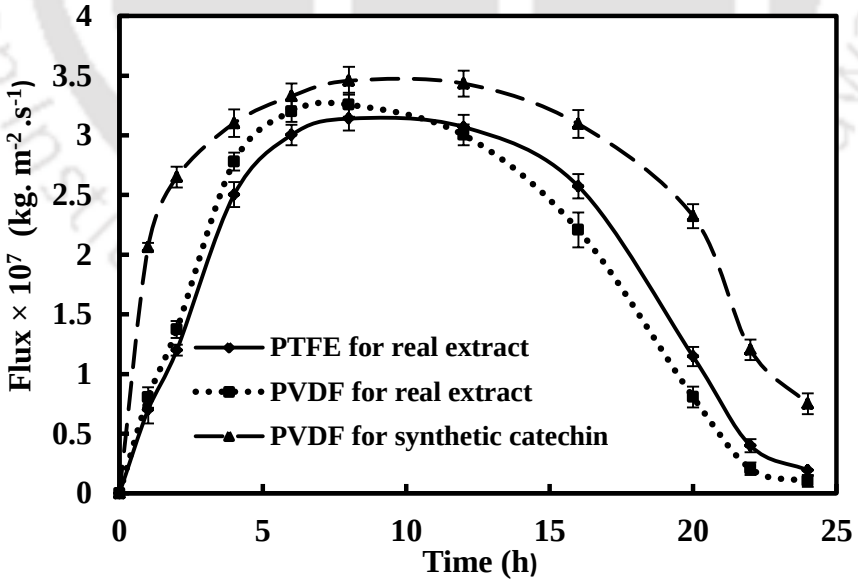


Figure 6.10: Comparison of fluxes between synthetic catechin and catechins from real extract (*feed concentration = 0.001 M and phase pH = 5.5, stripping solution concentration*

= 0.001 M, stripping solution pH = 1.55, stirring speed = 200 rpm, transportation time = 24 h)

6.3 Summary of the Fe-catechin complexation in SLM

The following are the salient points observed in this study

- The production of the enriched metal complex of bioactive catechins having medicinal importance through enhanced selective separation has been explored successfully.
- SLM based transport technique has been found to be an efficient means for metal (Fe) complexation of synthesized catechin prior to its precipitation and recovery.
- Catechin and its derivatives from tea leaves extract were also investigated for the purpose of complexation.
- The same membrane phase (TBP in *n*-decane) immobilized into micro-pores of polymeric PVDF and PTFE membranes have been found to be applicable for catechins recovery through iron complexation.
- Ferric nitrate, a catechin complexing agent has been utilized in stripping solution for complexation of catechin at the strip side interface.
- The optimum conditions for transportation studies have been found to be feed solution pH of 5.5, carrier concentration in the membrane phase of 1.2 M, stripping solution concentration of 0.001 M, stirring speed of 200 rpm and initial feed concentration of 0.001 M.
- The feed phase must be maintained in acidic pH range to ensure the catechin in its molecular *i.e.* un-ionized state to be extracted by a neutral carrier in *n*-decane.

- The extracted catechin was precipitated as complex in the stripping solution and recovered from there.
- The developed technique is quite applicable to the recovery of catechin compounds from hot water (60˚C) extracts of tea leaves without serious problems except the adsorption of the colored substances, tannins to the pore surface of the support material.
- The solution to this problem is a forthcoming challenge that will open wide the research prospect in separation/purification, recovery as well as the metal complexation of bioactive compounds from various medicinal plants.

Abbreviation

CPMAS 13C-NMR	cross-polarization magic angle spinning carbon-13 nuclear magnetic resonance
EDX	electron dispersive x-ray
(-)ECG	epicatechin gallate
(-)EGC	epigallocatechin
(-)EGCG	epigallocatechin gallate
FT-IR	fourier transform infrared
ML	membrane liquid
PTFE	polytetrafluoroethylene
PVDF	polyvinylidenefluoride
SLM	supported liquid membrane
SEM	scanning electron microscopy

TBP	tributyl phosphate
UV-vis	ultraviolet-visible
XRD	x-ray diffraction

Nomenclature

$[CatOH.2TBP]_{i,f}$	catechin-TBP complex at feed side interface
$[CatOH.2TBP]_{i,r}$	catechin-TBP complex at strip side interface
J_{org}	flux of catechin through membrane phase
Δ_{org}	resistance to the diffusion of complex
$X_{\max}$	mole fraction of catechin at maximum complexation
X	mole fraction of catechin
n	number of moles of catechin bonded with one metal atom

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CHAPTER-VII

Detailed Stability Analysis

CHAPTER-VII

Detailed Stability Analysis

An extensive experimentation on the stability of the SLMs was presented in this chapter. Various reasons for the instability of the SLMs were considered and the related parameters were experimentally investigated. The experiments were accomplished in the simplest configuration of SLM i.e. in FS-SLM. An electrolyte (NaCl) was added to the aqueous phases to increase the interfacial tension between membrane phase and any of the two aqueous phases. Surfactant with appropriate Hydrophilic-Lipophilic Balance (HLB) was added to the membrane phase to increase its hydrophobicity that resulted in lesser loss of membrane liquid (ML) from the pores of the support membrane into the aqueous phases. Various combinations of aqueous/organic phases and characterization of emulsion droplets formed thereby were analyzed, and that yielded the explanation of reduction of loss of ML. Minimum critical displacement pressures for the ML to come out of the pores of the support membrane were evaluated experimentally for various support materials based on their physical properties such as pore diameter, thickness, tortuosity and also the irregularity of the shapes of the pores.

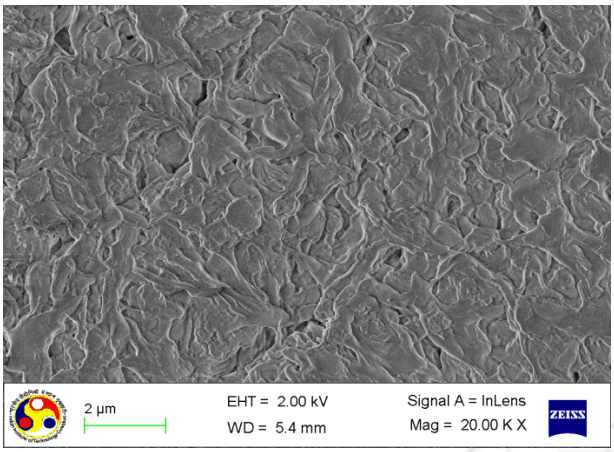
7.1 Procedures of transportation of catechin

All the stability criteria were checked and analysed in the process of permeation of catechin through FS-SLM. PVDF membrane was used in all transportation studies if not mentioned specifically. Catechin was transported from the feed solution of 100 $mg.L^{-1}$ catechin to the stripping solution of 0.4 M ethanol through the ML immobilized into the pores of size 0.2 μm. The permeation cell and the procedures of transportation were described in detail in Chapter-II.

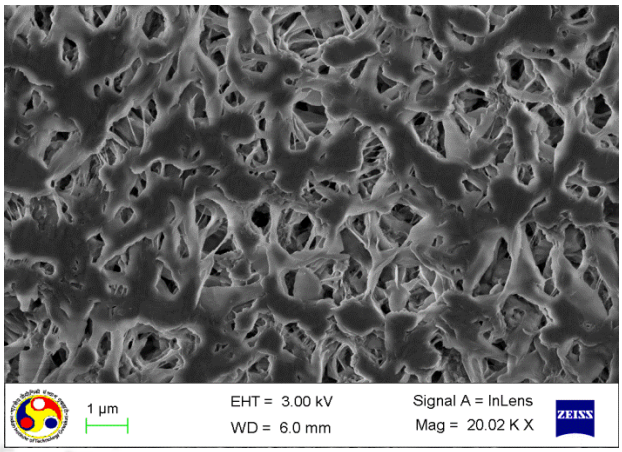
The procedure was repeated 5 times with the same LM (ML in to the pores of support membrane) but with fresh feed and strip solutions to check the extent of stability of the FS-SLM. All the experiments were acomplished thrice and the avearge values have been reported. Results have been shown with the help of standard error bars.

7.2 Results and discussions

The stability of the LM was studied in terms of the catechin transport efficiency for a period of 120 h in continuous run mode under the optimum conditions without re-impregnation of the ML in the support pores. The efficiency of the transport was measured either in terms of percentage transportation (% recovery) or that of flux of the catechin depending on the situation. As discussed before, the ML held in the pores of support comes out of the pores due to various reasons such as its solubility in the aqueous phases, emulsion formation at the interfaces, osmotic pressure differential across the interfaces, hydrodynamic forces *etc.* The FE-SEM analysis of the support material with ML in the pores after 24 h and after 120 h has been illustrated in Fig. 7.1 (a-b). Due to the loss of ML, the performance of the SLM deteriorates and the rate of catechin transportation decreases which is manifested in the catechin recovery as well as in the flux. The catechin recovery was measured after each 24 h whereas the values of fluxes of the 5^{th} run only were reported for estimation of stability of FS-SLMs. Depleted source and enriched strip solutions were replaced with fresh ones after each 24 h. Detailed analyses of the physico-chemical parameters that influence the stability of FS-SLM have been studied in the flowing sub-sections:



(a) Support with ML in the pores after 24 h run



(b) Support with less amount of ML after 120 h run

Figure 7.1(a-b): FE-SEM analysis of the support containing ML inside the pores after two different run times

7.2.1 Physical properties of ML

The ML was selected based on solute extracting capacity of carrier and the solvent-carrier compatibility based on their physico-chemical properties. Takahashi *et al.* [1] studied the stability of SLMs with various kinds of solvents as diluent and reported that the hydrocarbons with longer carbon chain are favorable. They reported that with solvents of same length of carbon chain, the stability is maximum for *n*-aliphatic followed by branched aliphatic, naphthenic and aromatic. Hence, two aliphatic solvents of straight chain with same length of carbon chain, *viz*. *n*-decane and *n*-decanol were used in the experimentation. On the other hand, carrier tributyl phosphate (TBP) has good extracting capacity of catechin and it was used in our previous work for LM based transportation of catechin in two different configurations *i.e.* bulk liquid membrane (BLM) and SLM [2, 3]. The amount of carrier (TBP) was varied for each solvent and thereby different compositions of ML were produced. The procedure of catechin transportation has been described in Section 2.3 of Chapter-II. Five runs of experiments were

carried out with same membrane but with fresh feed and strip solutions in each run. The stability was measured in terms of the maximum flux achieved in 5^{th} run. Table 7.1 shows the maximum flux in fifth run and the corresponding physical parameters related to stability of FS-SLM. The values of fluxes of catechin were better for every concentration of carrier in case of *n*-decane compared to that of *n*-decanol due to lower viscosity of ML comprising *n*-decane. The effective diffusivity of a solute-carrier complex is inversely proportional to its viscosity according to Stokes Einstein equation (Eqn. 1.1 of Chapter-I) [4] and it is supported by the experimental results. The maximum flux (16.34×10^{-8} $kg.m^{-2}.s^{-1}$) in 5^{th} run is 16% less compared to that in 1^{st} run where the flux is 19.45×10^{-8} $kg.m^{-2}.s^{-1}$ (Data is not shown) The values of interfacial tension were comparable but marginally greater for *n*-decane. The greater interfacial tension provides the greater stability and also contributes in yielding higher flux. Density of the ML plays a role too. If the density of ML is much different from the density of aqueous phases, then a buoyancy force affects the stability of the ML in the pores of the support. The density of the ML with *n*-decanol (872.6 $kg.m^{-3}$) was closer to the density of aqueous phases (1000 $kg.m^{-3}$) compared to that with *n*-decane (density = 802.8 $kg.m^{-3}$). So, the stability of LM should be higher for *n*-decanol compared to that for *n*-decane. However, the effect of lower viscosity and higher interfacial tension are dominant and the flux was better for *n*-decane as solvent. Hence, further stability analyses were accomplished with *n*-decane as the solvent. The change of viscosity with the TBP concentration in the ML and the effect of this on the transportation of the catechin were investigated. Solvent *n*-decane and carrier TBP have the viscosity of 0.92 mPa.s and 3.8 mPa.s respectively. So, the viscosity of ML increases with increasing concentration of TBP in ML (Fig. 7.2). Adding carrier in the ML has two opposite effects. With increasing TBP concentration in ML, percentage of transportation of solute increases up to 1.2 M TBP which is saturation

concentration of TBP in membrane phase [2], but the flux decreases as the viscosity increases. Hence, 1.2 M TBP was used in the subsequent experimentations. With increasing TBP concentration in ML, the mutual solubility of organic–aqueous phases may increase. The water content of ML was checked by the Karl Fisher Titration experiments described in Section 7.2.2.

Table 7.1: Physical properties of membrane liquid (ML) and corresponding flux

Solvent	TBP in LM (M)	Viscosity (mPa.s)	Interfacial tension (mN/m)	Density ($kg.m^{-3}$)	Maximum flux in 5th run $\times 10^8$ ($kg.m^{-2}.s^{-1}$)
n-decanol	0.75	8.08	9.3	858.3	2.12
	1.2	6.04	9.8	872.6	5.6
	1.5	4.81	10.1	886.9	3.4
n-decane	0.75	1.01	12.6	778.5	13.2
	1.2	1.20	11.8	802.8	16.34
	1.5	1.253	10.7	827.0	15.89

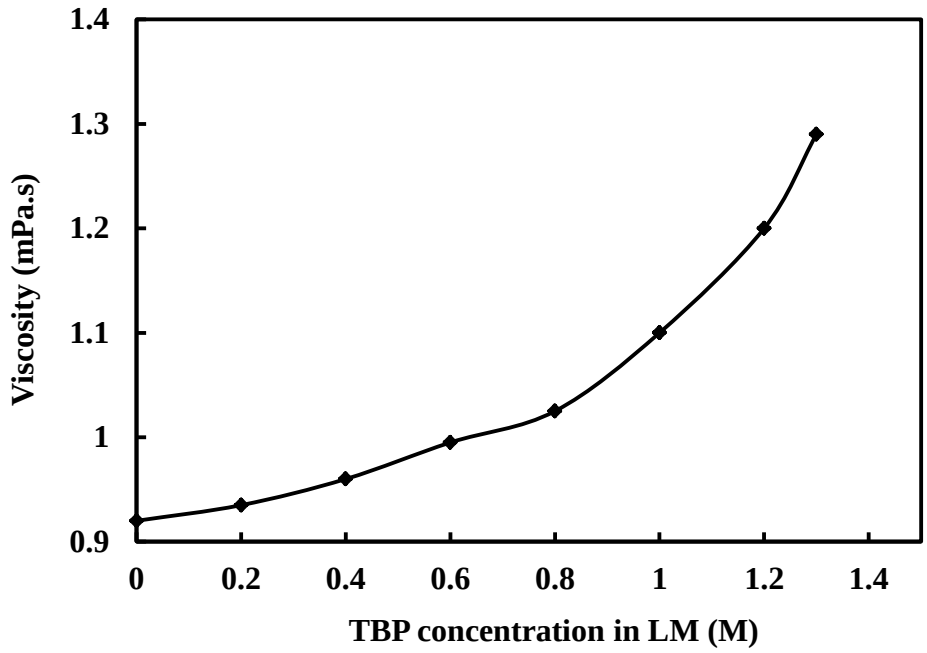


Figure 7.2: Change of viscosity of the LM with TBP concentration in it

7.2.2 Mutual solubility of phases

The mutual solubility of the membrane phase and aqueous phases affects the stability of ML in the pores of the support [5]. It was checked by two phase equilibrium studies. Various combinations of MLs and aqueous phases (either feed or strip) of volume 10 mL each, were equilibrated in Erlenmeyer flasks under the stirring speed of 200 rpm for 5 days. After the equilibrium was reached the two phases were separated by settling. The water contents dissolved in the ML were examined by Karl Fisher Titration method (787 KF Titrino, Metrohm) and the results are reported in Table 7.2. Solubility of water in the membrane phase with 1.2 M TBP were found to be 0.0012% and 0.002% when they were equilibrated with feed (100 $mg.L^{-1}$ catechin) and strip (0.4M ethanol) phases respectively. The solvent *n*-decane is insoluble in water and solubility of TBP in water is 160 ppm *i.e* 0.016%. With rigorous stirring, both the

aqueous phases (260 mL) can dissolute maximum 0.0416 mg TBP. The amount of ML used in the experiment was 55.4 mg and the amount of TBP in this ML was 22.04 mg. Hence, only 0.186% of TBP can be lost into the aqueous phases through dissolution under rigorous stirring condition. However, in practice the extent of dissolution may be much less than this value, because the exposed area of ML inside the pores to the aqueous phases is very small and the stirring in actual FS-SLM studies was not very rigorous. The details regarding the loss of ML from the pores of the support have been provided in Section 7.2.9. Nevertheless, this mutual solubility of the two phases can further be minimized by use of electrolyte in the aqueous phases and surfactant in ML.

Table 7.2: Mutual solubility of the organic-aqueous phases in various concentration of TBP

TBP in LM (M)	Solubility of water in ML(in %) with 100 mg.L^{-1} catechin in feed phase	Solubility of water in ML (in %) with 0.4 M ethanol in strip phase
0. 2	0	0
0.5	0	0.0007
0.8	0.0006	0.001
1.2	0.0012	0.002
1.4	0.0016	0.0025

7.2.3 Role of electrolyte in aqueous phases

As discussed before, addition of electrolyte in the aqueous phases reduces solubility of membrane phase in aqueous phases that consequently yields better stability of SLM. This fact is studied by adding NaCl (electrolyte) in aqueous phases. Initial studies were conducted to find the

effect of electrolyte on the SLM stability and the stability was measured in terms of % transportation *i.e*. % recovery. NaCl of concentration 0.5 *M* yields better result than 0.1 *M* NaCl (data not shown). Five runs, each of 24 hours, were performed with the same membrane (without re-impregnation) while feed and strip phases were renewed with fresh feed and strip solutions after each run. The experimental results (Fig. 7.3 and 7.4) clearly indicate that the membrane stability in terms of % catechin transportation was the best when NaCl was added to both the aqueous phases. The % transportation of catechin reduced to 38 % in 5^{th} run from 49 % after the 1^{st} run. About 6 % reduction in recovery was observed after 48 h and then it gradually increases up to 11 % at the end of 120 h. It can be shown from the Fig. 7.3 that the ML loss is more in the first 24 h due to initial adjustment of the interfaces and also for solubility effect (even it is very less) of membrane phase in the aqueous phases. The loss of ML gradually declines with time.

On the other hand, when no NaCl was added in either of the aqueous phases, the % transportation reduced to 18 % after 5^{th} run. The result also reveals that the addition of NaCl only to stripping phase yields better stability (34 % transportation) compared to when it was added to the feed phase only (28% transportation), because ML is more soluble in the stripping phase as ethanol (0.4 *M*) is more non-polar compared to pure water. The above experimental results can also be explained by the theory of surface tension gradient for the SLM instability proposed by Zha *et al.* [6]. The presence of electrolyte in aqueous phases enhances the interfacial tension between membrane phase and the aqueous phases along with the reduction of solubility of ML in aqueous phases and hence, the possibility of emulsion formation decreases. The results above further suggests that the presence of electrolyte in strip phase possibly has more positive effect on membrane stability as compared to that in feed phase and the SLM system becomes more of neutral surface tension gradient which is less prone to instability of SLMs [6].

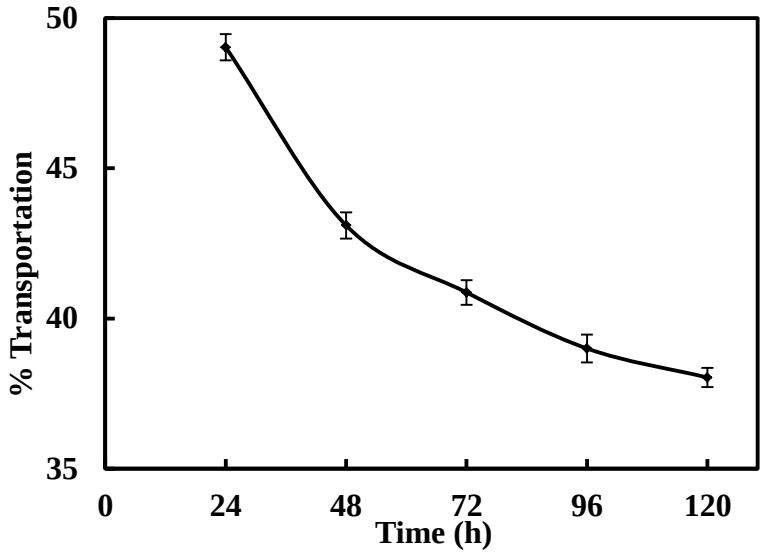


Figure 7.3: Long run stability behavior of SLM system as % transportation of catechin with time *(feed conc. = 100 mg L^{-1}, membrane phase=1.2 M TBP in n-decane, strip phase=0.4 M ethanol, stirring speed = 200 rpm, temperature = 25˚C, run time = 24 h)*

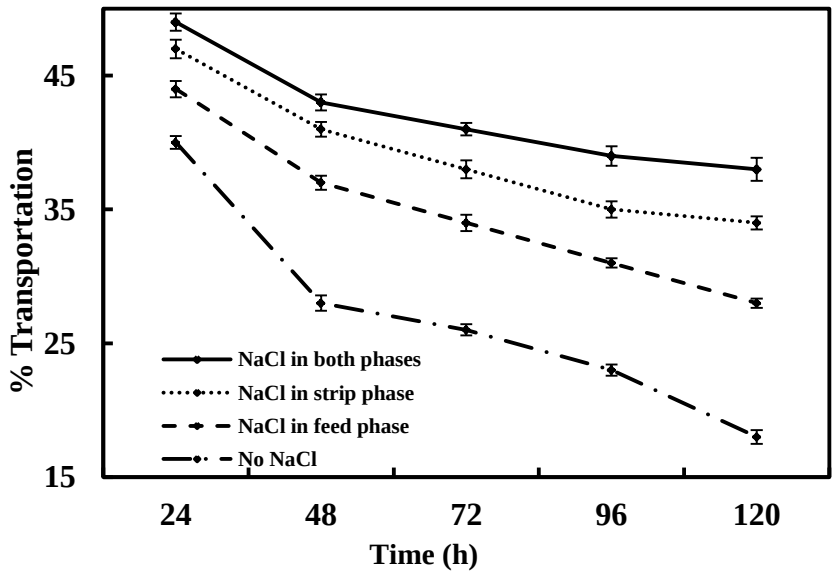


Figure 7.4: Role of electrolyte on membrane stability *(feed conc. =100 mg.L^{-1}, membrane phase=1.2 M TBP in n-decane, strip phase=0.4 M ethanol, stirring speed = 200 rpm, temperature = 25˚C, run time = 24 h)*

The reasons for the improved stability of SLM were investigated further in detail by finding the interfacial tension in various situations. Presence of electrolyte in the aqueous phases increases the interfacial tension between membrane phase and aqueous phases. Increasing interfacial tension (γ) increases the stability of the LM (vide the Eqn. 1.4). Various amounts of electrolyte (NaCl) were added both in feed and stripping phases in order to assess its role in increasing the stability of LM. Table 7.3 and Fig. 7.5 demonstrate the detail of the effect of NaCl in the aqueous phases with values of the interfacial tension between aqueous and organic phases against varying concentrations of NaCl. Interfacial tension increases moderately with increase in concentration of NaCl up to 0.5 M NaCl, and slowly beyond 0.5 M NaCl. The increase was only 20% (from 10.2 to11.8 mN/m) for feed phase and 22.02% (from10.4 to12.2 mN.m^{-1}) for strip phase. The flux of catechin was found to be maximum (18.06 × 10^{-8} kg.m^{-2}.s^{-1}) when 0.5 M NaCl was added in both the aqueous phases. Researchers have reported that one of the reasons for instability of SLM is the gradient of surface tension between feed and stripping phases and the SLM is more stable when this gradient is zero [6]. With 0.8 M NaCl in feed phase the interfacial tension of the ML with the feed solution is 11.7 mN.m^{-1}. The same value of interfacial tension between ML and the strip phase is obtained when 0.4 M NaCl is added in it. In other words, 0.8 M NaCl in feed phase and 0.4 M NaCl in the strip phase provide the zero gradient of surface tension in the system which ensures the best stability. Hence, the subsequent experiments were carried out with feed and strip phases containing 0.8 M NaCl in feed phase and 0.4 M NaCl in the strip phase respectively and the resulting flux of catechin was found to be 20.05 × 10^{-8} kg.m^{-2}.s^{-1}. This result

can also be explained as the zero gradient of osmotic pressure which provides the best stability of ML in the pores.

Table 7.3: Effect of NaCl in aqueous phases on flux of catechin

NaCl in feed phase [M]	Interfacial tension (γ) (mN/m)	NaCl in strip phase [M]	Interfacial tension (γ) (mN/m)	Maximum flux in 5th run × 10^8 ($kg.m^{-2}.s^{-1}$)
0.0	10.2	0.0	10.4	16.34
0.2	10.9	0.2	11.2	16.81
0.4	11.3	0.4	11.7	17.5
0.5	11.5	0.5	11.9	18.05
0.6	11.6	0.6	12.0	18.64
0.8	11.7	0.8	12.1	18.72
1.0	11.8	1.0	12.2	18.61
0.8	11.7	0.4	11.7	20.05

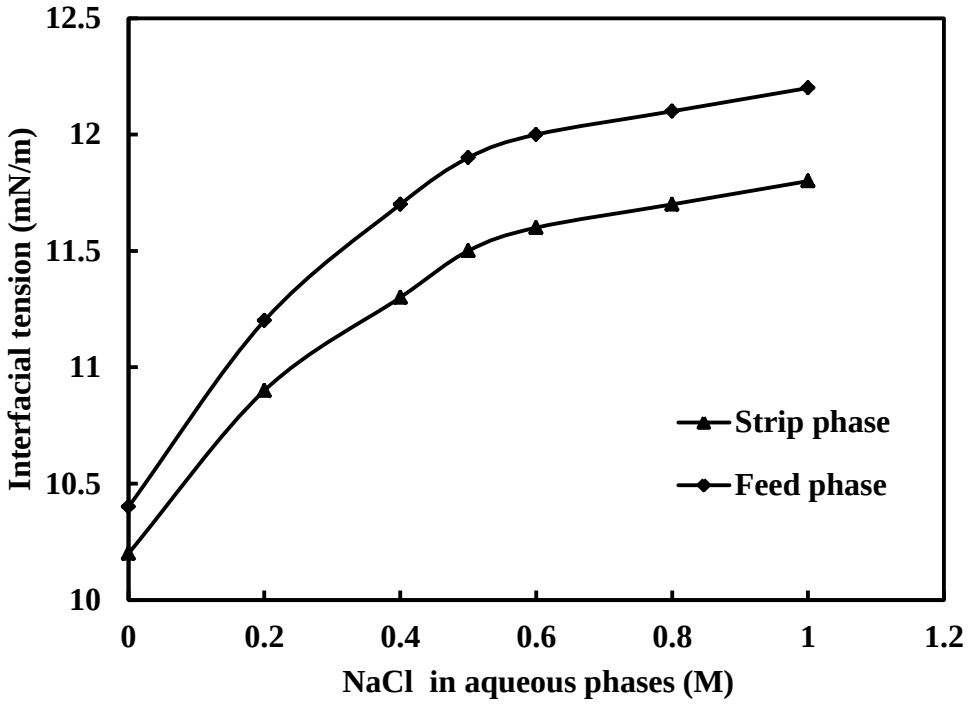


Figure 7.5: Interfacial tension between membrane phase and any of the aqueous phases containing various concentration of electrolyte (NaCl)

7.2.4 Role of surfactant

Various researchers reported the role of surfactants in the stability of SLM which depends on the hydrophilic-lipophilic balance (HLB) of the surfactants. Presence of surfactants with lower HLB values (3-5) in the ML increases its hydrophobicity [7]. The interfacial tension between ML and the aqueous phases increases with addition of suitable surfactant. As a result the stability of membrane phase inside the pores increases. On the other hand, the surfactants with higher HLB values (8-15) are not favorable for this purpose. In this work, 3 surfactants of different HLB values were considered for experimentation. In each run, one of them was mixed with ML for transportation of catechin. The flux was measured as described in Section 7.1. The maximum

flux, at the end of the 5th run, was measured and reported in Table 7.4. The flux was maximum (22.05×10^{-8} kg.m^{-}.s^{-1}) when 0.2 % (w/w) span 60 was used. Span 65 with HLB value of 2.1 was too hydrophobic to maintain the organic-aqueous interface for the transportation of catechin and as a result the flux was less. The flux of 22.05×10^{-8} kg.m^{-}.s^{-1} is certainly higher as compared to 20.05×10^{-8} kg.m^{-}.s^{-1} when the experiment was performed without any surfactant (Table 7.3). The surfactants with lower HLB value (3-5) provide the best stability as they form water in oil (W/O) emulsion and minimize the loss of ML. The surfactant with higher HLB value tends to form oil in water (O/W) emulsion and facilitates loss of ML, and the loss is even higher than the case when there was no surfactant in the ML.

Table 7.4: Role of surfactant on SLM stability

Surfactant in membrane 0.2% (w/w)	HLB value of surfactant	Viscosity (mPa.s)	Interfacial tension (mN/m)	Maximum flux at 5th run $\times 10^{8}$ (kg.m^{-2}.s^{-1})
None	-	1.20	11.8	16.34
Span 65	2.1	1.22	12.6	17.82
Span 60	4.7	1.204	12.3	22.05
Tween 80	15.0	1.23	0.6	Not measured

7.2.5 Characterization and stability of emulsions

One of the reasons of instability of the SLM is the formation of emulsion between the ML and aqueous phases. The probability of emulsification was studied in three conditions of the phases. The effect of emulsification in SLMs can be studied through the characterization of the emulsion droplets by their size, numbers and determination of stability as a function of process and

product variables prevailing in a particular SLM system [8]. Emulsions of ML in aqueous solutions were prepared by the ultrasonic agitation and the size distribution of droplets was measured in Delsa nano particle size analyzer. In order to obtain a precise measurement, the job was done immediately after agitation (emulsification) has been stopped. The solubility of ML was found more in strip phase as compared to that in feed phase, as observed in Section 7.2.2. Hence, the strip phase was selected as the aqueous phase for formation of emulsion. The volume (0.2 mL) of ML in all samples of aqueous phases, the time of emulsification and the power of ultrasonic agitation were kept constant. A comparison of the size distribution of droplets, at different conditions of organic/aqueous phases, provides a quantitative measure of dispersion of emulsions. Hence, the proper selection of aqueous/organic phase can be achieved and the factors applicable for reducing emulsification and increasing stability of membrane can be realized.

ML initially remains in the support pores (State I). During operation, if it comes to the aqueous phases (State II), the free energy change during formation of emulsion can be predicted as [7]:

$$\Delta G = (U_a^{II} + U_o^{II}) - (U_a^I + U_o^I) + \gamma_{ao}(A_o^{II} - A_o^I) - \gamma_{os}A_C^I - TS_o^{II} \quad (7.1)$$

where superscripts I and II represents states I and II, the subscripts *a* and *o*, represent the aqueous and organic phases respectively and γ the interfacial tensions between corresponding phases. *U* is the total bulk internal energy for respective states and phases. *A* is the interfacial area between the phases. T and S are the absolute temperature and the configurational entropy of the oil droplets formed, respectively. With various assumptions and simplification the Eqn. 7.1 can be resulted as [7]:

$$\Delta G = \gamma_{OS}(n\pi d^2) - \frac{4\varepsilon\delta A_m}{d_p}\gamma_{OS} + nkT\left[ln\phi_o + \left(\frac{1-\phi_o}{\phi_o}\right)\ln(1-\phi_o)\right] \quad (7.2)$$

Where n, ε, δ, A_m, k, d_p and ϕ_o are number of pores, porosity of support, thickness of support, interfacial area, the Boltzmann constant, pore diameter and the volume fraction of the membrane liquid in the aqueous solution, respectively. The first term of right hand side of Eqn. (7.2) is normally greater than the last two terms in absolute value unless the interfacial tension γ_{os} is very small, and therefore ΔG is mostly positive. Hence the emulsification in the aqueous phases is not a spontaneous process. The minimum external energy (equal to ΔG), which is required for the emulsification to take place, must be supplied to the system. Hence, the problem of emulsification can be avoided (or at least minimized) by minimizing the supply of energy from agitation (stirring and/or streams flow). On the other hand, minimum stirring is necessary for reduction of concentration polarization of solute (or solute-carrier complex) and thickness of laminar sub-layer at the interfaces. Hence, the stirring speed has been optimized as described in Section 3.2.6. The size of the droplets in emulsion was found to be largest when 0.2% (w/w) span 60 was added in ML and 0.4M NaCl was added in strip phase (Fig. 7.6). The stability of the emulsion decreases with increasing size of droplets and lower the stability of emulsion, higher is the stability of the SLM. Hence, best stability of SLM can be achieved when 0.4 M NaCl and 0.2% (w/w) span 60 were added in strip phase and ML, respectively. The advantage of addition of surfactant can be best realized by the reduction in loss of ML. The losses of ML from the pores of the supports used in the transportation process after 5 runs were calculated with best conditions of stability of SLM, as described above. The details have been reported in section 7.2.9.

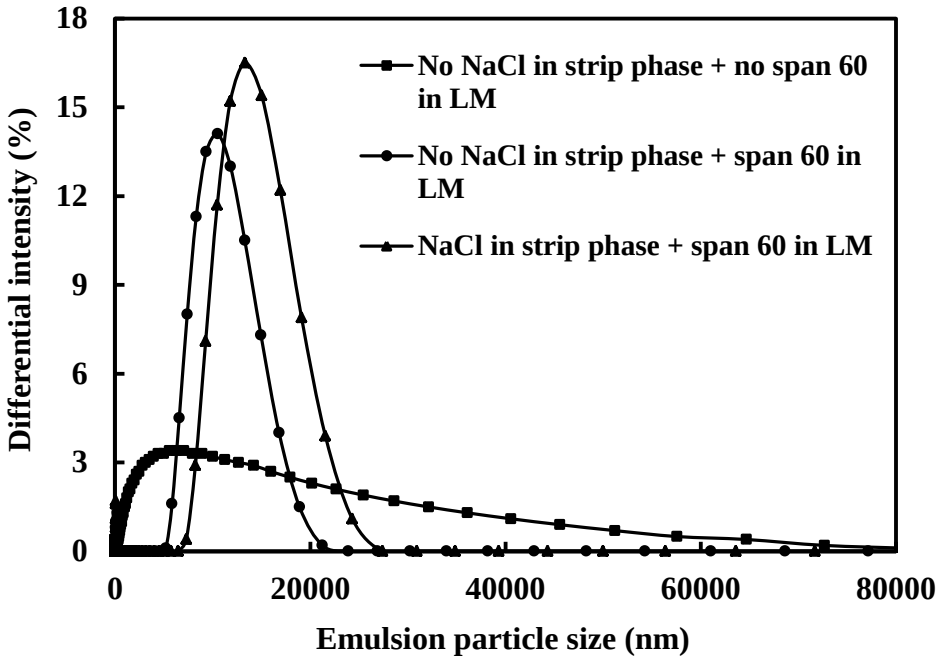


Figure 7.6: Effect of emulsification in varied conditions of organic-aqueous phases

7.2.6 Effect of stirring rate

The effect of stirring of the aqueous phases was studied at three different speeds of 150, 200 and 250 rpm. Stirring of the aqueous phases have two opposite effects. Vigorous stirring adversely affects the stability of membrane but yields the maximum flux of the solute in the transportation process. On the other hand, stability of SLM is higher when speed of stirring is low because the pressure experienced by the LM is less enough than the critical displacement pressure (Pc) in such situation. However, flux of the solute gets reduced at lower stirring speed. Fluxes of catechin in different stirring conditions were measured during the first run of transportation studies in order to optimize the speed of stirring. The stability of LM does not affect much in the subsequent runs if the optimized speed of stirring is maintained thereon. One run of 24 h was sufficient to check the effect of stirring. The maximum fluxes were found to be 23.4×10^{-8}, 22.8

$\times 10^{-8}$ and 18.7×10^{-8} kg.m^{-2}.s^{-1} for stirring speed of 250, 200 and 150 rpm, respectively (Fig. 7.7). There is not much improvement in the flux when speed of stirring was increased from 200 rpm to 250 rpm, whereas flux gets substantially reduced when the speed is lowered to 150 rpm. Hence, 200 rpm was selected as the optimum speed of stirring. It is argued that the stirring speed greater than the optimum value might always be very critical which might affect the stability of SLM adversely.

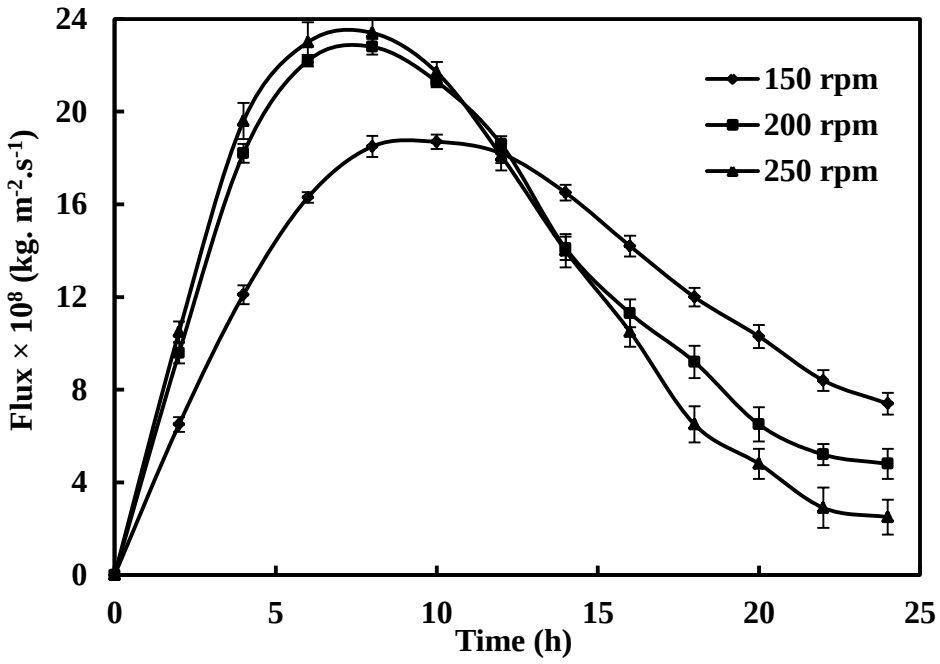


Figure 7.7: Effect of stirring speed on the flux of catechin

7.2.7 Significance of polymeric material as support for LM

The pore size of the support has a significant role in the stability of SLM. The ML can withstand only up to a maximum pressure, as given in the Eqn. (1.4). The critical displacement pressure, *Pc* is inversely proportional to the diameter of the pores and it is assumed that the pores are

perfectly cylindrical in shape. We considered four different polymeric materials as support for the ML and studied the influence of their diameters and shapes. All the four polymeric materials, *viz*. PVDF, PTFE, PES and Nylon, 6, had same average pore diameter of 0.2 µm. The maximum flux of catechin was obtained with PVDF as the support, followed by PTFE, PES and Nylon, 6. The thickness of the PTFE support is 77.2 µm and that of PVDF is 88.5µm. When all other parameters remain constant, the flux through the membrane is inversely proportional to the thickness of its support. Hence, PTFE support should have yielded more flux compared to PVDF. However, the structure of the pores also contribute in the stability of the SLM and consequently on flux of catechin. Fig. 7.8 (a-d) demonstrates SEM analysis of various polymeric supports. It is observed that the pore structures deviate from the regular cylindrical shape and the extent of irregularity is in the order of PTFE > Nylon, 6 >PES > PVDF. Hence, in the context of pore structure, PVDF support is better for stability of SLM which is consistent with the experimental results.

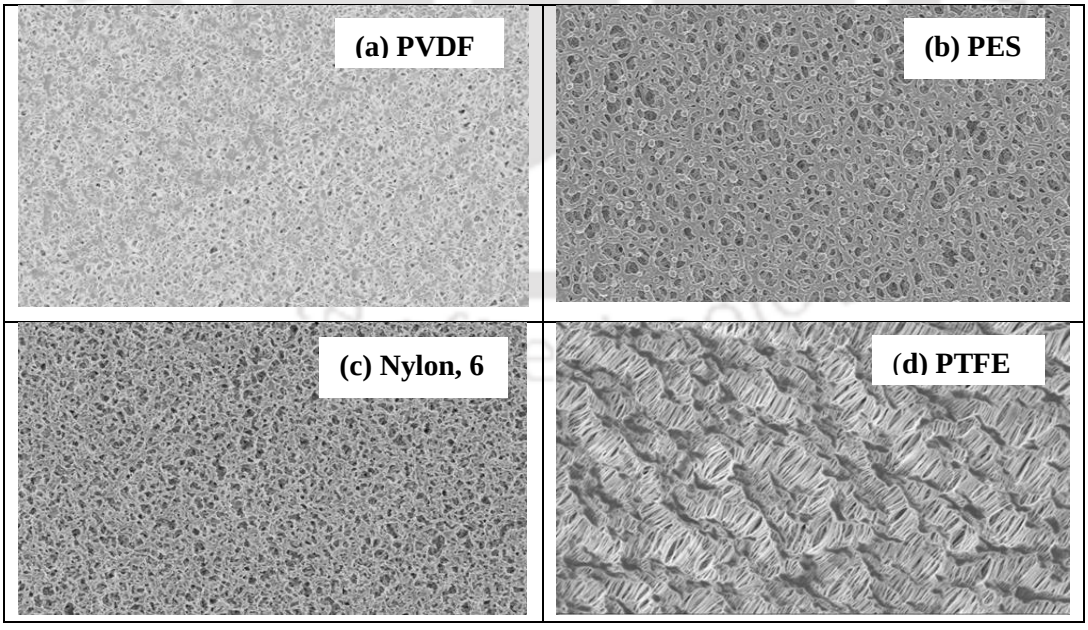


Figure 7.8(a-d): Scanning electron microscopic (SEM) images of membrane supports at magnification of 4.0 Kx each

7.2.8 Critical displacement pressure

Critical displacement pressures (P_c) that a LM (ML in the pores of support membrane) can withstand were determined through experimentation. The experimental set up is shown in Fig. 7.9. The procedure of measurement was similar to that followed by Zha *et al* [9]. The effective diameter of the measurement cell was 20 mm. Polymeric support materials, *viz*. PVDF, PES, PTFE each of 0.2 µm thickness and two Nylon, 6 supports with thickness 0.2 µm and 0.45 µm, were impregnated with ML consisting of 1.2M TBP in *n*-decane and 0.2% (w/w) span 60 (HLB value = 4.7). Impregnated supports by ML *i.e.* SLMs were placed in measurement cell. Same ML was kept in a glass tube of small diameter. The pressure at the aqueous side of the polymeric support was increased gradually by adjusting the regulator of gas (N_2) cylinder. Pressure was measured with mercury manometer. The lowest pressure, at which the ML in the glass tube starts rising up, was taken as the critical displacement pressure. Experiments with each support were repeated three times and the average value of critical displacement pressure was given in Table 7.5. Critical displacement pressure for PVDF support was maximum followed by PES, Nylon, 6 (of thickness 0.2 µm) and PTFE. The structure of pores in the PVDF support was most regular (close to cylindrical shape) and that of PTFE was least regular, as observed through SEM analysis. PTFE has "stripe type" network structure which is not favorable towards stability issue. Zha *et al*. proposed a theoretical expression for critical displacement pressure that is a function of complicated and varied pore structure of support [9]. They reported similar results whatever we observed through experimentation. Again according to Young-Dupre equation **(**Eqn. 1.4), the critical displacement pressure is inversely proportional to the radius of pore if other parameters

remain constant. For a particular support material (Nylon, 6) P_c will be greater as radius of pore is reduced. We used two different supports of Nylon, 6 having pore diameters of 0.2 µm and 0.45 µm, and *Pc* were found as 10.2 kPa and 4.7 kPa for supports with pore diameters of 0.2 µm and 0.45 µm, respectively.

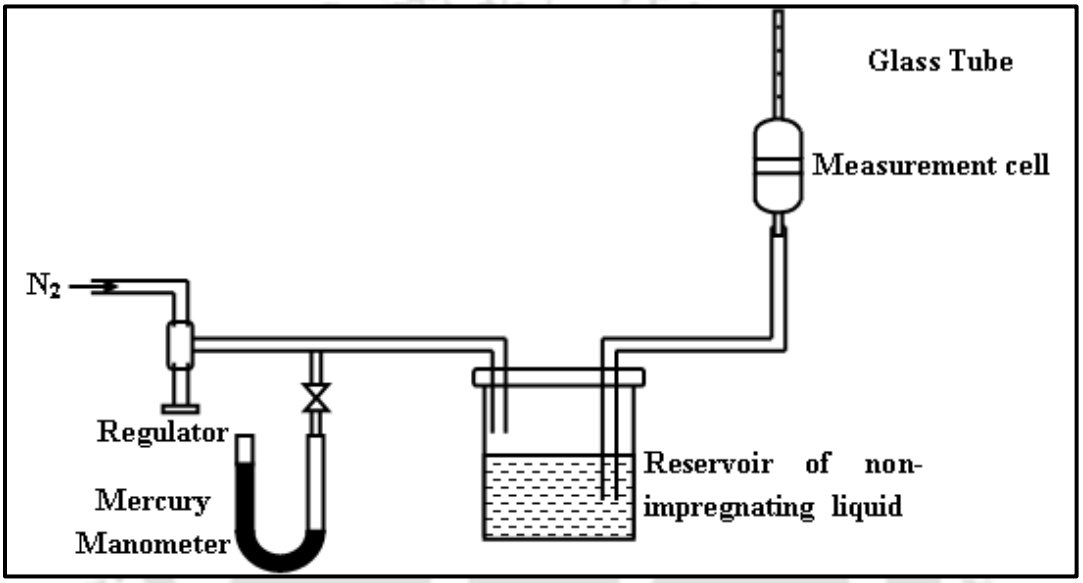


Figure 7.9: Schematic of the set up used for the measurement of critical displacement pressure for membrane supports

7.2.9 Loss of membrane liquid

Loss of ML from the pores of support, after incorporation of all the stability criteria, was measured after 120 h and the results are shown in Table 7.5. The loss was measured by the weighing method. The initial weight of the impregnated SLM support before the experiment and the final weight of removed support after the 5th run were measured in a weighing balance and the difference was calculated as the loss of LM. The calculation was done on water free basis. For supports with 0.2 µm pore size, the maximum loss was observed with PTFE (15.7%) and minimum loss was recorded for PVDF (7.62%). With bigger pore

size the loss increases and that is confirmed with the result of Nylon, 6 support of two different pore sizes. Losses of LM were found to be 12.3% and 30.2% for pore size of 0.2 and 0.45 µm, respectively.

Table 7.5: Critical displacement pressure for ML in the pores and loss of ML from the pores for various membrane supports

Membrane support	Average pore size (μm)	Thickness (μm)	Porosity (ε)	Tortuosity (τ)	Critical displacement pressure (P_c) (kPa)	Weight of support (10^{-3}*kg)	Weight of (support + ML) (10^{-3}*kg)	Weight of ML (10^{-3}*kg)	Loss of ML (10^{-3}*kg)	Loss (%)
PVDF	0.2	88.5	0.45	3.44	15.2	0.1082	0.1632	0.055	0.0056	7.62
PTFE	0.2	77.2	0.51	2.92	8.7	0.0783	0.1333	0.055	0.0086	15.7
PES	0.2	107	0.50	3.0	11.3	0.0768	0.1513	0.075	0.018	13.2
Nylon, 6	0.2	102	0.4	4.0	10.2	0.0675	0.1240	0.057	0.007	12.3
Nylon, 6	0.45	102	0.49	2.92	4.7	0.0551	0.1247	0.070	0.021	30.2

7.3 Summary of the detailed stability analysis

- The maximum flux of catechin after 120 h (*i.e.* at fifth run) was 16.34×10^{-8} $kg.m^{-2}.s^{-1}$ without use of electrolyte and surfactant. The same flux was increased to 20.05×10^{-8} $kg.m^{-2}.s^{-1}$ and 22.05×10^{-8} $kg.m^{-2}.s^{-1}$ after addition of only electrolyte in the aqueous phases and addition of both of the electrolyte in aqueous phases and surfactant in membrane phase respectively.
- The maximum flux in the 1^{st} run was found as 23.4 $kg.m^{-2}.s^{-1}$ with incorporation of all the stability criteria. The flux calculated at the 5^{th} run was 22.05 $kg.m^{-2}.s^{-1}$. Hence the stability of the SLM was obtained as 120 h with 5.8% reduction in flux.
- The loss of membrane liquid is found to be minimum for PVDF membrane support because of its most regular structures of the pores found from SEM analyses.

Abbreviations

BLM	bulk liquid membrane
FE-SEM	field emission scanning electron microscopy
HLB	hydrophilic-lipophilic balance
LM	liquid membrane
ML	membrane liquid
PTFE	polytetrafluoroethylene
PVDF	polyvinylidenefluoride
SEM	scanning electron microscopy
SLM	supported liquid membrane

TBP	tributyl phosphate

Nomenclature

T	absolute temperature
S	the entropy of the oil droplets formed
U_a^{II}	total bulk internal energy of aqueous phase in the emulsion
U_o^{II}	total bulk internal energy of oil (membrane phase)aqueous phase in the emulsion
U_a^I	total bulk internal energy of aqueous phase before formation of emulsion
U_o^I	total bulk internal energy of membrane phase (oil) before formation of emulsion
V	volume of aqueous solution
δ	thickness of the support membrane
ε	porosity
τ	tortuosity
γ_{OS}	Interfacial tension between the oil (membrane) and stripping phase
ΔG	free energy change
ϕ_o	volume fraction of membrane liquid

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CHAPTER-VIII

Conclusions

CHAPTER-VIII

Conclusions

The inferences drawn from the present research work were summarized in this chapter and recommendations towards future direction for recovery of bioactive and/or medicinal compounds (other than catechins) from other plants and herbs were also provided.

8.1 Conclusions

In the present work the performances of liquid membrane (LM) based processes in the recovery of medicinal catechins having nearly similar structures were investigated. The simplest structure of these plant polyphenols (organics) is the catechin. Hence all the experiments *viz*. two phase equilibrium studies, simplest three phase transportation studies through BLM, flat-sheet supported liquid membrane (FS-SLM), hollow fiber supported liquid membrane (HF-SLM) have been accomplished primarily with synthetic catechin. The important parameters were optimized for maximum yield of transportation. The optimized parameters were further applied for the transportation of the four catechins as an aggregate from the aqueous extract of the green tea leaves. The tea leaves were collected from the upper region of the state of Assam, India. The differences in the transportation yield between synthetic catechin and the other three catechin derivatives in real extract from green tea leaves were reported. The membrane fouling problem associated with the transportation of real extract was also studied and the extent of fouling was measured through the reduction of either percentage of recovery or flux of catechins. Consequently, an experimental membrane cleaning protocol was established. In context of fouling problem in case of real extract treatment in FS-SLM, investigations were performed to find out best support material and

PTFE was recommended as the best one for this purpose. The experimental results of HF-SLM were plotted in the well-established model equations for mass transfer in the liquid membrane techniques. Laboratory scale experiments were scaled up to a pilot scale operation for HF-SLM. The iron complexation of bioactive catechins was performed in their recovery process through FS-SLM technique. Various characterization techniques were applied to confirm the formation of the said complex of catechin only. The efficiency of recovery of catechins in various configurations of LM was then measured and compared through a series of experiments. The major conclusions are summarized below:

- Selection of LM suitable for the transportation of catechins was carried out through a series of equilibrium studies. The ML combination "*n*-decane-TBP" showed the encouraging result in the recovery of catechins. Hence the ML comprising of *n*-decane and TBP was selected as the suitable ML to study the performance of the various LM configurations
- Ethanol has been proved to be an excellent stripping agent for recovery of catechins from membrane phase
- The feed phase must be maintained in acidic pH range to ensure the catechins in their molecular *i.e.* unionized state to be extracted by a neutral carrier
- The use of physiologically benign vegetable oil (SFO) as solvent of ML for the recovery of bioactive substances, catechins has an enormous scope for pharmaceutical industries
- BLM separation study is very important as its findings help in understanding the separation feasibility of a LM process for any system of concern. It also provides the useful equilibrium and kinetic parameters necessary for mass transfer studies. Concentrations of strip phase and carrier, and stirring speed have significant impact on the transport of catechins

- The two phase studies demonstrate that the optimum extraction of catechin can be obtained at feed phase pH of 5.0 temperature of 25˚C, carrier concentration in the membrane phase of 0.8 M and initial feed concentration of 100 $mg.L^{-1}$ in an extraction time of 24 hours.
- An operation of the catechin transportation process in a fed batch manner has ensured the possibility of complete separation and pre-concentration of catechin with a relatively small amount of stripping agent
- BLM is, in general, useful only for lab scale study. The up-gradation of the technique to a pilot/commercial scale is ineffective due to much lower value of surface area to volume ratio. Hence, the efficiency of SLM modules both in flat sheet membrane and hollow fiber membrane modules were investigated in the recovery of catechins. Various supports impregnated with the ML, "*n*-decane and TBP" were examined to design a suitable SLM module. The SLM combination "PVDF-TBP-*n*-decane" is found to be suitable for recovery of synthetic catechin whereas PTFE-TBP-*n*-decane" was found better for recovery of catechins from real extract of green tea leaves
- Due to the strict environmental regulations it is important to look for an environment friendly ML for the above separation. The performance of sunflower oil (SFO) with carrier (TBP) as ML is investigated through BLM for this purpose. Transportation of catechin through BLM was found less based on flux and transportation efficiency due to high viscosity of the sunflower oil, hence the lower diffusivity of catechin in the resultant membrane phase. So, it was not considered further as the solvent of ML. Instead *n*-decane was employed for further studies in SLM. The SLM configuration "PVDF-TBP-*n*-decane" is found to be efficient in the recovery of catechins with higher flux
- SLM based separation technique has been found to be an efficient means for recovery of bioactive components, (+) catechin and its derivatives from tea leaves extract

Conclusions

- The optimum conditions for three phase studies have been found to be feed solution pH of 4.0, carrier concentration in the membrane phase of 1.2 *M*, ethanol concentration of 0.4 *M* in the stripping phase, stirring speed of 200 rpm and initial feed concentration of 100 $mg.L^{-1}$
- Stability is an important criterion of SLM for its application to be feasible. The present SLM "PTFE-TBP-*n*-decane" is found to be reasonably stable up to 24 hours of operation with membrane life time of 120 hours
- FS-SLM based transport technique has been found to be an efficient means for metal (iron) complexation of catechins prior to its precipitation and recovery
- The optimum conditions for transportation studies have been found to be feed solution pH of 5.5, carrier concentration in the membrane phase of 1.2 M, stripping solution concentration of 0.001 M, stirring speed of 200 rpm and initial feed concentration of 0.001 M
- Various catechins from real extract of green tea leaves have been recovered in HF-SLM module. TBP in *n*-decane immobilized into the micro-pores of polyethersulfone (PES) membrane have been found to be suitable carrier-solvent-support combination for the above purpose
- The established model equations available in the HF-SLM technique have been employed for the study of the experimental results
- The mass transfer parameters like solute diffusivity in the membrane phase (D_{ff}.), mass transfer coefficient in membrane phase (k_m), permeability (P) and the flux through strip side interface (J) *etc*. have been evaluated in case of HF-SLM
- A membrane cleaning protocol has been experimentally established and reported on the basis of measurement of fluxes

8.2 Recommendations towards future direction

- Development of mathematical model for transportation of catechins
- Determination of activity of Fe-catechin complex
- Further stability studies of SLMs
- Iron-catechin complexation through HFM module with necessary modifications
- Utilization of the developed methods and methodology for recovery of other bioactives from respective sources
- The use of sunflower oil as solvent in the present work is limited for the bulk liquid membrane only. It was not explored in SLM studies due to low permeability of catechin in membrane phase. It can be explored in future also in supported liquid membrane with suitable modifications *viz.* introduction of other diluents in the liquid membrane to reduce the effective viscosity of resultant liquid membrane, minimizing the liquid membrane thickness *etc.* Further research is necessary to develop an environment friendly liquid membrane based process for the recovery of catechins
- The flat sheet SLM module and hollow fiber SLM module designed in the present work show encouraging result in the separation and recovery of catechins from aqueous extract. The other supported liquid membrane configurations such as spiral wound and series of SLM modules may also be explored for same purpose in the same way and thus the process parameters can be optimized
- The skills and methodologies developed in this research can be utilized in a wider context. The recovery/purification of other bioactives may be achieved through metal complexation with various essential metals in a single step.
- All the experiments in this research work are carried out in batch mode. The efficiency of a continuous mode can be studied in future

APPENDIX

Appendix-I

Composition of common SFO

Sunflower oil is mainly a mixture of triglycerides with typical constituents as mentioned in Table AI.1. It is basically composed of long and medium chain triglycerides. It contains about 11% saturated fatty acids, 30% monounsaturated fatty acids (omega-9) and 59% polyunsaturated omega-6 fatty acids [1]. The fatty acid composition (according to *British Pharmacopoeia 2005*) of sunflower oil is tabulated in Table AI.1 [2].

Table AI.1 Composition of triglycerides SFO [2]

Name of components	Quantity (%)
Palmitic Acid (C16:0)	4.0-9.0
Stearic Acid (C18:0)	1.0-7.0
Oleic Acid (C18:1)	10.0-14.0
Linoleic Acid (C18:2 n6)	48.0-74.0

***[*0- saturated, 1- mono saturated, 2- poly saturated and n6- Omega-6*]**

Pharmacopoeia 2005) of sunflower oil is tabulated in Table AI.1 [2].

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Appendix-II

Leakage test for BLM set-up

The BLM cell as described in Chapter-II was tested for leakage in the following manner.

- ✓ One compartment of the cell was filled with clear water and the other with a colored (crystal violet) solution.
- ✓ Both the aqueous phases were stirred continuously for 24 hours at a stirring speed of 500 rpm.
- ✓ The intensity of color in the aqueous phases was then measured with UV-vis spectrophotometer at a wave length of 584 nm.

The results of the leakage test are furnished in the Table-A II.1 below:

Table A II.1: Result of leakage test of BLM set-up

Time (h)	Absorbance of clear water	Absorbance of colored water
0	0	0.001
24	0	0.002

The zero absorbance of the clear water confirmed that there was no leakage in the set-up.

List of publications

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