INTEGRATED BIOTECHNOLOGICAL APPROACHES FOR THE PURIFICATION AND CONCENTRATION OF LIQUID FOODS, PROTEINS AND FOOD COLORS

A thesis submitted to the

University of Mysore

For the award of the degree of

DOCTOR OF PHILOSOPHY

in

Biotechnology

by

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March-2004

Dedicated to My Beloved Parents……

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DECLARATION

I hereby declare that the thesis entitled **"Integrated Biotechnological Approaches for the Purification and Concentration of Liquid Foods, Proteins and Food Colors"** submitted to the **University of Mysore** for the award of the degree of **Doctor of Philosophy** in **Biotechnology**, is the result of the research work carried out by me in the Department of Food Engineering, Central Food Technological Research Institute, Mysore, India under the guidance of **Dr. KSMS Raghavarao**, during the period 2000-2004.

I further declare that the results of this work have not been previously submitted for any other degree or fellowship.

(Naveen Nagaraj)

Date:
Place: Mysore

Dr. KSMS Raghavarao
Head,
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Certificate from Guide

I hereby certify that this thesis entitled **"Integrated Biotechnological Approaches for the Purification and Concentration of Liquid Foods, Proteins and Food Colors"** submitted by Mr. Naveen Nagaraj for the degree of **Doctor of Philosophy** in **Biotechnology**, University of Mysore, is the result of the research work carried out by him in the Department of Food Engineering, Central Food Technological Research Institute, Mysore, India, under my guidance and supervision during the period 2000-2004.

(KSMS Raghavarao)
Research Guide

Date:
Place: Mysore

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SYNOPSIS OF THE THESIS

INTEGRATED BIOTECHNOLOGICAL APPROACHES FOR THE PURIFICATION AND CONCENTRATION OF LIQUID FOODS, PROTEINS AND FOOD COLORS

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SYNOPSIS

Recently, efforts are in progress by research and industrial community for the production of biological products through the application of biotechnology. However, the technology for downstream processing (DSP) of biomolecules from the broth has not kept pace with the advances in the upstream operations, despite the fact that in many cases DSP contributes major share (50-80%) of the total production cost. Existing DSP techniques such as chromatography, electrophoresis, precipitation etc., pose scale-up problems, and are prohibitively expensive on large-scale, unless the product is of high value. Therefore, current research in the area of DSP is directed towards the development of efficient and scaleable alternative bioseparation processes with flexibility for continuous operation.

Aqueous two-phase extraction (ATPE) has been recognized as superior and versatile technique for DSP of biomolecules. A wealth of information has been reported in the literature on various aspects of ATPE for the isolation and purification of proteins/enzymes and other biological materials. ATPE offers a better alternative to the existing methods of primary purification, providing low space-time yield, better enrichment of product, ease of scale-up and flexibility for continuous operation. This technique is effective also in removal of by-products such as other undesirable enzymes/proteins, unidentified polysaccharides and pigments. Furthermore, application of ATPE permits easy adoption of the equipment and the

methods of conventional organic-aqueous phase extraction used in the chemical industry. ATPE is recognized as a primary purification step in the overall protein recovery train, since it is not selective enough to provide the desired purity of the enzyme/protein. Use of ATPE enables the desired product (enzyme/protein) to partition into one of the phases, thus reducing the volume of the process stream to be handled during the subsequent purification steps. Hence, final purification can be accomplished by highly selective techniques such as chromatography, electrophoresis.

After successfully partitioning the biomolecule to one of the phases in ATPE, it is desirable to separate the phase forming components and concentrate the solution containing the biomolecules. Membrane processes such as ultrafiltration (UF), dialysis can effectively do this job. The integration of ATPE with any one of the above membrane processes holds considerable promise to increase the productivity of the overall process.

The subject matter of this thesis is presented in five chapters.

Chapter 1- This chapter comprises of General Introduction and scope of the present investigation, literature review pertaining to fundamentals and application of ATPE for the purification of biomoelcules. Further, application of osmotic membrane distillation (OMD) for the concentration of biomolecules/liquid foods has been discussed followed by possible integration ATPE with OMD process.

Chapter 2 – It comprises of preamble for aqueous two phase systems (ATPSs), and the major hindrances for the industrial application of ATPE. Further, this chapter is divided into five sub-sections consisting of methods to enhance phase demixing rate by the application of external fields followed by field assisted extraction of natural food colorants and polymer recovery from spent phases.

Section 2A – Acoustic field assisted demixing has been employed to enhance the phase demixing rate in ATPSs. Application of acoustic field has increased the phase demixing rate up to 3.2 fold by varying the axial distance from the acoustic transducer in polyethylene glycol/potassium phosphate system. The enhancement of phase demixing rate with varying axial distance from the acoustic transducer has been explained based on acoustic field and material of the contactor.

Section 2B – In this section, it has been demonstrated for the first time that electric field can be applied to enhance phase demixing rates even in polymer/salt systems which otherwise was thought not possible due to high conductivity of the phases. The electrokinetic demixing of polymer/salt has resulted in significant enhancement in demixing rates up to 4 fold. The effect of electric field polarity, electric field strength, volume ratio, phase composition on phase demixing has been studied. Further, the influence of

these parameters on phase demixing has been explained based on hydrodynamic flow electroosmotic flow (HEF) model.

Section 2C – In this section, microwave field has been explored for the first time to enhance the phase demixing rates (decrease the demixing times) in ATPSs. The microwave field assisted demixing process enhanced the phase demixing rates up to 4 fold in polyethylene glycol/potassium phosphate system and up to 6.5 fold in case of polyethylene glycol/maltodextrin system. The enhancement in demixing rate is explained based on dipole rotation, electrophoretic migration of free salts, multiple reflections at the interfaces, droplet-droplet collision and reduction in viscosity of the continuous phase that occur during the application of microwave field.

Section 2D – It was already shown that electric field can be successfully applied in enhancing the phase demixing of ATPSs. In the present study, an attempt has been made to apply electric field for the selective separation of betalaines (betaxanthin and betacyanin) derived from beet hairy roots.

Section 2E – Apart from slow rate of demixing, another major hindrance for the adaptation of ATPE on industrial scale is the high cost of phase forming polymers such as polyethylene glycol (PEG) and also, the environmental problems arising due to the disposal of PEG rich phase after the extraction of biomolecules. In order to overcome the above drawbacks, there is a need to

recover and recycle PEG from spent phases. In the present study an attempt has been made to separate and recover PEG from spent phases by the application of microwave field. The exposure of PEG rich phase to microwave field has resulted in phase separation of water (liquid phase) and PEG (solid phase). The separation of water from PEG rich phase is explained based on the decrease in PEG solubility at higher temperature and subsequent increase in PEG hydrophobicity. The separated PEG was dried to obtain it in the powder form. Studies were carried out to examine the physical and also chemical characteristics of PEG after recovery in order to ensure its suitability for reuse.

Chapter 3 – In recent years membrane based processes are gaining importance for the processing of biomolecules/liquid foods, in order to achieve value addition to the produce without product damage, to decrease the wastage and to facilitate preservation/transportation. Membrane processes like microfiltration (MF), ultrafiltration (UF) and reverse osmosis (RO) are advantageous as they operate under relatively lower temperatures, thus minimizing product damage unlike in the thermal evaporation. Also, the water is separated without phase change thereby conserving energy. However, the existing membrane processes suffer from the drawbacks of concentration polarization, membrane fouling and maximum achievable concentration (only up to ~ 25°B). Even, newer membrane process like

membrane distillation (MD) suffers from the drawbacks of low flux and temperature polarization. Hence, there is a need to develop an alternate/complementary process for the concentration of the solutions of proteins/natural food colors and other thermolabile biomolecules.

Osmotic membrane distillation (OMD) is a novel athermal membrane process that facilitates the concentration of solutions/liquids to the maximum achievable extent at mild operating conditions. In the present study, the effect of various process parameters such as type, concentration and flow rate of the osmotic agent, type and pore size of the membrane, temperature with respect to transmembrane flux was studied. Experiments were performed with real system (pineapple juice) in a flat membrane module. Osmotic agents (OA's) namely Sodium chloride and Calcium chloride dehydrate at varying concentrations are employed in the study. Higher transmembrane flux was observed at maximum osmotic agent concentration in case of both OA's. In comparison with sodium chloride, higher transmembrane flux was observed in case of calcium chloride. Experiments were carried out to study the effect of osmotic agent flow rate (25-100 ml/min) on transmembrane flux during concentration of pineapple juice by maintaining maximum osmotic agent concentration. Transmembrane flux increased with an increase in flow rate. A mass transfer-in-series resistance model has been developed considering the resistance offered by the membrane as well the boundary layers (feed and brine sides) in case of real

system for the first time. The model could predict the transmembrane flux and also the effect of different parameters studied on flux.

Like any other membrane process, OMD also suffers from relatively low flux. Studies have been undertaken to apply acoustic field for the enhancement of transmembrane flux. Acoustic field from an acoustic transducer having a frequency of 1.2 MHz was applied perpendicularly to the membrane. Experiments were carried out for 5M Sodium chloride/pure water, 5M Calcium chloride dihydrate/pure water, Sodium chloride/sugarcane juice and Calcium chloride dihydrate/sugarcane juice systems both in the presence and absence of acoustic field in lab-scale flat membrane test cell. An enhancement of 22 - 205% in transmembrane flux by the application of acoustic field was observed.

Chapter 4 – This chapter has been categorized into three sub-sections with Section 4A dealing with purification and concentration of C-phycocyanin, Section 4B on Concentration of pineapple juice by OMD and Section 4C on large-scale studies for the concentration of pineapple juice employing hybrid process.

Section 4A – C-phycocyanin is a natural blue colorant derived from blue-green algae which finds application in food coloring, cosmetics and therapeutic uses. C-phycocyanin extract when derived from its source has

high load of impurities and is in dilute form. Hence, C-phycocyanin needs to be purified and concentrated, so as to obtain C-phycocyanin suitable for food/pharmaceutical applications. Conventional methods employed for the purification of C-phycocyanin are inefficient, expensive and cumbersome due to involvement of more number of unit operations. Further, C-phycocyanin which is also protein needs to be concentrated under mild operating conditions since it is sensitive to heat/shear. Hence, studies have been undertaken to purify C-phycocyanin with lesser number of unit operations. Further, concentration of C-phycocyanin to higher levels has been undertaken by employing OMD process under mild operating conditions.

Section 4B – Pineapple is a popular non-citrus tropical and seasonal fruit. Pineapple has refreshing sugar-acid balance, attractive flavor and aroma. The fruit needs to be preserved suitably preferably in the form of fruit juice so as to cater to the consumer demand throughout the year all over the globe. Conventional thermal concentration process employed for concentrating pineapple juice leads to loss of color, flavor and aroma resulting in low quality end product. Hence, concentrated pineapple juice having all the organoleptic properties of the original fruit can find applications in the production of juice blends, carbonated soft drinks etc. Hence, alternate/complementary membrane process like OMD has such potential since it facilitates the concentration of pineapple juice to higher levels with minimal product

damage. Studies have been undertaken to concentrate pineapple juice (>60°B) employing OMD process in a flat membrane module. The concentrated pineapple juice obtained from OMD process was analyzed for its sensory qualities by Kramer's rank sum method which confirmed that there was no difference in the quality of juice when compared to control sample.

Section 4C – OMD has low transmembrane flux like any other membrane process. Hence, it becomes inherently uneconomical to operate OMD process as a single step unless the product is of high value. In view of the above there is a need to enhance the overall productivity during large-scale processing of biomolecules/liquid foods. Attempts have been undertaken to concentrate pineapple juice on large-scale by employing hybrid membrane process involving UF, RO followed by OMD.

Chapter 5 – It comprises of other applications/constraints, suggestions for future work, followed by References.

Chapter 1

General Introduction

1. A. AQUOEUS TWO PHASE EXTRACTION

In recent years, application of biotechnology for the production of biomolecules by research and industrial communities has increased. Downstream processing (DSP) forms an integral part of any biological product development and the final cost of the product depends largely on the cost incurred for DSP. The conventional filtration process employed for solid-liquid separation is not suitable for the bioseparation, where the size of the microorganisms to be separated is small, especially when the cells are disintegrated to release the intracellular biomolecules resulting in a system of increased viscosity (Huggins, 1978; Mosqueria, 1981). In case of conventional methods, like centrifugation, and even modern methods such as electrophoresis or column chromatography, scale-up problems are considerable making them uneconomical unless the product is of high value. Hence, there is a need to develop simple, efficient, economical, environmentally benign DSP methods for the recovery of biomolecules with flexibility for continuous operation. Extraction using aqueous two-phase systems (ATPSs) is one such method. Although, this technique was developed by Albertsson during 1950's, its importance and applications have been recognized only in the recent years.

Liquid-liquid extraction using organic-aqueous phase systems is extensively used in chemical industry. In spite of all the advantages, this method has not gained wide industrial recognition in the field of

biotechnology. In fact, the commonly used organic solvent systems are unsuitable for the intended purpose as the biomolecules in general are either insoluble or become denatured in organic solvents. Aqueous two-phase extraction (ATPE) has been able to overcome the limitations of conventional organic-aqueous extraction, since both the phases are aqueous. Thus, ATPE has been recognized as a superior and versatile technique for the extraction and purification of biomolecules (Walter *et al.,* 1985; Albertsson, 1986; Zaslavsky, 1996). ATPE has shown its utility in the extraction and purification of biological materials such as enzymes/proteins, nucleic acids, viruses, cell organelles etc. A wealth of information was reported in the literature on various aspects of ATPE and its applications (Kula *et al.,* 1982: Walter *et al.,* 1985; Albertsson, 1986, Diamond and Hsu, 1992; Zaslavsky, 1995; Raghavarao, *et al.,* 1998, Raghavarao *et al.,* 2003). A few other liquid-liquid extraction methods employed for the extraction and purification of various biomolecules are reverse micellar extractions (Luisi and Magid, 1986), cloud point extractions (Hinze and Pramauo, 1993), and micellar extractions (Scamehorn *et al.,* 1988). However, ATPE is better alterative to these existing methods of primary purification due to high capacity, better yield/purity of product, low space time yield, biocompatible environment, lower process time, low energy and ease of scale-up. Furthermore, application of ATPE permits easy adaptation of the equipment and the methods of conventional organic-aqueous systems used in the chemical

industry. This technique is effective in removal of by-products such as undesirable enzymes/proteins, unidentified polysaccharides and pigments. Broth can be directly subjected to ATPE by the addition of desired quantities of phase forming polymers and salts. ATPE can be designed such that the desired biomolecule selectivity partitions to one of the phases in a concentrated form, with considerable reduction in the volume of the stream to be handled during the subsequent purification steps. Hence, ATPE is complementary to other methods employed for biomolecule purification.

Thus, ATPE has been recognized as a primary purification step in the overall enzyme/protein recovery train (Sikdar *et al.,* 1991). For a successful extraction of the desired biomolecule from fermentation broth or plant extract, various process parameters have to be optimized in such a way that the cell debris along with some contaminating biomolecules, partitions to one phase and the desired biomolecule partitions to other phase. In addition, to obtain desired recovery and purity, attention needs to be paid towards the partition coefficient of the desired biomolecule, volume ratio and phase composition of the system (Hustedt *et al.,* 1985). Partition coefficient of a biomolecule is defined as the ratio of the equilibrium concentration of the biomolecule in the top phase to that in the bottom phase. It determines the selective distribution of the desired product in ATPE. The exact mechanism governing the partition coefficient is yet to be completely understood. Qualitatively, it can be said that the molecule partitions in such a way that the maximum number of

interactions are possible and the minimum energy state of the system is achieved. Early attempts to explain this mechanism of partitioning was made by Bronsted (1931) which predicts the partition coefficient based on size and charge of the solute molecule. In case of biomolecules, the partition coefficient is affected almost individually by different factors such as size and charge of the protein, choice and molecular weight of the polymer, phase composition, pH of the system, type and concentration of the additives and temperature, which were discussed in detail by Raghavarao *et al*. (1995).

Using ATPE, effective isolation and purification of various proteins have been demonstrated (Kula *et al.,* 1982; Tjerneld *et al.,* 1987; Hustedt *et al.,* 1988). It may be noted that over a fairly wide concentration range and scale of operation, partition coefficient is practically independent of initial protein concentration. Hence, for commercial applications, ATPE can be directly employed for large-scale operations based on the experimental results obtained during small scale studies. Large scale isolation and purification of formate dehyrogenase from yeast and lactate dehydrogenase from bacteria, respectively was reported by Cordos and Kula, 1986; Schutte *et al.,* 1983. Pilot scale extraction study of a recombinant protein in polyethylene glycol (PEG)/salt system with an overall recovery of 37% has been reported by Strandberg *et al.,* (1991). Many more large scale purification studies of the enzymes/proteins was undertaken (Kroner *et al.,* 1978; Veide *et al.,* 1983; Kroner *et al.,* 1984; Boland *et al.,* 1991;

Papamichael *et al.,* 1992). Recently, ATPE has been employed on large scale for the isolation and purification of human insulin like growth factor I (Hart *et al.,* 1994).

Apart from the large-scale extraction and purification of extracellular enzymes as well as recombinant proteins, ATPE also finds applications in many other fields such as: (i) extraction and purification of intracellular and membrane proteins (Sivars and Tjerneld, 1997) (ii) concentration and purification of viruses (Albertsson, 1986), nucleic acids (Cole, 1991; Walter *et al.,* 1985) and plant proteins (Persson and Johansson, 1989; Vilter, 1990) (iii) partitioning and separation of microbial cells (Albertsson, 1986) as well as animal cells (Hamamoto *et al.,* 1996) (iv) in food industry, for the clarification of cheddar whey (Chen, 1989) and isolation of high phytin containing particles from rice bran (Ogawa *et al.,* 1975) (v) in the measurement of relative hydrophobicity and approximate isoelectric pH of biomolecules (Zaslavsky, 1995) (vi) in bioremediation (Rogers, 1997) (vii) purification and concentration of food colorants (Rito Palomares *et al.,* 2001).

In spite of several advantages offered by ATPE, the high cost of phase forming polymers and slow rate of phase demixing are the major hindrances for its wide industrial adoption. In the present study, attempts have been made to address these problems.

After successfully partitioning the desired biomolecule to one of the phases in ATPE, it is desirable to separate it from the phase forming polymer

component of that phase (polymer or salt) and concentrate the biomolecule. Membrane processes such as ultrafiltration (UF)/dialysis holds considerable promise in this regard. The integration of these membrane processes with ATPE will enhance the overall productivity. Vaide and co-workers (1989) have coupled ATPE with diafiltration to separate and recover β–glactosidase from PEG. ATPE in combination with UF was employed for the concentration and purification of amyloglucosidase produced by solid state fermentation (Tanuja *et al.,* 2000). The feasibility of coupling ATPE with UF during purification and concentration of plant peroxidase *(Ipomoea palmetta)* was undertaken to enhance the overall productivity in terms of purity, recovery and concentration (Srinivas *et al.,* 2002).

1. B. OSMOTIC MEMBRANE DISTILLATION

Conventionally, filtration refers to the separation of solid from liquid or gaseous streams. Membrane filtration extends this application further to include the separation of dissolved solutes in liquid streams and for separation of gas mixtures. The primary role of a membrane is to act as a selective barrier which permits the passage of certain components and retain certain other components of a mixture. Lakshminarayanaiah (1984) has defined membrane as a “phase that acts as a barrier to prevent mass movement but allow restricted and/or regulated passage of one or more species through it. By this definition, a membrane can be gaseous, liquid or

solid or combination of these. Membranes can be further classified by (a) nature of the membrane – natural or synthetic (b) structure of the membrane-porous versus non-porous (c) application of the membrane - gas-gas or gas-liquid or liquid-liquid or solid-liquid separations (d) mechanism of membrane action-adsorptive versus diffusive. The membrane separation processes are classified based on particle or molecular size. The major separation processes are microfiltration (MF), ultrafiltration (UF), reverse osmosis (RO), diafiltration and electrodialysis (Cheryan, 1986).

In recent years, membrane based separation processes are gaining importance over the conventional methods such as evaporation/freeze concentration during the processing of thermolabile biomolecules such as protein solutions/natural food colorants, since most of them are shear/heat sensitive. Freeze concentration has major drawback in terms of maximum achievable concentration (only up to ~ 40°B). Moreover, both these processes are energy intensive since phase change is involved.

Membrane processes such as MF, UF and RO are advantageous since these processes operate at ambient temperature (20-27°C), thereby reducing thermal damage to the product and do not involve phase change thus conserving energy. However, these membrane processes suffer from the drawbacks such as maximum achievable concentration (only up to ~ 25°B), membrane fouling and shear damage to the product (say proteins). Hence, there is a need to develop an alternate/complementary process

technology for the concentration of the solutions of proteins/natural food colors and other thermolabile biomolecules. Osmotic membrane distillation (OMD) has such potential since it facilitates the concentration of aqueous solutions under mild operating conditions. Application of OMD enables to achieve maximum concentration (>60 ^{0}B) without thermal damage to the product. Further, absence of shear damage makes it an attractive alternative for the concentration of solutions of proteins/natural food colors, pharmaceuticals and other biological products which are thermally sensitive (Hogen *et al.,* 1998).

Integration of ATPE with membrane processes such as OMD will enhance the productivity considerably during the purification and concentration of proteins/natural food colors. The use of ATPE will enable desired products (enzyme/protein) to partition to one of the phases and the impurities to the other phase, while purifying and reducing the volume of the process stream to be handled further. OMD process can be used as pre-concentration step to reduce the water load on subsequent concentration steps such as freeze drying and to reduce the quantum of the process stream to be handled during purification steps such as electrophoresis, chromatography etc. Hence, it is essential to study the effect of various process parameters on transmembrane flux during the concentration of protein solutions/liquid foods using OMD.

1. C. AIM AND SCOPE OF THE PRESENT WORK

The present study is presented in five chapters. In the first chapter, a General Introduction about ATPE, athermal membrane process, namely OMD and integration of ATPE with membrane processes such as OMD has been documented, highlighting advantages and general applications.

In Chapter 2, attempts to address the major hindrances for the industrial application of ATPE have been elucidated. Field assisted methods (acoustic, electric and microwave fields) were employed to increase the rate of phase demixing. Further, electroextraction studies to increase the selectivity of ATPE and microwave field assisted recovery of PEG from spent phases are also presented.

The application of OMD enables to achieve maximum concentration of thermolabile biomolecules under mild operating conditions without product damage. Very little information is reported in literature about this process. In third chapter effect of various process conditions followed by modeling of mass transfer in OMD for real systems is described. Like any other membrane process, OMD also suffers from low transmembrane flux. An attempt to enhance the transmembrane flux by the application of acoustic field on lab scale is presented.

Fourth chapter mainly deals with feasibility studies of OMD application on lab scale for the concentration of natural food colors and liquid foods (which are heat/shear sensitive). Further, large scale studies have been

undertaken by integrating other membrane processes such as UF/RO with OMD to arrive at a hybrid process.

Finally, integration of ATPE with OMD is explored for the purification and concentration of biomolecules. Various other applications of OMD, constraints of ATPE/OMD along with suggestions for future work have been presented, followed by references.

Chapter 2

Application of External Fields

2. Preamble

2.1 Aqueous two-phase systems

2.1.1 Formation of *aqueous two-phase systems:*

Aueous two phase systems (A TPSs) are mainly of two types, namely polymer-polymer and polymer-salt. A TPSs are formed by the addition of two water-soluble polymers or a polymer and salt to aqueous media above a critical concentration. Among polymer-polymer systems, the extensively studied system is polyethylene glycol (PEG)/Oextran (Ox) and among polymer-salt systems most popular system is polyethylene glycol/potassium phosphate. For large scale applications, polymer/salt systems are preferred over polymer- polymer ones, because they are easy to prepare, demix quickly and offer better selectivity for protein extraction. Some of the phase systems are shown in Table 2.1 and Table 2.2.

2.2. *Factors affecting the aqueous two-phase systems*

Molecular weight, hydrophobicity and concentration of the salts, type and concentration of the externally added salts and temperature affect the formation of ATPSs. A phase diagram for typical ATPS (PEG/potassium phosphate) is shown in Figure 2.1. The polymer and salt are separately miscible in water in all proportions and at low polymer-salt concentrations with each other. As the concentration of the phase forming components

increases above a certain critical value, phase separation occurs with the formation of a PEG-rich upper phase and salt-rich phase, each containing more than 80% water. Below critical concentration, the system exists in the homogeneous form as indicated by the point H1 in Figure 2.1. The critical concentration of the phase forming components always lies on the binodal. The concentration of the polymer required for phase separation will be lower at the higher molecular weight of the polymer and vice-versa (Diamond and Hsu, 1989 a, b). Higher the hydrophobicity of the polymers in polymer-polymer systems, lower is the tendency for the phase formation rtsson, 1986). Temperature has a considerable effect on phase diagram Walter *et al.,* 1991). At lower temperature, polymer-polymer systems form at lower concentrations of the phase forming components. However, polymer-salt systems require higher concentrations of the phase forming components to separate into two phases at lower temperature (Zaslavsky, 1995). The type (univalent or multivalent) and concentration of salt have considerable effect on the phase system (Zaslavsky, 1995). In case of univalent salts, in the PEG/Dx system, increasing their concentration up to 0.1 M will change the composition of the phases, however, the position of the binodal will not alter significantly. On the other hand, use of multivalent salts in the same system have a tendency to partition into Dx (bottom) phase with an increase in salt concentration, away from the critical point. As the concentration of the salt is increased up to 0.1 M in polyethylene/dextran system, the binodal gets

shifted to lower phase composition along with the change in position of the binodal (Zaslvasky, 1986).

2.3 Physical properties of *the two-phase systems*

The physical properties of A TPSs, such as density, viscosity and interfacial tension are helpful in determining the phase demixing behavior and also contribute to the biomolecule partitioning behavior.

2.3.1 Density

The phase demixing rate is an overall effect of factors such as density difference between the phases, viscosity of individual phases and interfacial tension. Although viscosity of the phases is low, close to the critical point, the demixing rate will be low due to low density difference. Away from the binodal, although the density difference is larger, demixing rate is very low due to high v.iscosity of the phases, which is generally observed in polymer / polymer systems (Kula *et al.,* 1982).

2.3.2. Viscosity

The viscosity of the phases increases with increasing polymer concentration anc molecular weight. This increase is, however, partly compensated by the fact that lowei concentration of polymers with larger molecular weights is sufficient for the phase formation. At the same time the density difference between the phases increases with the tie line length. (Raghavarao *eta!.,* 1995).

The phase viscosities play an important role in biomolecule partitioning in large scale operations in which large volumes of phases are to be handled. Viscosity plays an important role also in continuous-flow apparatus, such as toroidal coil in which effective mass transfer between the phases and efficient retention of the stationary phase is essential in order to take advantage of very high theoretical separation efficiencies (Raghavarao et. Al., 1995) .

Further, phase volume ratio is another factor which influences the demixing rate. The phase demixing rate depends on the volume of the phase which forms the continuous phase. If more viscous phase forms the continuous phase, then phase demixing rate will be lower when compared to situation wherein the more viscous phase forms the dispersed phase.

2.3.3 Interfacial Tension

When two mutually insoluble liquid phases are brought together, the interface possesses a definite amount of free energy per unit area, by virtue of the unbalanced force field acting on the surface molecules (Treybal, 1963). Each unit area of interface has some definite quantity of free energy and, as a result, the interface tends to contract. Thus free energy mathematically is equivalent to interfacial tension. The source of interfacial free energy is an unequal attractive force exerted on the interfacial layer by molecules of the two separate phases. The attractive force between the two liquid phases would be greater than that between liquid and a gas phase.

This presumption postulates a positive attraction between the molecular species and is to be expected simply because of the greater number of molecules per unit volume in a liquid phase. Accordingly, the interfacial tension between the two liquid phases is always lower than the individual surface tensions of both the liquids.

Interfacial tension, despite being an important physical parameter having a decisive influence on the partitioning behavior of particulates/biomolecules (Albertsson, 1986), did not receive its due attention in terms of measurement. Interfacial tension between the two aqueous phases is usually very small, often in the range of 0.0001 to 0.1 dyne/cm, which is very difficult to measure with sufficient accuracy by standard methods such as the capillary or Dunoy ring method. The role of interfacial tension in the partitioning of biomolecules is not yet completely understood.

Similar phase densities, high phase viscosities and low interfacial tension lead to slow rate of phase demixing in A TPSs which has been the major hindrance for large scale adoption of A TPE (Raghavarao *et a/.,* 1995). Conventionally, phase demixing is achieved by gravity settling, which is a very slow process (Hustedt *et a/.,* 1985). Asenjo and coworkers (1995) studied in detail *the* phase separation kinetics in polymer/salt (polyethylene glycol (PEG-4000)/potassium phosphate) under gravity. Their investigations provided a background for the design of large-scale gravitational separators for A TPSs. An alternative method that has been practiced is centrifugation,

which becomes prohibitively expensive on large scale (Hustedt *et al.,* 1989). Hence, efforts have been directed towards enhancing the phase demixing rates by the application of external fields. Larsson and co-workers (Wikrostrom *et al.,* 1987) have explored the possibility of applying magnetic field externally to enhance phase demixing in polymer/polymer systems. Enhancement of demixing rate in polymer/polymer systems by the application of electric field was reported. The observed enhancement was attributed to the increased mobility of phase droplets in the presence of electric field and was explained based on electroosmotic model (Raghavarao et al., 1998). Application of acoustic field at high frequency (MHz range) showed significant enhancement in demixing rates for both polymer/polymer systems and polymer/salt systems (Raghavarao and Todd, 2000; Srinivas *et al.,* 2000a, b; 2002). The application of acoustic field results in mild circulation currents in the phase dispersion, which has increased the probability of droplet coalescence. This in turn leads to the enhancement in demixing rate. In ATPSs, both the phases are electrically conductive, application of electric fields in these systems gives rise to electrokinetic mass transfer of charged species. Thus, A TPS function as a medium for electrophoretic separation, facilitating product recovery. Theos and Clark (1995) have reported that proteins are transferred electrophoretically into either top or bottom phase of A TPSs employing electric field perpendicular to the phase interfaces.

Another major hindrance for large scale application of A TPE is high cost of phase forming polymers. ATPE of biomaterials (e.g. proteins/enzymes/natural food colorants) leads to the partitioning of the desired product to one of the phases which usually contains 5-25% polymer components. Even if the extracted material can be stored in such an environment (the polymers are often biocompatible in nature), a separation of biomaterial and phase forming polymers is often desirable. During DSP, reutiliization of polymer, is essential for economic and environmental reasons.

In this regard various methods have been adopted for the ery/recycling of the recovery/recycling of the phase forming components. Separation of PEG from the protein can be achieved by addition of a new salt so as to form a new PEG/salt system, wherein the desired biomolecule partitions to the salt phase in which the concentration of PEG is very low. Such a residual amount of PEG can some times be tolerable, or if desired can be removed along with the salt by ultrafiltration or diafiltration (Hustedt *et al.,* 1985; Hummel *et al.,*1985, Srinivas *et al.,* 2002). A simple dialysis can remove the salt from the biomolecule. PEG can be separated by chromatographic adsorption on hydroxypatite or ion-exchange columns (Albertsson, 1986, Kula *et al.,* 1982). PEG has been recovered by employing conventional techniques such as vacuum evaporation/drying, precipitation, recrystallization (Harris and Yalpani, 1986). An attempt has been undertaken to study the effect of polymer recycling on protein recovery and its activity (Rito-Palomares and

Lyddiatt, 1996; Rito-Palomares *et al.,* 2000; Wu *et al.,* 2001). Further, Tjernald and co-workers (1985) have employed a new ATPSs comprising of thermosetting polymers for the purification of proteins and recycling of polymer**s.**

In the following chapters attempts to explore the application of microwave, electric and acoustic fields to enhance the demixing rate are discussed. Further, studies have been undertaken to explore the application of external fields to the extraction of natural food colorants and to recover polymer from spent phases.

Table 2.1 Components of Polymer-Polymer Phase Systems

Polymer 1	Polymer 2
olyethylene glycol (PEG)	Dextran
	Ficol/
	Pull ulan
	Polyvinyl alcohol
Polypropylene glycol (PPG)	Dextran
	Hydroxypropyl dextran
	Polyvinyl pyrrolidone
	Polyvinyl alcohol
	Polyethylene glycol
	MethoxYPolyethylene glycol
Polyvinyl alcohol	Dextran
	Hydroxypropyl dextran
	Methyl cellulose
Polyvinyl pyrrolidone	Dextran
	Hydroxypropyl dextran
	Methyl cellulose
Methyl cellulose	Dextran
	Hydroxypropyl dextran
Ethyl hydroxy ethyl cellulose	Dextran
Hydroxypropyl dextran	Dextran
Ficol/	Dextran

Table 2.2 Components of Polymer-Salt Phase Systems

Polymer	Salt
Polyethylene glycol (PEG)	Potassium phosphate
	Sodium sulphate
	Copper sulphate
	Sodium citrate
	Ferrous sulphate
	Ammonium sulphate
	Magnesium sulphate
Polypropylene glycol (PPG)	Potassium phosphate
MethoxYPolyethylene glycol	Potassium phosphate
Polyvinylpyrrolidone	Potassium phosphate

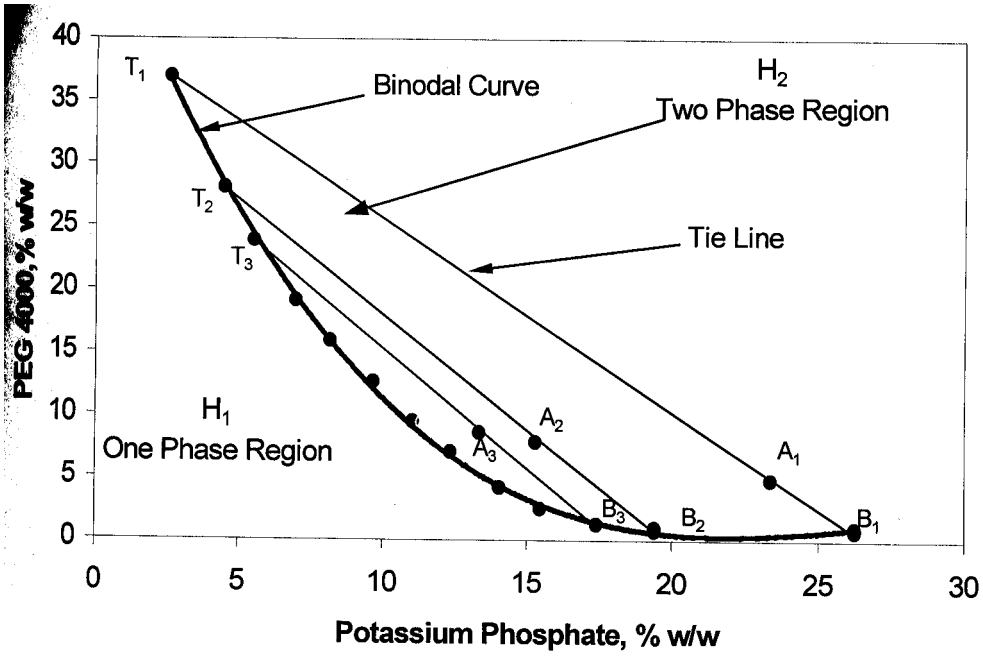


Figure 2.1. Phase Diagram for PEG 4000/potassium phosphate

Section 2A

Acoustic field assisted phase demixing

2A.1. INTRODUCTION

Aqueous two-phase extraction (ATPE) is finding applications in the area of biotechnology (Albertsson, 1986; Walter *et al.,* 1985; Zaslavasky, 1995, Raghavarao *et al.,* 1998). Major hindrances for the large-scale applications of this technique are high cost of the phase forming polymers and slow demixing of the equilibrated phases. The former aspect has been addressed to a great extent by adapting temperature induced phase separation for recovery and recycling of the polymers (Galaev and Mattiasson, 1993; Johansson *et al.,* 1997).

On the other hand, relatively less attention has been paid to the slow demixing rate in ATPE. Slow demixing rate of the thoroughly mixed phases after the extraction is due to small difference in densities between the phases, high viscosity of the individual phases and low interfacial tension.

To address slow rates of phase demixing, attempts have been made to enhance the demixing rates in ATPSs by the application of external fields each having its own drawbacks. Larsson and coworkers (Wikrostrom *et al.,* 1987) introduced magnetic field assisted demixing process by the addition of iron particles or ferro-fluids to the system which significantly enhanced the demixing rates in polyethylene glycol (PEG)/dextran system. However, this technique was not found useful when the PEG phase was dispersed. Raghavarao et al. (1998) have extensively studied electrokinetic demixing process which resulted in the enhancement of demixing rate markedly in

polymer/polymer systems, and is also not without limitations. In this case, there is need for fabrication of special equipment and addition of chemicals such as salts to the system and furthermore, the technique is not applicable for PEG/salt systems.

Recently, acoustic field assisted demixing was shown to result in 2 to 3 fold increase in demixing rate (Raghavarao and Todd, 2000; Srinivas *et al.,* 2000a, b, 2002). Conventional wisdom predicts that application of acoustic field causes mixing rather than demixing in the system, since it imparts energy to the system in order to achieve dynamic agitation, shear, cavitation, heating etc. This is the reason for the application of acoustic field for surface cleaning and disruption of microbial cell walls. However, this is not the case with respect to acoustic fields of higher frequency (MHz range) (Raghavarao and Todd, 2000) and in contrast it has resulted in mild circulation currents in the phase dispersion, which has increased the probability of droplet coalescence, eventually resulting in enhanced demixing rate. The method is simple, easy to scale-up and economical to operate (the acoustic transducer being high voltage and low current devices). Furthermore, readily available ultrasonic transducers could be employed.

Based on the results obtained during previous studies, it was thought desirable to examine if further enhancement in demixing rate is possible. Hence, the effect of varying the axial distance from the transducer on demixing rate was studied. The approach of the present study undertaken is

in contrary to the conventional understanding of acoustic effect with varying the axial distance from the transducer. Since, conventional wisdom predicts that acoustic intensity decreases with an increase in axial distance from the source of energy. In other words, phase demixing rate is expected to decrease with an increase in the axial distance. However, this was not the case in the preliminary experiments. Therefore, the effect of varying the axial distance from the transducer on demixing rate with respect to polymer/salt (PEG/potassium phosphate) and polymer/polymer (PEG/MDX) systems are undertaken.

2A.2. Theoretical aspects

Some of the researchers have assumed that approximately the Stoke's law (which was developed for a rigid sphere) can describe phase demixing under unit gravity;

$$V_s = \frac{D^2 \Delta\rho g}{18\ \mu_C} \qquad (2A.1)$$

where '*D*' is the droplet diameter, '$\Delta\rho$' is the density difference between the phases, 'μ_C' is the dynamic viscosity of the continuous phase, '*g*' is the acceleration due to gravity and 'V_s' is the droplet rise/fall velocity.

It is rightly indicated that for a swarm of droplets considerable deviations from the Stoke's law can be expected (Srinivas *et al.,* 2000b). As the droplets are not rigid the circulation inside them (induced by the drag of

the continuous phase) has to be taken into account as given by the Hadamard-Rybzcynski equation

$$V_s = \frac{D^2 \Delta\rho g}{18\mu_C}\left(\frac{3\mu_D + 3\mu_C}{3\mu_D + 2\mu_C}\right) \qquad (2A.2)$$

where 'μ_C' and 'μ_D' are the viscosities of the continuous and dispersed phases respectively.

Phase demixing can be seen as a combined effect of droplet rise/fall and droplet coalescence. If a single droplet is considered then the two steps are clearly in series. The droplet has to rise/fall to the interface and there it coalesce with the interface (Kaul *et al.,* 1995). In this situation, droplet migration will be the controlling step in the overall demixing process. In ATPSs, this situation can be seen when the phase volume ratios are either very high or very low. The time required for the separation of the two phases at this situation can be represented by equation (2A.2). However, this may not be the case generally. The presence of multiple droplets leads to considerable droplet-droplet interaction, which leads to coalescence as they rise/fall. This will increase the droplets size and in turn alters their rise/fall velocities (proportional to the square of the droplet diameter). In the present study, retardation of the drop rise velocity, due to high viscosity of continuous phase was found to play a major role on the demixing behavior.

To understand the enhancement of phase demixing rate with variation in axial distance from the transducer, it is necessary to understand the propagation of ultrasonic wave through a medium/dispersion. It is often assumed that ultrasonic wave travels with diameter equal to that of acoustic transducer. The field produced by an ultrasonic transducer can be divided into two regions as shown in Figure 2A.1; the near field and the far field. The near field extends to a distance 'x' from the front of the transducer and depends on the diameter of the transducer and the wavelength of the ultrasound.

$$x = \frac{d^2}{4\lambda} \qquad (2A.3)$$

In this region, the diameter of the ultrasonic beam is approximately equal to the diameter of the transducer. In the far field, the ultrasonic beam diverges (Figure 2A.1) leading to diffraction effects and ultrasonic reflections from the side walls of the material containing the sample. This results with the interference with the waves passing directly through the sample leading to appreciable decrease in efficacy of acoustic energy (McClements, 1997)

2A.3. Materials and methods

2A.3.1. Chemicals

Polyethylene Glycol (PEG; MW 6000) was purchased from Sisco Research Laboratories, Mumbai, India and Maltodextrin (MDX; MW 105000) was procured from Laxmi Starch Private Limited, Mumbai, India. Potassium

phosphate was procured from Sd Fine Chemicals, Mumbai, India and Sodium chloride was purchased from Qualigens Fine Chemicals, Mumbai, India.

2A.3.2. Phase system preparation

Phase systems were prepared by adding pre-determined weighed quantities of phase forming polymers and polymer-salt to distilled water, allowing them to dissolve for 2 hours and then mixed well for 1 hour using a magnetic stirrer. The well-mixed phases were allowed to separate into two phases in a separating funnel. The equilibrated and separated phases were collected and used as stock for demixing experiments. In this way 500 g of systems were prepared in each case.

2A.3.3. Phase demixing experiments

Figure 2A.2 shows the schematic diagram of the acoustically assisted demixing process. Demixing experiments were carried out at intermediate phase compositions of polymer/salt (PEG/potassium phosphate; phase composition 15/11) and polymer/polymer (PEG/MDX; phase compositions 10/30) systems at all the three volume ratios (30/70, 50/50, 70/30) by varying the axial distance from the transducer (0.0-5.2 cms). For these experiments demixing contactor made of polycarbonate (PC) and polypropylene (PP) (H/D ratio 1.46:1) were employed along with glass contactor. For, polymer/salt system ultrasonication was provided continuously from the

bottom, since the phase demixing rate being less resulted in no heat generation within the dispersion. In case of polymer/polymer system, for all the experiments ultrasonication was provided from the bottom in repeated cycles of 5 minutes sonication followed by 5 minutes under gravity. This was done to avoid excess heat generation in the system due to continuous application of acoustics. All the experiments were carried out with traveling wave mode of ultrasonication. The dispersion height was defined as the height of the non-separated dispersion (cloudy region). The time for complete phase demixing was taken, as the time required for clear horizontal interface to be formed. All the experiments were repeated thrice and average values reported. The readings were found well within ± 5% of error. Phase density and viscosity measurements were carried out as mentioned elsewhere (Srinivas *et al.,* 2000b) and the values are reported in Table 2A.1 and Table 2A.2.

2A.4. Results and discussion

In case of PEG/potassium phosphate system, demixing experiments were performed by varying the axial distance from the transducer and results are presented in Table 2A.3. It was observed that at this condition demixing rates enhanced up to 3.2 fold (only about 2-fold enhancement in demixing rate observed in case of PEG/potassium phosphate system without varying the axial distance; Srinivas *et al.,* 2000a, b). This is due to increase in the

intensity of acoustic field with varying the axial distance (axial distance varied up to 5.2 cms from the transducer), which is contrary to the conventional understanding. Another noteworthy observation made is that enhancement in demixing rate with variation in axial distance is well within the near field (in the present case near field distance from the transducer $L= d^2/4\lambda$ = 5.6 cm from equation 2A.3). The increase in intensity of acoustic field with increase in axial distance from the transducer is attributed to the lesser amount of ultrasonic wave reflections from the bottom surface of the demixing contactor. This results in less interruption for the propagation of acoustic field into the dispersion, thereby enhancing the demixing rate. It may also be noted that the enhancement in demixing rate is also considerably influenced when the material of demixing contactor is Polycarbonate/and Polypropylene instead of glass under otherwise similar conditions. This is attributed to higher transmissivity for acoustic energy through the demixing contactor. From the studies carried out it is evident that materials such as Polycarbonate and Polypropylene are more transparent to acoustic energy than glass, as they allow higher amount of acoustic energy to pass through it. Hence, all these factors contributes significantly towards hastening rate of coalescence between the droplets, increasing the buoyant velocity of the coalesced droplets (since velocity is proportional to the square of the droplet diameter), eventually enhancing the demixing rate in aqueous phase dispersions.

For PEG/MDX system, there was no appreciable enhancement in demixing rates under otherwise similar conditions. Under these conditions, acoustic intensity is reduced when the wave propagates through more denser/viscous dispersion. This results in attenuation of acoustic field in polymer/polymer system, with no further enhancement (above 2-fold) in demixing rate is observed.

2A.5. Conclusions

Acoustically assisted process has significantly enhanced the phase demixing rate and the rate increased with variation in axial distance from the transducer. Application of acoustic field has induced mild circulation currents in the phase dispersion, which in turn increased the probability of droplet coalescence, eventually resulting in enhanced demixing rate. The method appears to be simple, economical (low current and high voltage device), easy to scale-up and readily available ultrasonic transducers could be employed.

Table 2A. 1. Viscosity and density values for PEG/MDX system

Phase composition (PEG/MDX % w/w)	Viscosity (mPa. s)		Density (kg m^{-3})		Δ Density (kg m^{-3})
	Top	Bot.	Top	Bot.	
10/30*	13.5	85.1	1107.0	1226.4	119.4

Table 2A.2. Viscosity and density values for PEG/potassium phosphate system

Phase composition (PEG/potassium phosphate % w/w)	Viscosity (mPa. s)		Density (kg m^{-3})		Δ Density (kg m^{-3})
	Top	Bot.	Top	Bot.	
15/11*	34 .8	2.2	1080	1130	50

* rest is water

Bot.=Bottom

Table 2A. 3. Effect of varying the axial distance from the transducer on demixing time

Phase system: PEG/potassium phosphate: phase composition 15/11

Material of demixing contactor	Demixing times with increase in distance from the transducer (min)				
Volume ratio 30/70					
	Gravity (min)	0 cms	3 cms	3.8 cms	5.2 cms
Glass	8	5.1	--	--	--
Polycarbonate	2.45	2.34	2.28	2.13	1.34
Polypropylene	2.27	2.27	2.14	2.03	1.34
Volume ratio 50/50					
	Gravity (min)	0 cms	3 cms	3.8 cms	5.2 cms
Glass	10.29	5.32	--	--	--
Polycarbonate	3.24	2.5	2.4	2.29	2.29
Polypropylene	4.32	2.5	2.35	2.32	2.32
Volume ratio 70/30					
	Gravity (min)	0 cms	3 cms	3.8 cms	5.2 cms
Glass	5.22	3.29	--	--	--
Polycarbonate	2.0	1.6	1.56	1.5	1.23
Polypropylene	2.52	2.2	2.1	2.09	1.11

Mode of application of acoustic field is continuous

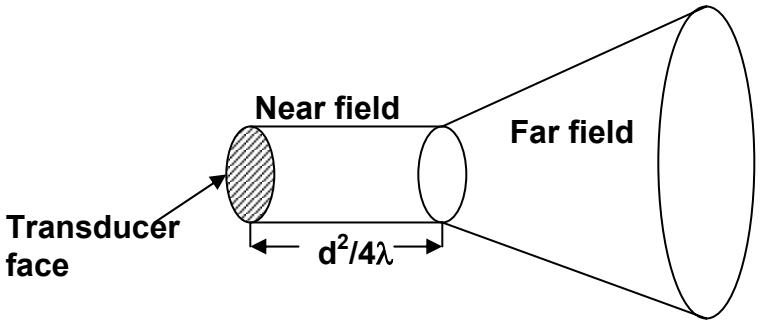


Figure 2A.1. Schematic representation of ultrasonic wave propagation (McClements, 1997)

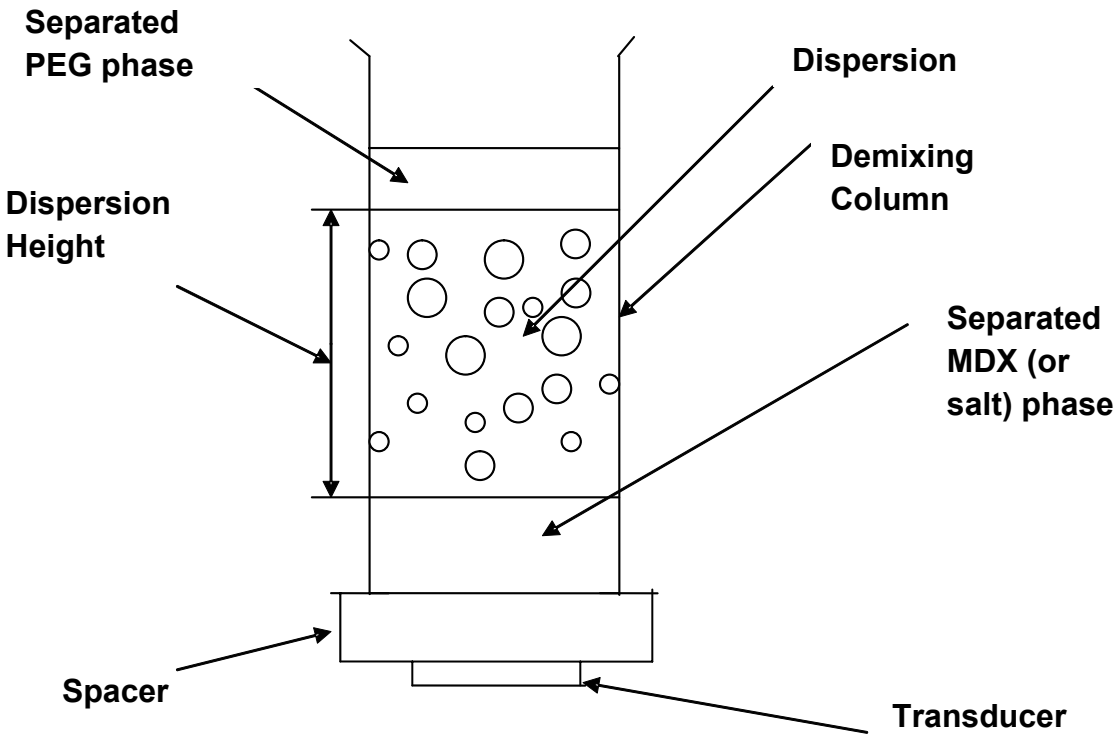


Figure 2A. 2. Schematic diagram of the acoustically assisted demixing process

SECTION 2B

Electrokinetic phase demixing

2B.1. Introduction

In the previous section (2A) of this chapter, the problems encountered due to slow rate of phase demixing in ATPSs, different methods and approaches available in literature to enhance the slow rate of demixing rate, drawbacks of these methods and reasons which necessitates the need for newer methods have been discussed. It was shown that acoustic field assisted demixing has enhanced the demixing rates significantly in aqueous polymer-salt two-phase systems (by about 3.2 fold enhancement). However, it is essential to examine if further enhancement in demixing rates is possible. In this regard electrokinetic demixing is attempted specially with polymer/salt systems for the first time. Polymer/salt systems are preferred industrially over polymer/polymer systems due to the advantages such as ease of handling, low phase viscosities, low cost and low demixing time. Conventional wisdom anticipates that electrokinetic demixing may not be feasible in case of PEG/salt systems due to high conductivity (which induces convective currents) and inherently high demixing rates of the phases. However, the present study revealed the opposite, with very interesting and unexpected results of faster demixing rates in presence of electric field. Encouraged by the initial results, a detailed study of electrokinetic demixing of polymer/salt systems has been undertaken. It is known that the kinetics of phase demixing strongly depends on physical properties such as density, viscosity and interfacial tension of the phases. These physical properties can

be varied by changing the phase composition of the system. Hence, the effect of phase composition on demixing times in zero electric field (gravity demixing for comparison) and in presence of applied electric field was studied. It is evident that volume ratio plays an important role in phase demixing and in concentrating the target solute in small volume, hence its effect was investigated in the presence and absence of electric field.

In case of polymer/salt systems, electric field polarity and electric field strength play a vital role in influencing the phase demixing rate. Therefore, the investigations were carried out at both the field polarities (normal and reverse) and at different field strengths. In the present study hydrodynamic flow electroosmotic flow model (HEF) which could explain clearly the influence of these parameters in enhancing the demixing rate of electrokinetic demixing of ATPSs.

2B.2. Theoretical Aspects

A colloidal particle having a positive surface charge is known to move towards cathode in the presence of an electric field (as depicted in the Figure 2B.1 (a)). Surprisingly opposite was observed in case of aqueous two-phase systems. In presence of electric field, PEG droplets having net positive charge moved towards anode and Dextran droplets having net negative charge moved towards cathode, contrary to the anticipated direction of their mobility (Raghavarao *et al.,* 1998; Brooks and Bamberger, 1982). This could be explained by electroosmotic flow model. Unlike the fixed (external) double

layer of a colloidal particle, a phase droplet will have an additional internal diffuse double layer. This causes electroosmotic flow inside the droplet, in the presence of electric field, schematically represented in the Figure 2B.1 (b). The electroosmotic circulation flow inside the droplet causes a tractor-treading motion that dominates over the electrophoretic motion and the droplet moves towards the electrode that is opposite to that predicted by conventional wisdom. For instance, PEG droplet moved to anode although conventional electrokinetics predicts it to migrate towards cathode in presence of electric field. In a column contactor (shown in Figure 2B.2) due to buoyancy the PEG droplet moves up. This causes hydrodynamic reaction flow in the salt continuous phase that is surrounding the droplets. Due to drag (or skin friction) this in turn induces hydrodynamic circulation flow inside the PEG droplet (shown in Figure 2B.1(c)).

Similarly, the electrophoretic motion of drop also causes reaction flow in the continuous phase surrounding the droplet. The electroosmotic and hydrodynamic circulation flows inside the droplet will be in the same direction or in opposite direction depending on the polarity of the electric field vis-à-vis, the dispersed phase. Similar is the case with reaction flows in the continuous phase (induced by buoyant motion and electrophoretic motion of the drop) will be in the same direction or in opposite direction to each other depending on the polarity vis-à-vis, the dispersed phase. The intensities of all these flows increase with an increase in drop diameter.

This hydrodynamic flow electroosmotic flow model which explains more clearly the electrokinetic demixing of ATPSs, in column where gravity (buoyancy) also comes into picture even in presence of the electric field is developed (unlike microelectrophoresis). Above a critical droplet size buoyancy dominates and below it electrokinetics dominates the demixing process in a column. In order to appreciate this it is required to develop a model equation for critical droplet diameter by considering both buoyancy and electrokinetic components.

When a polymer and salt are dissolved in water above the critical concentration, phase separation occurs. Both the phases contain three components although one will be rich in polymer (with less salt concentration) while the other will be rich in salt (with less polymer concentration). The unequal distribution of salt between the phases leads to an electrical potential across the interface and an apparent electrokinetic potential at the surface of the dispersed phase droplets (Brooks *et al.,* 1984). As a consequence of the latter, these droplets move in the continuous phase in the presence of an electric field (Levine, 1982; Van Alstine; 1987; Rudge and Todd, 1990).

This electrophoretic mobility is defined by

$$\mu_E = \frac{\upsilon_E}{E} \qquad (2B.1)$$

where 'μ_E' is the electrophoretic mobility ($cm^2/V.s$), 'υ_E' the droplet

electrophoretic velocity (cm/s) and 'E' the electric field strength (V/cm). This mobility is exploited for the electrophoretic phase demixing. The velocity of a suspended dispersed phase droplet is proportional to the force on it (Robinson and Stokes 1960; Cussler, 1984)

$$-\upsilon = \left(\frac{1}{3\pi\eta_c d}\right)\left[\frac{\partial\mu}{\partial z}\right] \qquad (2B.2)$$

where 'υ' is the velocity, 'μ' is the chemical potential, 'η_c' is the viscosity of the continuous phase, '*d*' is the diameter of the droplet.

The chemical potential accounts for all types of forces acting on the droplet namely, Brownian, gravitational and electrokinetic (Belter *et al.,* 1988). Substituting the expression for 'μ' in equation (2B.2), accounting for the internal circulation of the droplet as per Hadmard and Rybczynski's equation (Levich, 1962), the equation for the motion of the droplet under these forces can be written as

$$-\upsilon = \left(\frac{k_B T}{3\pi\eta_C d}\right)\frac{1}{C}\frac{dC}{dz} + \left(\frac{d^2(\rho_D - \rho_c)g}{18\eta_c}\right)\left[\frac{3\eta_D + 3\eta_C}{3\eta_D + 2\eta_C}\right] + \left(\frac{d\,\sigma_E E}{2\left(3\eta_D + 2\eta_C + \frac{\sigma_E^2}{\lambda}\right)}\right) \qquad (2B.3)$$

where 'ρ_D' is the dispersed phase or droplet phase density, '*E*' is the electric field strength (V/cm), 'σ_E' is the surface charge density (C/cm^2), 'k_B' is the

Boltzman constant, 'η_D' is viscosity of the continuous phase and 'λ' is a function of the conductivities of the dispersed and the continuous phases.

In the present study it was observed that size of the droplet is much larger than the droplets of microelectrophoresis or typical colloidal particles, and the drops grow in size due to coalescence during electrokinetic demixing. Hence, the contribution from (first term) diffusion can be ignored. The buoyancy/gravity as well as electrophoretic forces will be acting during phase demixing in the column even in presence of an electric field (equation 2B. 3). When the electric field is applied in normal polarity (anode at the top), the gravity and electric field work in the same direction. In this situation probability of droplet coalescence will be relatively less (Raghavarao *et al.,* 1998). In contrast, when the electric field is applied in reverse polarity, these fields work opposite to each other and the droplets are held in the dispersion zone against gravity. In this situation droplet coalescence will be higher when compared to the previous situation due to higher contact time and high rate of droplet collisions. It may be noted that the buoyant velocity is proportional to *'d^2'* (square of the diameter) while electrophoretic velocity is proportional only to'd' (unit power). Hence above critical droplet diameter buoyancy forces dominate over the electrophoretic forces. This critical drop diameter (d_{CR}), can be obtained by equating the buoyant component (second term) to the electrophoretic component (third term) of the velocity as given by equation 2B.4.

$$d_{CR} = \frac{3\sigma_E E \eta_C (3\eta_D + 2\eta_C)}{g(\rho_D - \rho_C)(\eta_D + \eta_C)(3\eta_D + 2\eta_C + \frac{\sigma_E^2}{\lambda})} \qquad (2B.4)$$

2B.3. Materials and methods

Polyethylene glycol (PEG; molecular weight 6000) was procured from Loba Chemie, Mumbai, India. Potassium dihydrogen phosphate, di-Potassium hydrogen phosphate and sodium chloride were obtained from Ranbaxy Chemicals, Punjab, India. Platinum electrodes and electrical power pack (Model: 8E102-38) were purchased from Bangalore Genie, Bangalore. The phase systems were prepared using distilled water from Millipore Inc., distillation unit.

2B.3.1. Preparation of phase systems

Phase systems comprising of PEG/potassium phosphate (K_2HPO_4: KH_2PO_4 :: 1.82:1) (7/11, 15/11, 35/11) was prepared by adding a known quantity of distilled water, so as to make the total composition of the system 100% (w/w). After dissolving the components, the system was mixed thoroughly for about 60 minutes and allowed to equilibrate overnight in a separating funnel. The equilibrated top and bottom phases were then separated and used as stock solutions for further experiments. Around 3000 g of systems were prepared for each phase composition. Phase dispersion was prepared at

different volume ratios of 15/35, 25/25 and 35/15 for conducting the phase demixing experiments.

2B.3.2. Phase demixing experiments

A schematic representation of the experimental apparatus is shown in Figure 2B.1. All the demixing experiments were carried out in a water-jacketed electrophoresis column filled with 50 ml of freshly prepared dispersion of various volume ratios. The dispersion column is in contact with buffer solutions reservoirs, which has provision to house side-arm platinum electrodes. These electrodes support and contact the phase dispersion through 15% polyacrylamide gel plugs. The platinum electrodes in the side arms are in turn connected to electrophoresis power pack for electrical power supply. The demixing experiments were carried out both in normal polarity (upper electrode is held positive) and reverse polarity (upper electrode is held negative) in triplicate and average values are reported.

2B.3.3. Estimation of physical properties

Density and viscosity of the individual phases were measured using specific gravity bottles and an Ostwald U–tube viscometer of 10-ml capacity, respectively. All the measurements were carried out in triplicate at 27± 1°C and average values are reported.

2B.4. Results and discussion

2B.4.1. Effect of electric field polarity

In the present study electrokinetic demixing experiments were carried out in presence of electric field at both normal and reverse polarities for three selected phase systems (Table 2B.2 and Table 2B.3) at three different volume ratios namely 15/35, 25/25 and 35/15. At low phase composition (7/11), effect of electric field strength was significant at normal polarity when PEG rich phase is the dispersed phase (volume ratio 15/35). Here, the electric field and the buoyancy act in the same direction. Further, in normal polarity, hydrodynamic flow electroosmotic flow (in the phase droplets) and the reaction flow (outside the droplet) will be in the same direction (as explained in Section 2B.2). As a consequence the droplets are pulled rapidly resulting in enhancement of phase demixing rate by about 67% (Table 2B.2). At these conditions, as observed the electric field was very effective at the reverse polarity also. Here, the electric field and the buoyancy work in the opposite direction due to which hydrodynamic flow electroosmotic flow (inside the droplets) are opposite to the reaction flows (outside the droplets). This effect pulls the droplets against their natural buoyancy and holds them in the dispersion zone, which enables the droplets to coalesce and thereby increases the droplet diameter (as explained in Section 2B.2). Once the droplet attains the critical diameter *'d_{CR}'*, as given by equation (2B.4), the buoyancy takes over (since migration velocity is proportional to the square of

the droplet diameter) resulting in faster demixing rate (by about 62-74%; Table 2B.3). In contrast, when salt rich phase is the dispersed phase (volume ratio of 25/25 and 35/15), the effect of the electric field is very less in case of normal polarity (only 12.5% increase in demixing rate at the volume ratio of 35/15; Table 2B.2). The high viscosity of the PEG continuous phase, causes higher drag, which has resulted in the breakage of dispersed (salt) phase droplets mainly due to the low interfacial tension (as the phase system is very close to the binodal) thereby decreasing the effective electric field strength. When the electric field is changed to reverse polarity, the demixing rate decreased further, since the electric field at this polarity retards their natural buoyant motion of the droplets and higher continuous phase viscosity retards their coalescence. This can be observed from the fact that at these conditions the demixing rate is lower than the demixing rate under gravity.

At intermediate phase composition (15/11), the effect of electric field is similar to that of low phase composition when PEG rich phase is the dispersed phase, at both normal (by about 53.7%; Table 2B.2) and reverse polarity (about 56.9%; Table 2B.3). However, the behavior changed significantly when salt rich phase formed the dispersed phase, at both the field polarities (normal and reverse). In normal polarity, due to the increase in phase composition the interfacial tension increased and the occurrence of drop breakage (observed at low phase composition) did not take place. Hence, electric field and buoyancy act in synergy enhancing the phase

demixing rates (by about 69%; Table 2B.2). When the electric field is changed to reverse polarity, the demixing rate increased further (by about 77%; Table 2B.3) for the similar reasons as described earlier in this section.

At high phase composition (35/11) also, the effect of electric field strength is similar to that at low and intermediate phase composition. At low volume ratio (15/35) of this phase composition, the demixing rate has decreased in presence of electric field instead of increasing, at both normal and reverse polarity. This is due to the high conductivity of the salt rich continuous phase (since salt concentration increases with increase in phase composition), which reduces the effective electric field strength. The high quantum of continuous phase having high conductivity causes enormous heating of the dispersion. This leads to generation of intense natural convective currents causing churning (mixing) rather than demixing of the phases, resulting in the observed behavior. When PEG rich phase formed the continuous phase the observed demixing trend is similar to that of intermediate phase composition at both the polarities. However, the extent of demixing rate has decreased (only 18.5% and 22.3% at 25/25 volume ratio, irrespective of the polarities; Table 2B.2 and Table 2B.3). The effect of electric field is less mainly due to the high viscosity of the continuous phase, which retards the buoyant motion of the phase droplets. This effect could be observed prominently (at the volume ratio of 35/15), when the electric field is changed to reverse polarity. At this polarity even though the droplets are held

in the dispersion by the electric field against the buoyancy for the reasons as explained earlier. The droplets are not able to collide with each other due to high continuous phase viscosity, thereby retarding the probability of droplet coalescence. Hence, the demixing rate is low when compared to that at intermediate phase composition (the demixing rate is lower than that of gravity in presence of electric field by about -51.51%).

2B.4.2. Effect of electric field strength on demixing

Electrokinetic demixing experiments were carried out at both the field polarities (normal and reverse) by varying the electric field strength in the range of 0-4.4 V/cm. At low phase composition (7/11), when PEG forms the dispersed phase (15/35 volume ratio) at normal polarity, the demixing rate has decreased slightly at lower field strengths and has increased with further increase in the electric field strength (Figure 2B.3). This initial decrease in demixing rate at lower field strength is attributed to the domination of electric field over buoyancy of the droplets, as their size is small (greater than critical diameter; equation 2B.4) In other words, the electric field is pulling the droplets too rapidly without allowing them to grow by coalescence in the dispersion zone. As the field strength increases, the droplets are pulled more rapidly to the interface where they accumulate and coalesce faster as there are more droplets in the vicinity of one another. As the droplet attains critical diameter buoyancy takes over resulting in faster demixing rate (effect of field strength is proportional to droplet diameter; equation 2B.4; Raghavarao *et*

al., 1998). At these conditions, in case of reverse polarity, the demixing rate has decreased continuously with an increase in electric field strength (at 15/35 volume ratio; Figure 2B.4). Here, electric field and buoyancy act in opposite direction, which has already been explained based on hydrodynamic flow electroosmotic flow model. Under these circumstances the electric field pulls the droplets against their natural buoyancy and holds them in the dispersion zone. This results in increased droplet collision and in turn higher probability of droplet coalescence, thereby enhancing the demixing rate, which has already been explained based on hydrodynamic flow electroosmotic flow model. At the volume ratios of 25/25 and 35/15 where the high viscous PEG rich phase forms the continuous phase, the demixing rate has practically decreased with an increase in electric field strength at both the polarities. In case of normal polarity, initially there is a decrease followed by an increase in demixing rate with an increase in electric field strength (Figure 2B.3). This is due to the synergistic effect of electric field and buoyancy (which are acting in the same direction) explained based on hydrodynamic flow electroosmotic flow model. When the field polarity is reversed, the demixing rate decreased continuously with an increase in electric field strength (especially at 35/15 volume ratio). As already explained, the decrease in demixing rate at this condition is due to retardation of droplet natural buoyant motion by the electric field and high viscous continuous phase retards the droplet coalescence. Further, the

extent of reduction in demixing rate was higher in reverse polarity when compared to normal polarity. It is due to the inability of the electric field to hold the droplets in the dispersion zone against their natural buoyancy, and also due to viscous resistance offered by the high viscous continuous PEG phase. This results in less frequency of droplet collision at low electric field strength, thereby decreasing the probability of droplet coalescence. In other words, at lower electric field strength, neither buoyancy nor electric field is aiding the demixing rate. However, at higher field strength the electric field is able to overcome the viscous resistance offered by PEG continuous phase. As the electric field overcomes viscous resistance, the droplets are forced to remain in the dispersion zone, which results in the droplet growth by coalescence (already explained based on hydrodynamic flow electroosmotic flow model in Section 2B.2). Once the droplet attains critical diameter (equation 2B.4) the buoyancy dominates over the electric field causing faster demixing rate (Figure 2B.4). At high volume ratio (35/15) also, PEG rich phase being the continuous phase the demixing behavior with respect to electric field strength is similar to that of 25/25 volume ratio. At these conditions, in case of normal polarity the effect of electric field on demixing rate is low (Figure 2B.3). Low interfacial tension of the system and higher drag offered by high viscous PEG continuous phase has resulted in drop breakage of dispersed (salt) phase droplets. This phenomenon has made the electric field less effective, resulting in lower demixing rate. Under otherwise

similar conditions, in case of reverse polarity, the demixing rate has further decreased since the electric field at this polarity retards the droplet natural buoyant motion and high viscosity of the continuous phase retards the droplet mobility/collision and in turn droplet coalescence (Figure 2B.4). In addition, at this volume ratio (35/15) due to high quantum of PEG continuous phase the electric field is not able to overcome the viscous resistance as in the case of 25/25 volume ratio. This results in a gradual decrease in demixing rate (without reaching the maxima) with an increase in field strength as shown in Figure 2B.4.

At intermediate phase composition (15/11), at volume ratio of 15/35, in case of normal polarity the demixing behavior pattern (Figure 2B.5) is similar to that at low phase composition (7/11) for the same reason (synergy) as explained in Section 2B.4.1. In case of reverse polarity, under similar conditions the extent of decrease in demixing rate at this volume ratio is due to high conductivity of the salt continuous phase which makes the electric field less effective. This is due to inability of the electric field to hold the droplets against their natural buoyancy in the dispersion zone. At higher electric field strength the ability to hold the droplets in the dispersion zone increases, causing faster demixing rate (Figure 2B.6) as explained in Section 2B.4.1. At the volume ratios of 25/25 and 35/15, when PEG forms the continuous phase the demixing rate has shown an increasing trend under similar conditions at both the polarities. It is primarily due to increase in

interfacial tension (due to the increase in phase composition) and no drop breakage of dispersed (salt) phase droplets (unlike at 7/11 phase composition under otherwise similar conditions). Hence, it is the synergy between electric field and buoyancy, which has enhanced the demixing rate at normal polarity. When the polarity is reversed the demixing rate has increased continuously (Figure 2B.5 and Figure 2B.6) for the reasons as described in previous section.

As the phase composition increases to 35/11, at the volume ratio of 15/35, (PEG rich phase forming the dispersed phase), the demixing rate has decreased initially and then has increased with an increase in electric field strength at both the polarities as shown in Figure 2B.7 and Figure 2B.8. This trend is similar to that observed at low and intermediate phase compositions. However, under the similar conditions the extent of demixing rate has decreased, due to high conductivity of salt continuous phase, which reduces the effective electric field strength. The high quantum of continuous phase due to high conductivity causes enormous heating of the dispersion setting in natural convective currents, which in turn causes churning (mixing) instead of demixing. When high viscous PEG rich phase forms the continuous phase (25/25 and 35/15 volume ratio), the demixing rate has increased further with an increase in electric field strength at normal polarity. It is due to synergy between electric field and buoyancy which aid in phase demixing as explained earlier. However, when compared to low and intermediate phase

compositions under otherwise similar conditions, the extent of demixing rate has decreased at both the field polarities. The effect of electric field is less due to high viscosity of the PEG continuous phase, which retards the natural buoyant motion of the phase droplets. In fact, this phenomenon is more prominent at reverse polarity (volume ratio of 35/15) where the droplets are held together in the dispersion by electric field against their natural buoyancy. The droplet mobility/collision is retarded due to viscous resistance offered by the PEG continuous phase. This result in reduction of demixing rate due to lower droplet coalescence, in fact the extent of demixing rate is much lower than gravity. However, the electric field at the volume ratio of 25/25, overcomes the viscous resistance being offered, thereby causing faster demixing rate with an increase in electric field strength.

2B.4.3. Effect of volume ratio on phase demixing

From the demixing experiments carried out (at zero field and in presence of electric field) it can be noted that volume ratio plays a significant role in phase demixing as seen from Figure 2B.9 and Figure 2B.10. At all the three phase compositions, under gravity conditions (Table 2B. 4) the demixing rate has decreased initially and then has increased with an increase in volume ratio. The demixing rate is the lowest at the volume ratio of 25/25 (phase inversion point that is when dispersed phase changes to continuous phase). At this volume ratio, visual observations indicated assembling of dispersed droplets near the interface forming a densely

packed zone without coalescing with already formed PEG layer. The formation of the densely packed zone is due to lower droplet coalescence with the interface, when compared to migration rate of the droplets, thereby resulting in lower demixing rate at the volume ratio of 25/25.

At low phase composition of 7/11, the demixing rate has increased when the continuous phase changed over from salt rich phase (15/35 volume ratio) to PEG rich phase (35/15 volume ratio). This is due to larger size of the dispersed phase droplets which results in higher frequency of droplet collision. This in turn results in higher probability of droplet coalescence, thereby enhancing the demixing rate. In contrast, under similar conditions the demixing rate decreased when phase inversion occurred (change of volume ratio from 15/35 to 35/15) at intermediate and higher phase composition. This is because of the resistance offered by high viscous PEG rich continuous phase (due to increase in phase composition) which subsequently results in lower droplet coalescence. In presence of electric field, in normal polarity at low phase composition (7/11), the demixing rate has decreased with an increase in volume ratio as shown in Figure 2B.9. Under similar conditions in case of reverse polarity, the decrease in demixing rate with an increase in volume ratio is much higher (Figure 2B.10). In fact the demixing rate is lower than that of gravity for the same reasons as Section 2B.4.1 and Section 2B.4.2.

In case of 15/11 phase composition with respect to normal and reverse polarity, the demixing rate is high when compared to gravity with an increase in volume ratio for the reasons as explained earlier. The observed demixing trend is similar to that of gravity demixing with an initial decrease and an increase in demixing rate with increase in volume ratios as shown in Figure 2B.9 and Figure 2B.10. At high phase composition (35/11) with increase in volume ratio the demixing rate has increased in presence of electric field with respect to normal polarity (Figure 2B.9). However, under similar conditions the demixing trend at reverse polarity was different. In fact with increase in volume ratio the demixing rate has decreased much below gravity for the reasons explained earlier sections (Figure 2B.10).

2B.4.4. Effect of phase composition on demixing

An increase in phase composition increases the interfacial tension, density difference and the phase viscosities. The combined effect of these properties results in an increase in average droplet size with increase in phase composition. This results in faster rise/fall of droplets thereby increasing the demixing rate as shown in Table 2B.4 under gravity (zero field).

In contrast as observed in presence of electric field (both normal and reverse polarity) from Table 2B.2 and Table 2B.3, the demixing rate has decreased with an increase in phase composition. The decrease in demixing rate is due to combined effect of volume ratio and phase composition. When

salt rich phase is forming the continuous phase, the conductivity of the dispersion increases with an increase in phase composition. This increase in conductivity of the dispersion results in reducing the effective electric field strength as explained in Section 2B.4.1 and Section 2B.4.2. When PEG rich phase is the continuous phase, the phase viscosity increases with an increase in phase composition. The high viscosity of the continuous phase results in the retardation of droplet buoyant motion (more prominent in reverse polarity). Further, electric field reaches attenuation (at high phase composition), thereby reducing the effect of electric field intensity. It may be concluded that phase composition and volume ratio act synergistically in influencing the demixing rate in presence of electric field also, as observed in the present study.

2B. 5. Conclusions

Application of electric field has enhanced the phase demixing rate significantly even in polymer/salt systems, which was not expected keeping in view of high conductivity of the salt phase. The phase volume ratio and phase composition were found to play a decisive role in kinetics of phase demixing in presence of electric field also. The effect of various process parameters such as electric field polarity, electric field strength, phase volume ratio and phase composition in influencing the demixing rate during electrokinetic demixing of ATPSs could be explained based on hydrodynamic flow-electroosmotic flow model.

Table 2B.1. Density and viscosity measurements for the phase compositions studied

Phase compositions	Viscosity m Pa s		Density kg/m^3		Density difference
	Top	Bottom	Top	Bottom	
7/11	12.5	1.3	1082.0	1113.9	31.9
15/11	28.9	1.4	1093.0	1130.0	37.0
35/11	110.1	1.5	1098.6	1390.0	291.4

Table 2B.2. Reduction in demixing at varying field strength for different phase compositions and phase volume ratio (normal polarity).

Phase composition 7/11					
Volume ratio 15/35		Volume ratio 25/25		Volume ratio 35/15	
Field strength, V/cm	% reduction	Field strength, V/cm	% reduction	Field strength, V/cm	% reduction
0.0	---	0.0	---	0.0	---
0.87	-15.76	0.87	-43.93	0.87	-108.33
1.74	-10.25	1.74	-36.36	1.74	-184.72
2.61	+17.86	2.61	-36.36	2.61	-94.44
3.48	+44.87	3.48	+35.61	3.48	-52.77
4.35	+66.92	4.35	+69.69	4.35	+12.5
Phase composition 15/11					
Volume ratio 15/35		Volume ratio 25/25		Volume ratio 35/15	
Field strength, V/cm	% reduction	Field strength, V/cm	% reduction	Field strength, V/cm	% reduction
0.0	---	0.0	---	0.0	---
0.87	-45.03	0.87	+28.33	0.87	+22.17
1.74	-8.609	1.74	+33.33	1.74	+41.98
2.61	+19.21	2.61	+50.0	2.61	+48.11
3.48	+27.15	3.48	+58.83	3.48	+68.16
4.35	+53.64	4.35	+66.66	4.35	+68.86
Phase composition 35/11					
Volume ratio 15/35		Volume ratio 25/25		Volume ratio 35/15	
Field strength, V/cm	% reduction	Field strength, V/cm	% reduction	Field strength, V/cm	% reduction
0.0	---	0.0	---	0.0	---
0.87	-26.42	0.87	+13.0	0.87	+1.818
1.74	-48.21	1.74	+9.708	1.74	+3.64
2.61	----*	2.61	+14.0	2.61	+6.06
3.48	----*	3.48	+19.41	3.48	+9.09
4.35	----*	4.35	+18.45	4.35	+33.33

∗mixing observed instead of demixing

Table 2B.3. Reduction in demixing at varying field strength for different phase compositions and phase volume ratio (reverse polarity).

Phase composition 7/11					
Volume ratio 15/35		Volume ratio 25/25		Volume ratio 35/15	
Field strength, V/cm	% reduction	Field strength, V/cm	% reduction	Field strength, V/cm	% reduction
0.0	---	0.0	---	0.0	---
0.87	+0.77	0.87	-112.12	0.87	-108.33
1.74	+22.82	1.74	-81.81	1.74	-52.77
2.61	+22.82	2.61	-21.21	2.61	-163.88
3.48	+61.96	3.48	-21.21	3.48	-191.66
4.35	+73.53	4.35	+61.12	4.35	-108.33
Phase composition 15/11					
Volume ratio 15/35		Volume ratio 25/25		Volume ratio 35/15	
Field strength, V/cm	% reduction	Field strength, V/cm	% reduction	Field strength, V/cm	% reduction
0.0	---	0.0	---	0.0	---
0.87	-45.7	0.87	+16.33	0.87	+24.52
1.74	-40.72	1.74	+30.0	1.74	+52.83
2.61	-5.62	2.61	+58.16	2.61	+66.98
3.48	+40.4	3.48	+58.83	3.48	+70.75
4.35	+56.92	4.35	+66.66	4.35	+76.41
Phase composition 35/11					
Volume ratio 15/35		Volume ratio 25/25		Volume ratio 35/15	
Field strength, V/cm	% reduction	Field strength, V/cm	% reduction	Field strength, V/cm	% reduction
0.0	---	0.0	---	0.0	---
0.87	-83.92	0.87	-9.0	0.87	-30.30
1.74	-53.57	1.74	+2.91	1.74	-30.30
2.61	----*	2.61	+22.33	2.61	-27.27
3.48	----*	3.48	+16.5	3.48	-30.30
4.35	----*	4.35	+22.3	4.35	-51.51

* mixing observed instead of demixing

Table 2B.4. Demixing times at different phase compositions and volume ratios under gravity

Phase composition 7/11		
Volume ratio 15/35	Volume ratio 25/25	Volume ratio 35/15
Demixing time, min	Demixing time, min	Demixing time, min
9.07	13.2	7.2
Phase composition 15/11		
Volume ratio 15/35	Volume ratio 25/25	Volume ratio 35/15
Demixing time, min	Demixing time, min	Demixing time, min
3.02	6.0	4.24
Phase composition 35/11		
Volume ratio 15/35	Volume ratio 25/25	Volume ratio 35/15
Demixing time, min	Demixing time, min	Demixing time, min
2.8	5.15	3.3

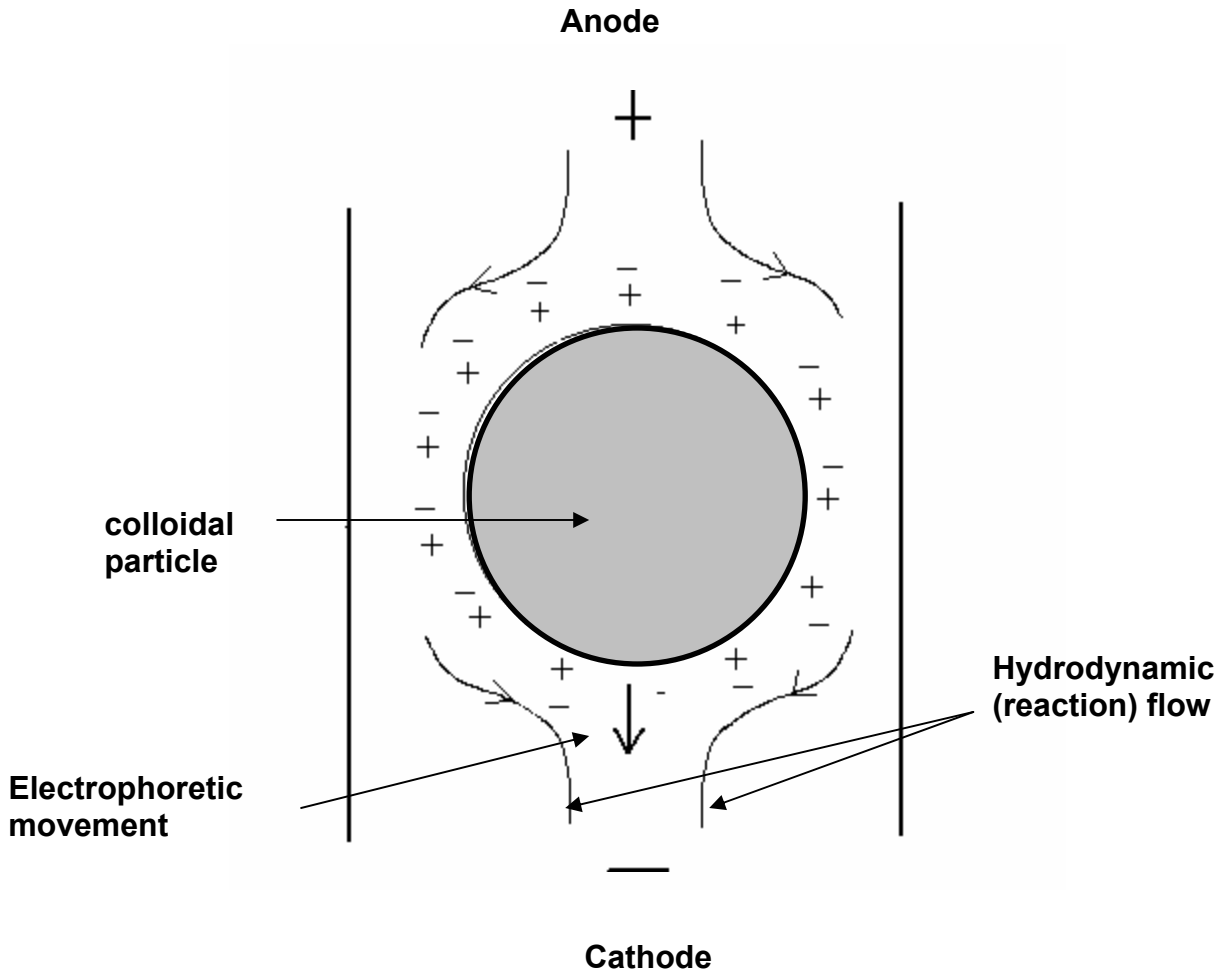


Figure 2B.1 (a). Conceptual diagram of rigid colliodal particle movement in presence of electric field

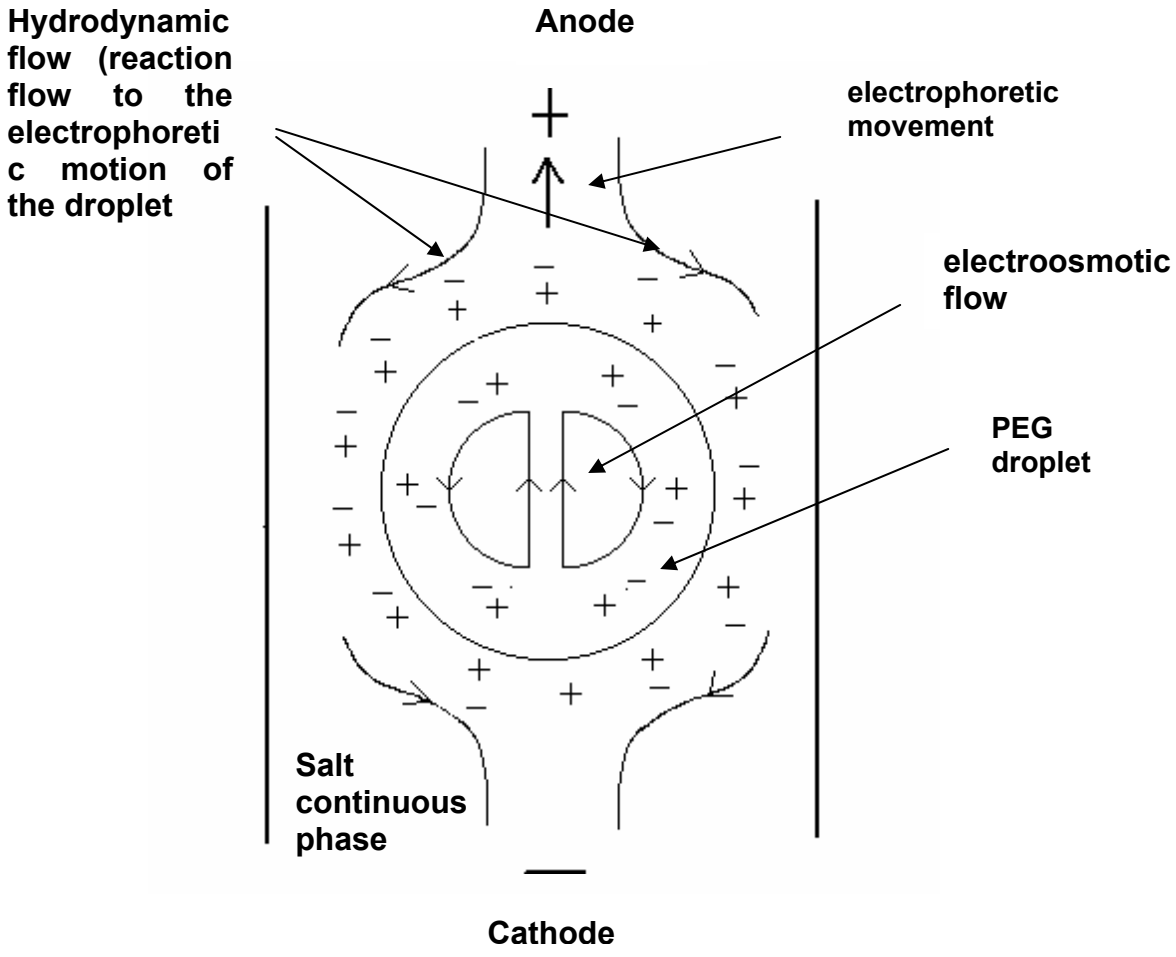


Figure 2B. 1(b). Conceptual diagram of electrophoretic flow in the phase droplet in presence of electric field

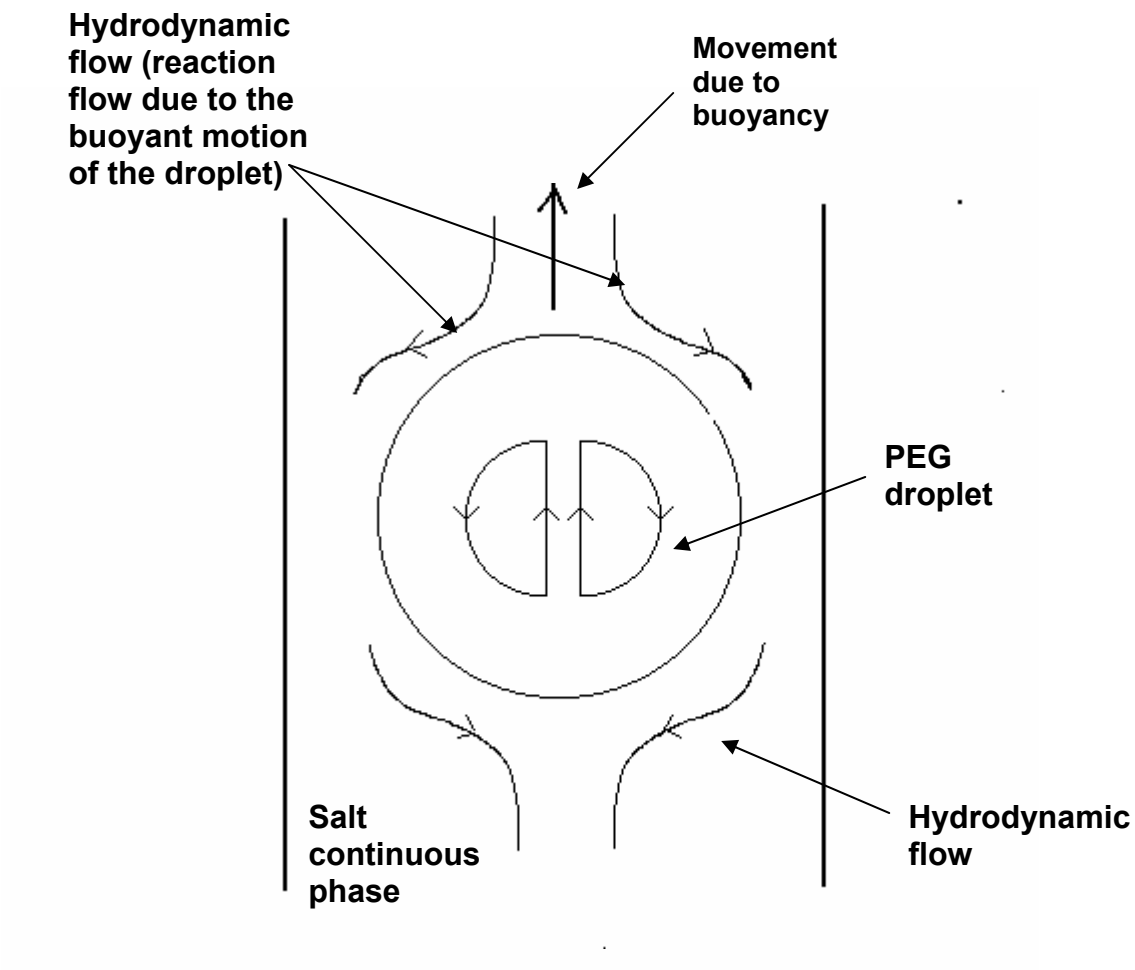


Figure 2B.1(c). Conceptual diagram of hydrodynamic flow in the phase droplet

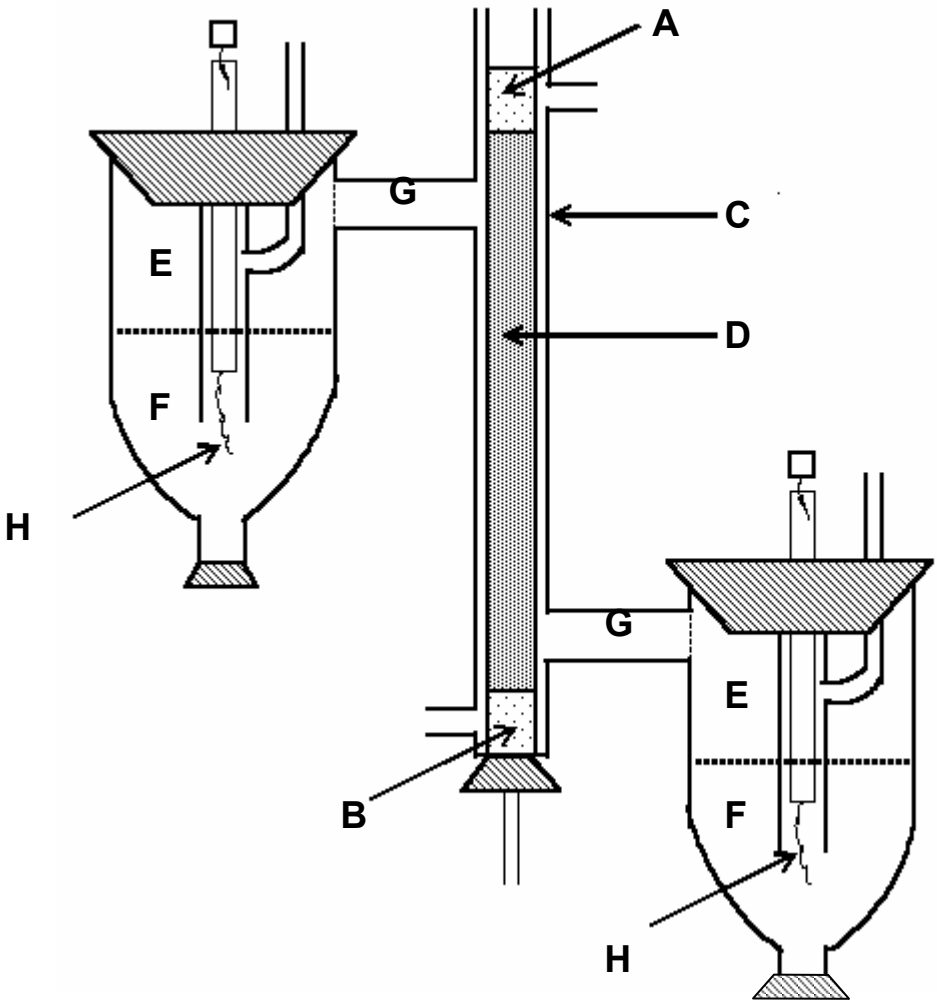


Figure 2B. 2. Schematic representation of experimental set-up for electrokinetic demixing of aqueous two-phase systems

A - Separated top phase; B - Separated bottom phase;
C-Cooling jacket; D-aqueous two-phase dispersion;
E-potassium phosphate buffer; F-saturated sodium chloride;
G-15% polyacrylamide gel plug; H-Platinum electrodes

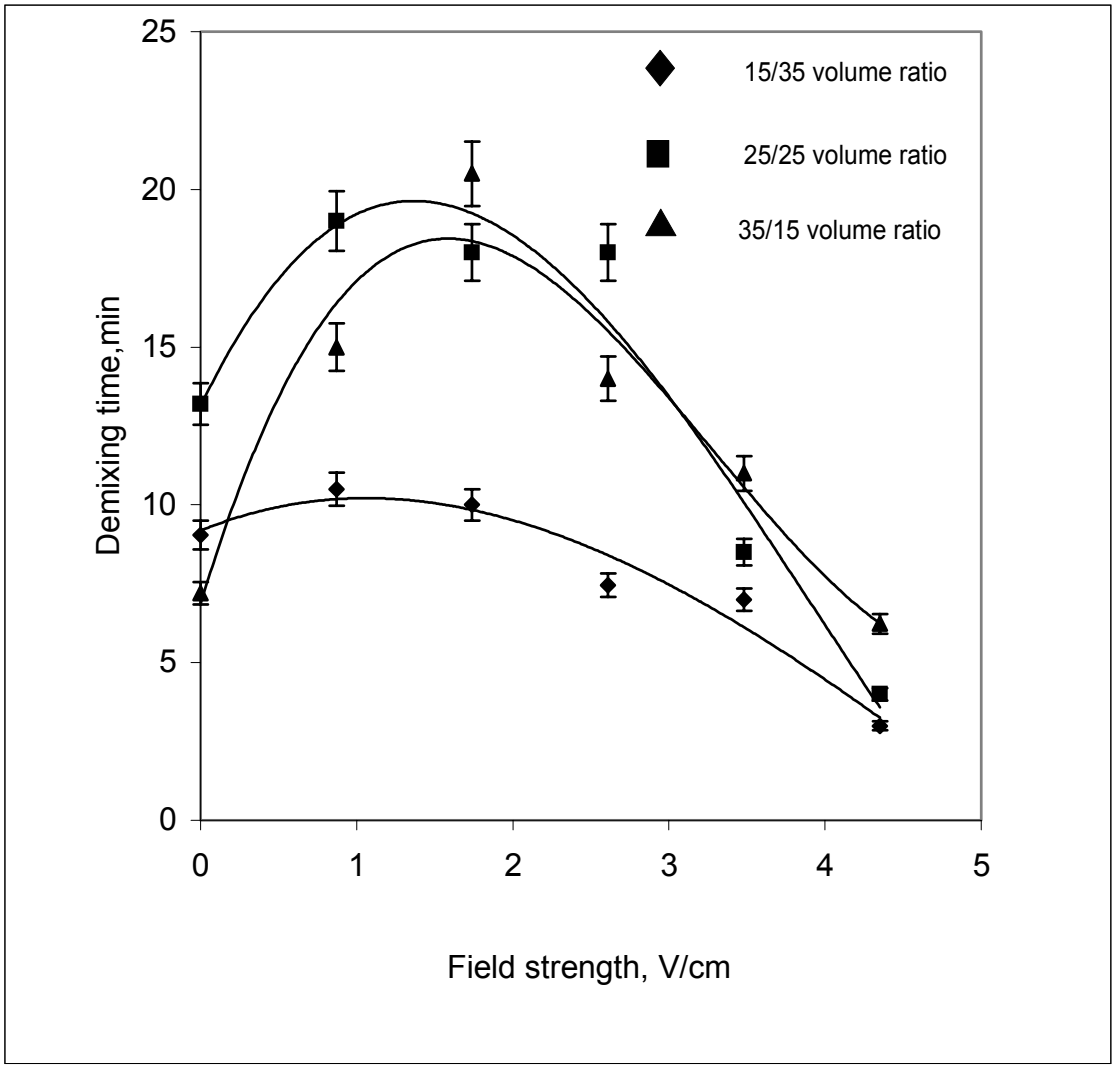


Figure 2B.3. Effect of field strength on demixing time at 7/11 (normal polarity)

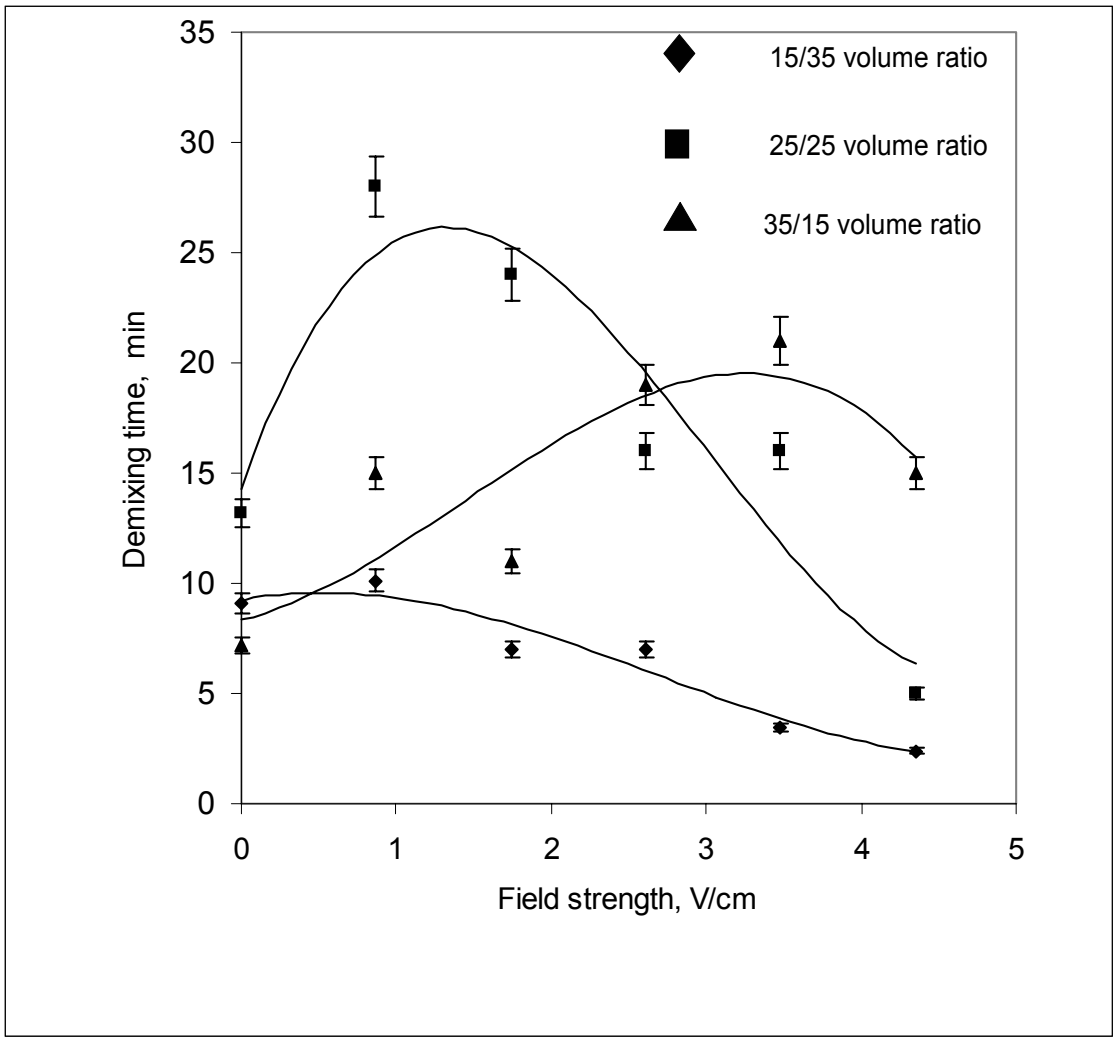


Figure 2B.4. Effect of field strength on demixing time at 7/11 (reverse polarity)

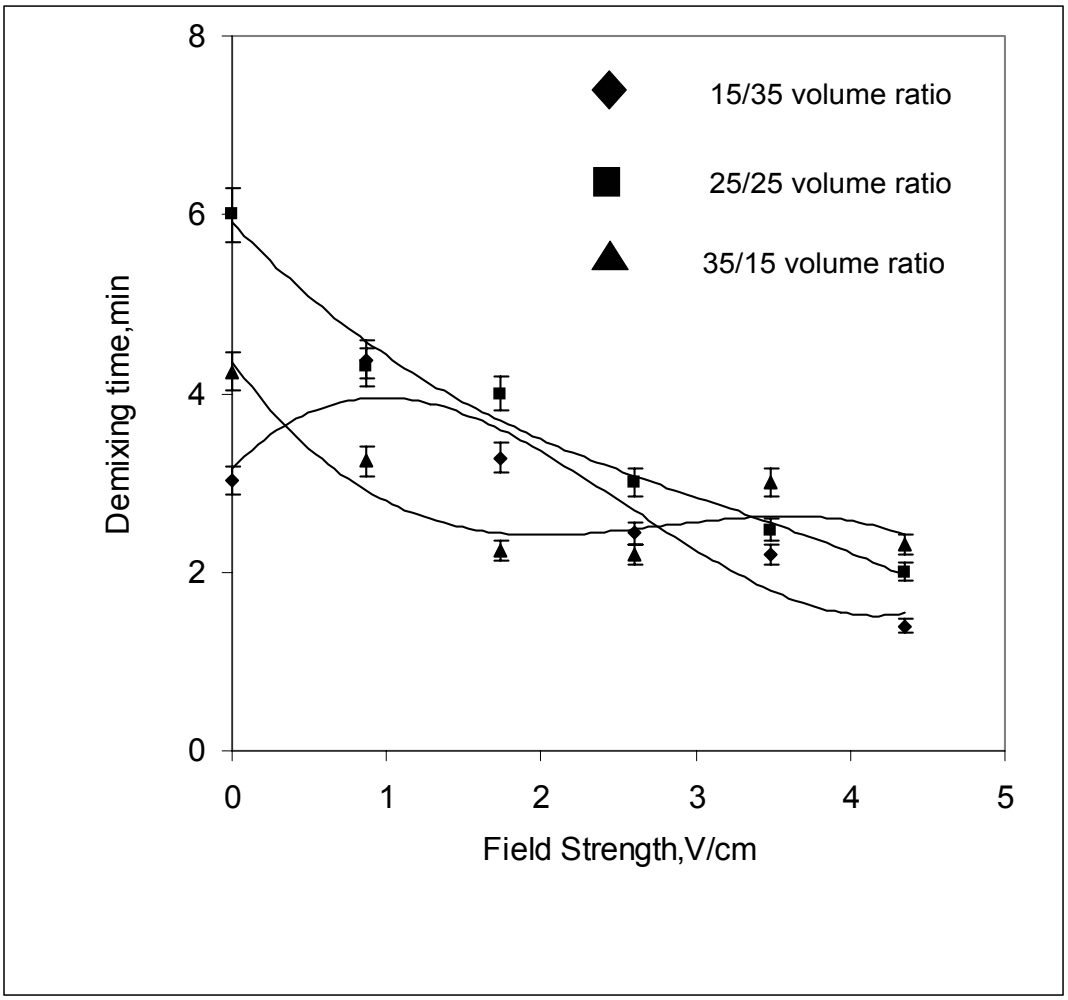


Figure 2B.5. Effect of field strength on demixing time at 15/11 (normal polarity)

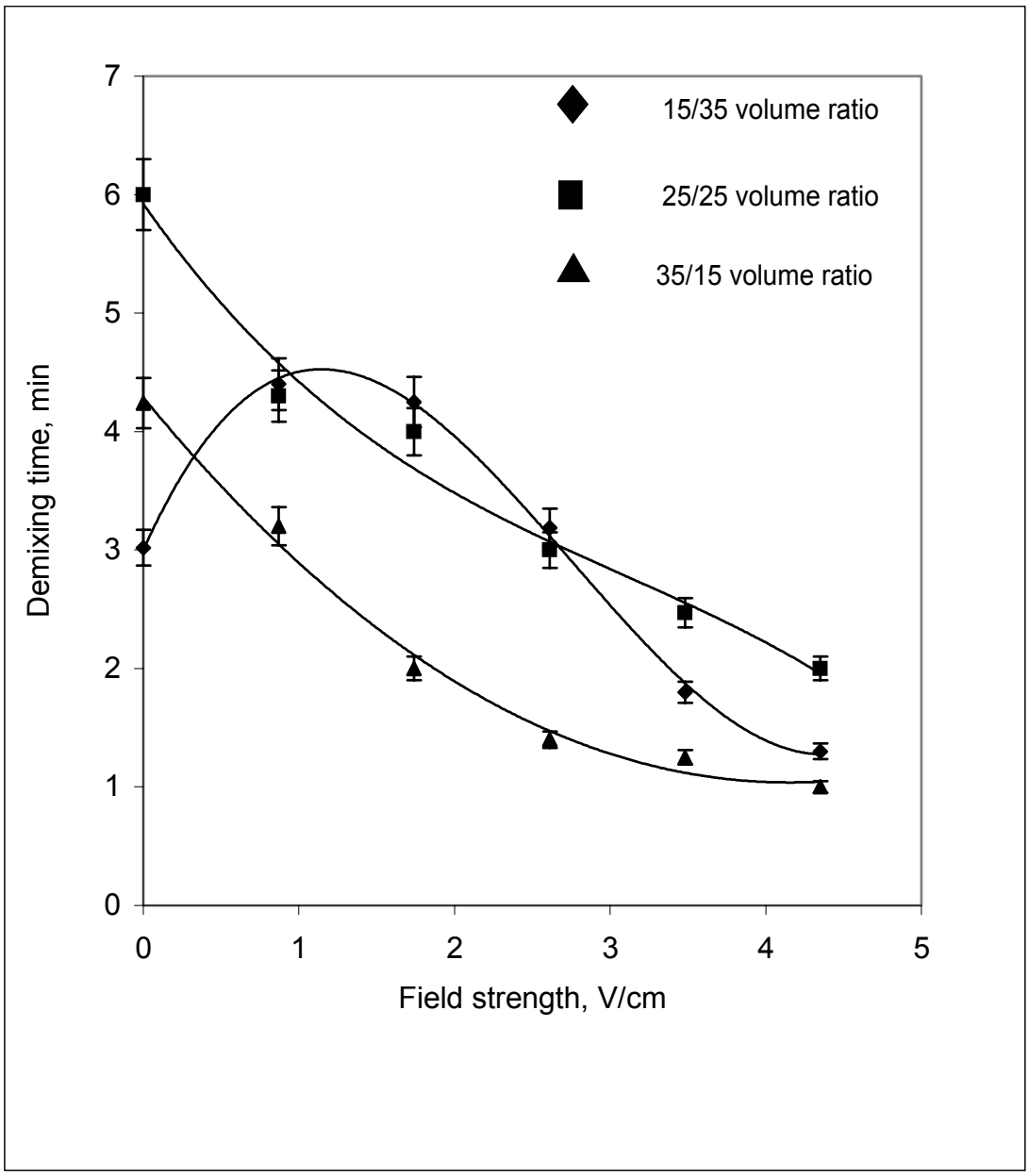


Figure 2B.6. Effect of field strength on demixing time at 15/11 (reverse polarity)

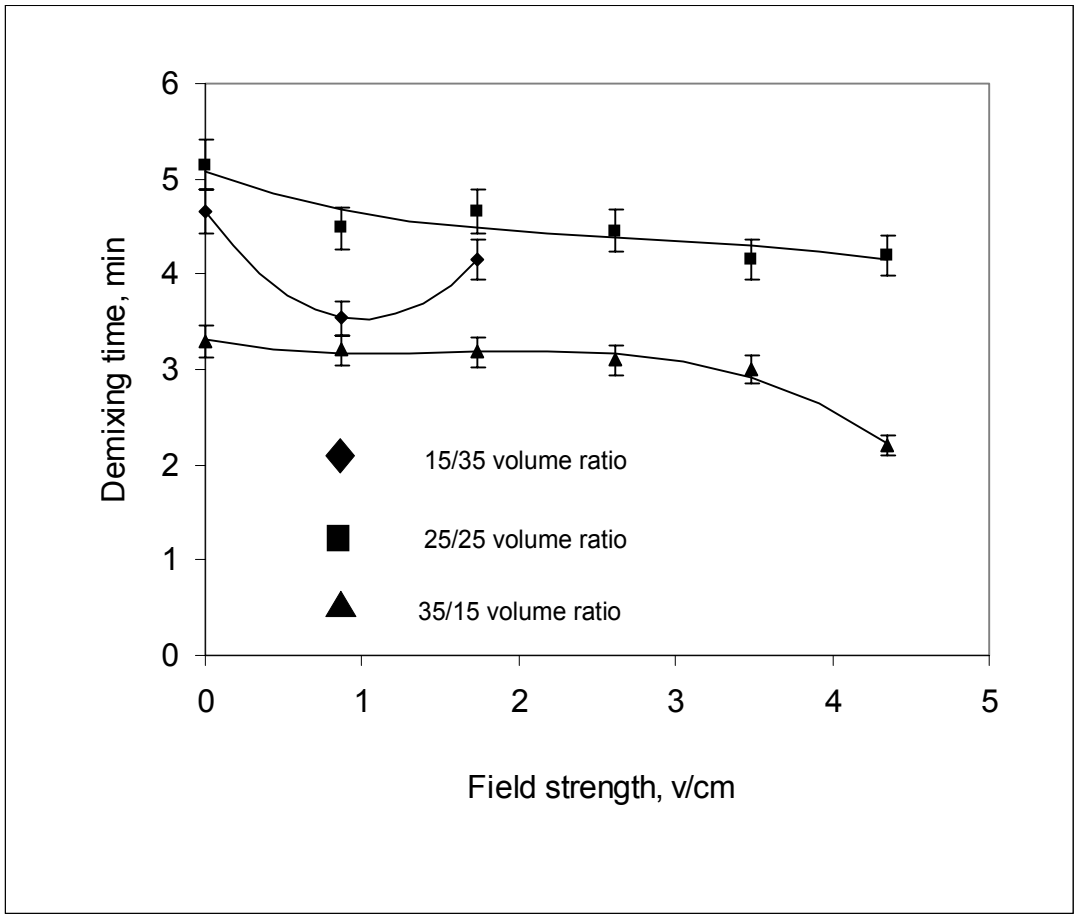


Figure 2B.7. Effect of field strength on demixing time at 35/11 (normal polarity

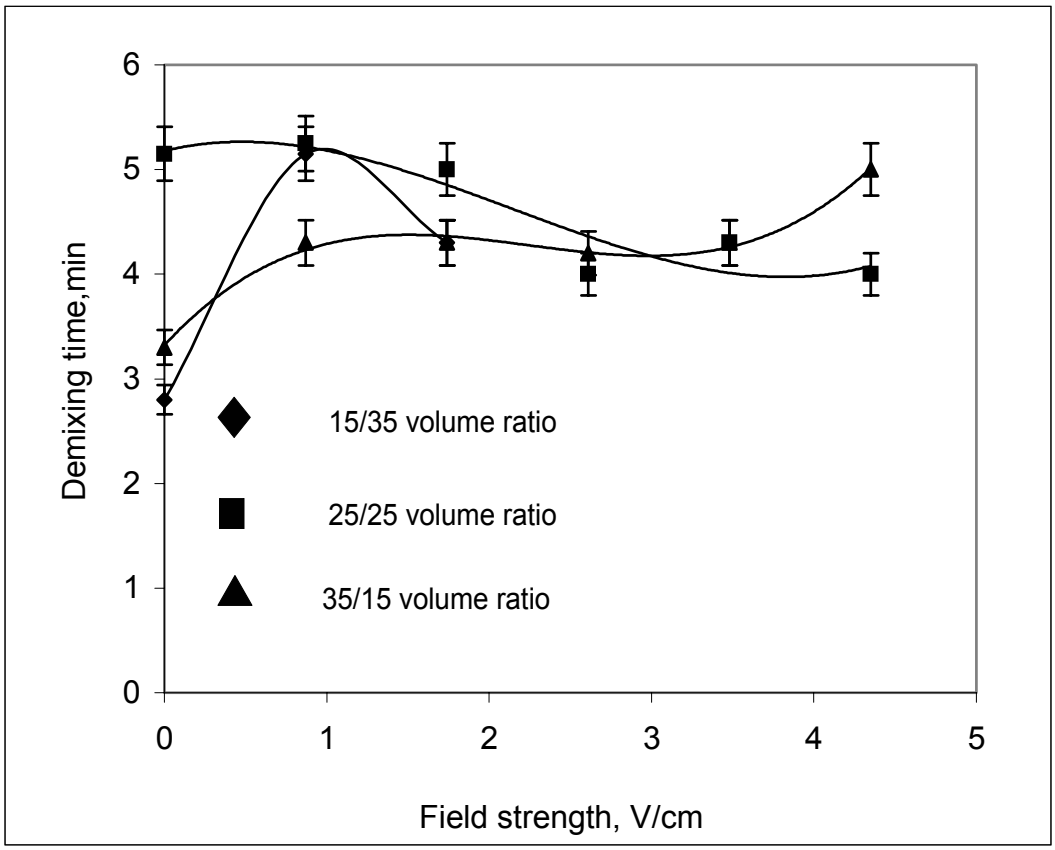
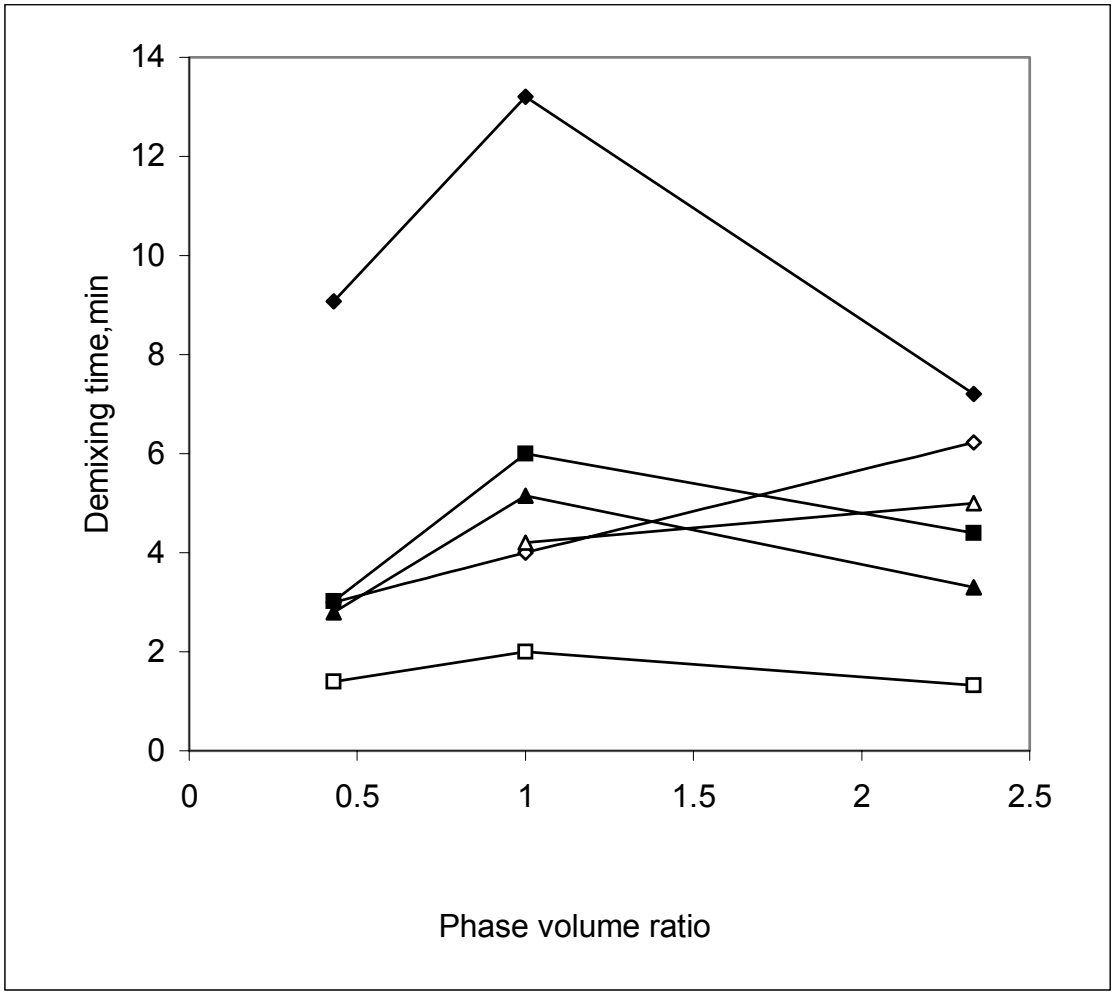


Figure 2B.8. Effect of field strength on demixing time at 35/11 (reverse polarity)



◇ 7/11 phase composition

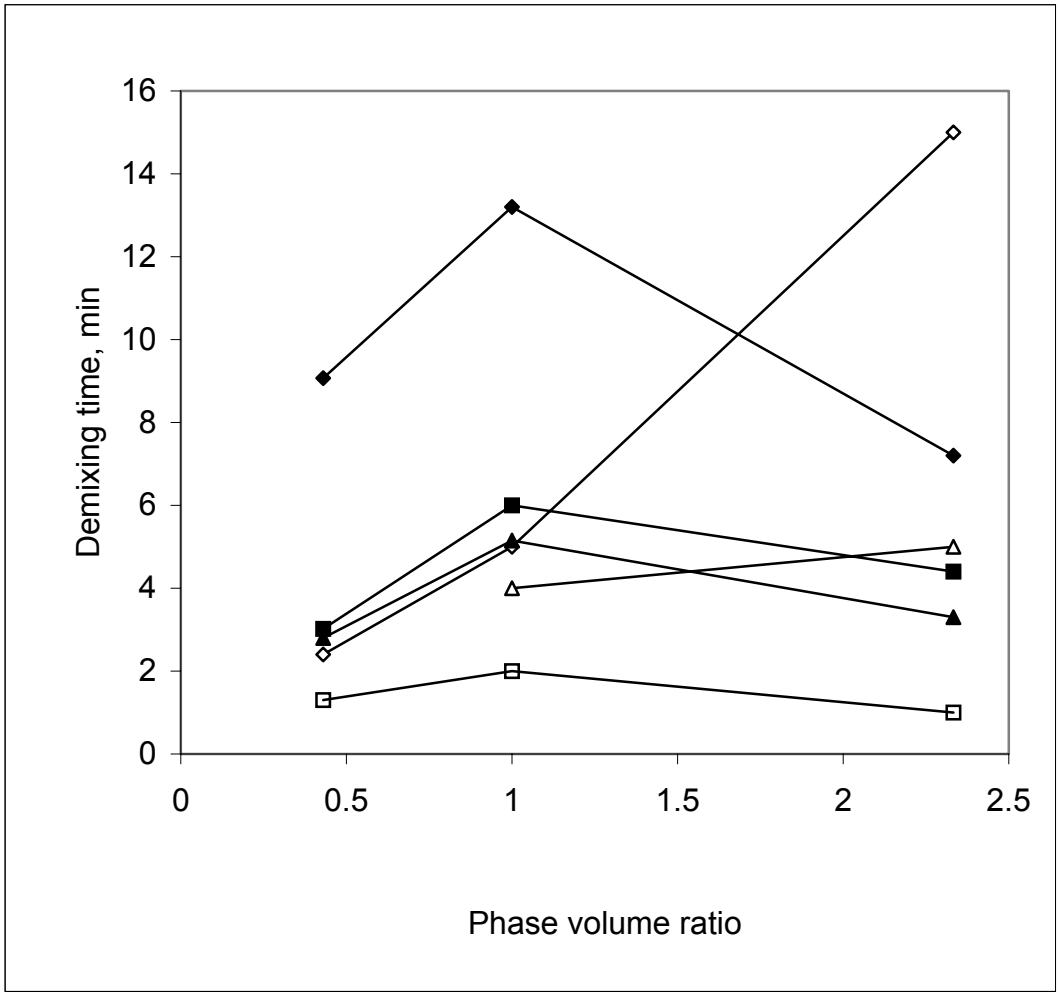
□ 15/11 phase composition

△ 35/11 phase composition

Closed symbols: demixing under zero field

Open symbols: demixing under electric field

Figure 2B.9. Effect of phase volume ratio on demixing time (normal polarity)



◇ 7/11 phase composition

□ 15/11 phase composition

△ 35/11 phase composition

Closed symbols: demixing under zero field

Open symbols: demixing under electric field

Figure 2B.10. Effect of phase volume ratio on demixing time (reverse polarity)

SECTION 2C

Microwave field assisted demixing

2C.1. INTRODUCTION

Acoustic and Electric fields are not very attractive for the enhancement in demixing rate in aqueous polymer-polymer two-phase systems as discussed in the previous sections (2A and 2B). For instance, continuous application of acoustic field to polymer-polymer systems has resulted in temperature rise with only 2 fold enhancement. To perform electrokinetic demixing of polymer-polymer systems there is a need for specialized equipment and addition of chemicals such as salts to the system. Hence, search for an alternative method continued. In this section, microwave field assisted demixing of ATPSs is reported for the first time.

During the last few decades applications of microwave field has been restricted to food processing industry (Datta, 1990). In recent years application of microwave is gaining importance in material processing and chemical synthesis (Ayappa, 1997). Our main focus in the present study was to explore the application of microwave field to enhance the demixing rates of ATPSs.

2C.2. Theoretical Aspects

During heating of liquids, microwave power distributed volumetrically gives rise to complex convection patterns in the liquid, which may increase or decrease the uniformity of temperature in the liquid. Due to spatial density difference in heated liquids, the energy balance must incorporate heat transfer due to convection as well. The energy balance is

$$\rho_o C_p (\partial T/\partial t) + \rho_o C_p v \nabla T = \nabla K \nabla T + p(r, T) \quad (2C.1)$$

The momentum and continuity equations for a Newtonian fluid are respectively,

$$\rho_o (\partial v/\partial t) + \rho_o v.\nabla v = -\nabla p + \mu \nabla^2 v + g \rho_o [1-\beta(T-T_o)] \quad (2C.2)$$

$$\nabla v = 0 \quad (2C.3)$$

In the above equations 'ρ_o' is the liquid density at the reference temperature 'T_o', 'β' is the volume expansion coefficient, 'v' is the velocity, 'μ' the viscosity, 'p' the dynamic pressure, 'g' the acceleration due to gravity, 'k' the thermal conductivity and 'C_p' the specific heat capacity. In the momentum balance the variation in density appears only as a body force and the viscosity is assumed to be constant. This is known as the Boussinesq approximation and is widely employed during the study of natural convection of liquids. At the sample boundaries the appropriate thermal boundary conditions are specified depending on whether the boundary is maintained at a fixed temperature or if heat is being lost by convection to the surroundings (Ayappa, 1997).

For convection, the dimensionless number commonly used in the presence of source term is the modified Greshoff number (Gartling, 1982).

$$Gr = \frac{\rho^2 g \beta L^5 \overline{P}}{\mu^2 k} \qquad (2C.4)$$

where $\overline{P}$ is the spatially averaged microwave power absorbed by the sample.

This can be calculated by using the following equation, (Datta, 1990).

$$\overline{P} = 2\pi f \varepsilon_O \varepsilon'' E^2 \qquad (2C.5)$$

where electric field *'E'* is determined by

$$E = \sqrt{\frac{\rho\ c_p \frac{\Delta T}{\Delta t}}{2\pi f \varepsilon_o \varepsilon''}} \qquad (2C.6)$$

where '*f* ' is the frequency of microwave field - 2450 MHz, 'c_p' specific heat , 'ε_0' is the dielectric constant of free space, 'ε" ' is the loss factor for the dielectric material being heated.

2C.3. Materials and methods

2C.3.1. Chemicals

Polyethylene glycol (PEG MW 6,000) was purchased from Loba Chemie (Mumbai, India). Sodium chloride (NaCl) and Potassium phosphate were purchased from Ranbaxy Fine Chemicals (Punjab, India). Maltodextrin (MDX) was procured from Laxmi Starch Private Limited (Mumbai, India).

2C.3.2. Methods

2C.3.2.1. Preparation of the phase systems

Phase systems comprising of PEG/potassium phosphate (15/11% w/w) and PEG/MDX (10/30% w/w) were prepared by dissolving the components in distilled water for about one hour in case of PEG/ potassium phosphate system and about 2 hours for PEG/MDX system. These phase compositions, corresponding to the intermediate tie lines of the respective phase diagrams, were selected based on our earlier studies on phase demixing of ATPSs. Around 600g of each phase systems were prepared and allowed to separate overnight in a separating funnel. Equilibrated and separated phases were collected and used as stock for the preparation of phase dispersion. Phase dispersion was prepared at three different volume ratios namely 30/70, 50/50, 70/30.

2C.3.2.2. Phase demixing experiments

The phase-demixing experiments were performed in a demixing contactor of 100 ml volume (height to diameter ratio 6.4:1) which was filled with 100 ml of freshly prepared, thoroughly mixed (for 10 minutes) phase dispersion. The dispersion was subjected to microwave field at a frequency of 2450 MHz, in low power mode (power equivalent to 175 watts) in a BPL-SANYO, BMC-900T microwave oven as shown schematically in Figure 2C.1. PEG/potassium phosphate system was subjected to microwave field at different time intervals (viz. 2, 4, 6, 8 and 10 seconds) and allowed to demix

under gravity alone. For, PEG/MDX, the dispersion was exposed in repeated cycles of microwave field (2 to 10 seconds) followed by gravity demixing, to avoid excessive heat generation. Demixing experiments were carried out under gravity in the absence of microwave field for both phase systems at all three-volume ratios. For all experiments, the time for complete phase separation was taken, as the time required for a clear horizontal interface to be formed. All experiments were carried in triplicate and average values are reported. The dispersion height was recorded as a function of time. This has been plotted as dimensionless height versus dimensionless time. Dimensionless height is defined as ratio of height of dispersion at any time "t" to total height of dispersion and Dimensionless time is defined as ratio of time of separation to time required for complete separation. Phase density and viscosity measurements were carried out as mentioned elsewhere (Srinivas *et al.,* 2000a, b).

2C.4. Results and discussion

Phase demixing kinetics (dimensionless height versus dimensionless time) for PEG/potassium phosphate (15/11) and PEG/MDX (10/30) systems, both in presence and absence of microwave field at the different volume ratios of 70/30, 50/50, and 30/70 are shown in Figure 2C.2 and Figure 2C.3. Densities and viscosities of the systems selected to examine the effect of phase composition on demixing rate are given in Table 2C.1.

Table 2C.2 and Table 2C.3 show the demixing time for all the three volume ratios, both in the presence and absence of microwave field. PEG/potassium phosphate system was exposed to microwave field for different time intervals as indicated in the Table 2C.2 and was followed by demixing under gravity. Rise in temperature was also recorded and has been reported in the Table 2C.2. At the end of each experiment observation showed that there was practically no change in the volume ratio (maximum change in the volume is $\pm$ 1ml). It can be seen that as time interval of exposure to microwave field increased, demixing rate enhanced significantly. There was about 4 fold enhancement in demixing rate when PEG/potassium phosphate system was exposed to microwave field for duration of 10 seconds, followed by gravity demixing.

Phase dispersion of PEG/MDX was also exposed to microwave field at various intervals of time, followed by demixing under gravity. Rise in temperature was also recorded and has been reported in Table 2C.3. It was observed that there was considerable increase in temperature (up to around 24°C) at the exposure time of 60 seconds at all phase volume ratios.

Such rise in temperature is unfavorable for the processing of macromolecules. Keeping this in mind, demixing experiments were conducted at various modes of repeated intervals of microwave field followed by gravity, selecting 30/70 phase volume ratio (which showed lowest demixing rate under gravity) as reported in Table 2C.4. It was observed that

10 seconds exposure to microwave field followed by 10 minutes gravity mode has resulted in about 5 fold reduction in demixing time. The rise in temperature at various modes as indicated in Table 2C.4, are the initial and final temperatures of the dispersion at the beginning and completion of demixing experiments. Figure 2C.4 shows the kinetics of phase demixing for PEG/MDX system, at 30/70 volume ratio. For this volume ratio, demixing experiments were performed in different operational modes of repeated cycles of exposure to microwave field followed by gravity demixing. Visual observations indicated vigorous movement of droplets in the dispersion when exposed to microwave field. Both in the case of PEG/potassium phosphate and PEG/MDX systems, the enhancement of demixing rate is associated with an increase in temperature. Hence, it was thought desirable to eliminate the effect of temperature on demixing rate in order to analyze exclusively the role of microwave field on phase demixing rate.

Hence the demixing experiments were carried out for PEG/potassium phosphate system (at 30/70 and 50/50 volume ratios) in temperature controlled water bath, to analyze the effect of temperature on demixing rate. The temperature controlled water bath was maintained at different temperatures namely 32°C, 36°C and 38°C (which corresponds to the average temperature rise at 2, 6 and 10s exposure to microwave field, as shown in Table 2C.5). At 30/70 volume ratio, for PEG/MDX system bath temperature was maintained at 40 and 50°C respectively (corresponding to

average temperature rise at 20 and 60s exposure to microwave field as shown in Table 2C.6). For the phase systems, dispersion was pre-equilibrated in the water bath to the above temperatures for one hour, mixed thoroughly for 10 minutes and demixing experiments were carried out in the water bath at the above mentioned temperatures.

In case of PEG/potassium phosphate system, the enhancement in demixing rate varied from 1.0 - 1.2 fold (as against 2.0 – 4.0 fold in presence of microwave) in temperature controlled water bath (Table 2C.5). In case of PEG/MDX, the enhancement in demixing rate varied from 1.3 – 1.5 fold (as against 1.3 – 3.0 fold in presence of microwave). In temperature controlled water bath, visual observations indicated that there was no vigorous movement of droplets under otherwise similar conditions (Table 2C.6). This difference in enhancement in the extent of demixing rate clearly indicates that in addition to rise in temperature, there is an additional effect of microwave field in enhancing the demixing rate.

Enhancement in demixing rate in presence of microwave field in case of PEG/potassium phosphate system could be attributed to the interactions of ions in the phase dispersion in presence of electromagnetic field. Due to these interactions there is heat generation within the dispersion due to “molecular friction”, primarily by the disruption of weak hydrogen bonds associated with the dipole rotation of free water molecules and with the electrophoretic migration of free salts in an electrical field of rapidly changing

polarity (Mudgett, 1985). This internal heating causes two-fold effect in the phase dispersion. Firstly, it sets in natural convection currents in the dispersion represented mathematically by equations 2C.1-2C.3. Secondly, it reduces the continuous phase viscosity with rise in temperature (Tello *et al.,* 1994), which in turn facilitates the faster mobility of the dispersed phase droplets in the direction in accordance to their buoyancy. As a result droplet – droplet collisions occur at an increased rate, giving rise to higher probability of droplet coalescence due to increase in collision frequency, hastening phase demixing due to increased migration of the larger droplets.

In case of PEG/MDX system, the mechanism of enhancement in demixing rate due to application of microwave field is more due to reduction in the continuous phase viscosity. The reduction in continuous phase viscosity is mainly due the dipole rotation of water molecules present along with the polymers in the dispersion. This result in internal heating reducing the continuous phase viscosity associated with rise in temperature, which facilitates faster mobility of the droplets, thereby hastening the phase demixing. The viscosity of the continuous phase being high in case of PEG/MDX systems, when compared to PEG/ potassium phosphate, the effect of microwave field is more significant in the case of former only when mode of operation is changed (Table 2C.4).

Another noteworthy observation from Table 2C.2 and Table 2C.3 is the effect of microwave field is much higher at 50/50 volume ratio when

compared with other volume ratios (30/70 and 70/30) both in case of PEG/potassium phosphate and PEG/MDX phase systems. At 50/50 volume ratio, in case of PEG/potassium phosphate enhancement in demixing rate was up to about 4 fold and for PEG/MDX, it was around 3 fold. At this volume ratio, continuous phase has higher concentration of dispersed droplets. This leads to more number of interfaces surrounding any given droplet when compared with other volume ratios. These interfaces reflect incident microwave radiation, giving rise to multiple internal reflections among the interfaces surrounding the droplets, which increases the rate of microwave power absorption (Barrienger *et al.,* 1995). This results in higher frequency of droplet rotation leading to higher droplet – droplet interaction, which in turn hastens the rate of droplet coalescence, resulting in increased droplet size. Bigger droplets migrate faster to the interface, eventually resulting in enhanced rate of demixing.

To account for the effect of microwave field quantitatively, values of the power absorption and Grashoff's number are estimated using equations 2C.4-2C.6 and the results are shown in Table 2C.7. In case of PEG/potassium phosphate system, at all the three volume ratios studied, corresponding to increase in demixing rate, values of power absorption and Greshoff number showed an increasing trend with increase in duration of microwave field exposure. This increase in Greshoff number clearly indicates that natural convective currents facilitate the droplet-droplet interactions as

explained earlier. Another observation made in this case is, at higher exposure time (10 s) the values of power absorption and Greshoff number decreased at all volume ratios. This decrease in Greshoff number is mainly due to the possible saturation level reached by the dispersion to absorb microwave power. However, enhancement in demixing rate under otherwise similar conditions is mainly due to reduction in continuous phase viscosity associated with rise in temperature, at higher exposure time in microwave field. In case of PEG/MDX system, values of power absorption and Greshoff number decreased (Table 2C.7). This decrease could be attributed to low dielectric properties of the polymers, which results in low power absorption of the dispersion reaching the saturation level at low exposure time itself.

2C.4.1. Effect of addition of neutral salt

Further, experiments were carried out to study the effect of addition of neutral salt (NaCl) to the PEG/MDX phase system at varying concentrations as shown in Table 2C.8. For the present study 30/70 volume ratio was selected since it had the lowest demixing rate under gravity. It was observed that demixing rate enhanced by up to 6.5 fold for 4% NaCl concentration in presence of microwave field when compared with gravity demixing without NaCl addition.

Addition of NaCl increases the dielectric loss of the water phase thereby increasing the electrophoretic migration of dissolved ions in applied field. This results in interactions between the dissolved ions and solvent

water molecules resulting in increased rate of microwave power absorption causing internal heating of the dispersion resulting in the development of natural convection currents in dispersion (Barrienger *et al.,* 1995). Internal heating also results in reduction in continuous phase viscosity (associated with rise in temperature) which facilitates the buoyant mobility of droplets. This leads to increase in frequency of droplet collision thereby increasing probability of coalescence of the droplets. Thus, synergistically enhances the demixing rate.

2C.5. Conclusions

Microwave field assisted demixing is studied for the first time to enhance the rate of phase demixing. Effect of microwave field on demixing rates at different phase volume ratios for intermediate phase composition and addition of neutral salt have been studied. The enhanced demixing rates (reduced demixing time) could be explained based on dipole and ionic interactions of the droplets, droplet-droplet collision and reduction in viscosity of the continuous phase. The enhanced phase demixing rate was explained based on the droplet-droplet coalescence in case of PEG/potassium phosphate system and reduction in continuous phase viscosity in case of PEG/MDX system.

Table 2C.1. Composition, density and viscosity of aqueous two-phase systems.

Phase System	Composition (top/bottom) w/w %	Viscosity, mPa s		Density,kg/m^3	
		Top	Bottom	Top	Bottom
PEG/ Potassium phosphate	15/11	28.92	1.389	1092.73	1103.82
PEG/ Maltodextrin	10/30	13.99	91.273	1111.0	1199.9

Table 2C.2. Demixing times at different volume ratios of 15/11 PEG/potassium phosphate system

Volume ratio Top/bottom Phase	Demixing time (min)					
	Exposure to microwave followed by gravity (seconds)					
	Gravity (min)	2s	4s	6s	8s	10s
30/70	7.3	7.0	5.3	4.0	3.3	3.1
50/50	8.3	8.2	5.15	3.1	2.5	2.2
70/30	6.4	5.3	4.3	4.2	2.5	2.2
Rise in temperature,°C	0	1-2	2-3	5-6	7	8

Room Temperature: 30°C

Table 2C.3. Demixing times at different volume ratios of 10/30 PEG/MDX system

Volume ratio Top/bottom Phase	Demixing time (min)				
	Exposure to microwave followed by gravity (seconds)				
	Gravity (min)	10s	20s	30s	60s
30/70	250	240	237	226	188
50/50	80	40	37	32	26
70/30	70	65	56	47	35
Rise in temperature, °C	0	5-7	8-11	10-15	22-24

Room Temperature: 30°C

Table 2C.4. Demixing times at 30/70 phase volume ratio of phase composition PEG/MDX at various duration of application of microwave/gravity in cycles

Phase composition	Microwave/gravity (seconds/min)										
	Gravity (min)	2/10	2/20	4/10	4/20	6/10	6/20	8/10	8/20	10/10	10/20
10/30	240	110	120	100	120	80	108	63	90	50	82
Temperature rise, °C	0	1-3	1-2	2-5	2-4	3-10	3-10	5-13	8-10	7-16	5-10
No. of exposure cycles	-	12	7	11	7	9	8	5	4	6	5

Room temperature: 30°C

Table 2C.5. Effect of temperature on phase demixing rate*

Volume ratio Top/bottom phase	Temperature of water bath ^{0}C	Fold reduction in demixing time
30/70	32	1.09
	36	1.31
	38	1.53
50/50	32	1.08
	36	1.51
	38	1.88

* temperature controlled water bath for 15/11 PEG/potassium phosphate system

Table 2C.6. Effect of temperature on phase demixing rate*

Volume ratio Top/bottom phase	Temperature of water bath °C	Fold reduction in demixing time
30/70	40	1.31
	50	1.57

* temperature controlled water bath for 10/30 PEG/MDX system

Table 2C.7. Values of power absorption and Greshoff number at different volume ratios

PEG/potassium phosphate system (15/11)						
Time of exposure in microwave (s)	Power absorbed x 10^{-6} W			N_{Gr} x10^{-10}		
	Volume ratio			Volume ratio		
	30/70	50/50	70/30	30/70	50/50	70/30
2	2.1	2.08	1.93	3.2	1.29	0.647
4	3.15	3.13	2.9	4.8	1.94	0.972
6	3.5	3.48	3.22	5.33	2.15	1.07
8	3.67	3.65	3.38	5.6	2.26	1.13
10	3.36	3.34	3.09	5.12	2.07	1.03

PEG/MDX system (10/30)

Time of exposure in microwave (s)	Power absorbed x 10^{-6} W			N_{Gr} x10^{-9}		
	30/70	50/50	70/30	30/70	50/50	70/30
10	1.531	1.64	1.75	1.83	3.19	6.63
20	1.22	1.3	1.376	1.45	2.53	5.25
30	1.064	1.14	1.22	1.26	2.22	4.61
60	0.978	1.05	1.12	1.17	2.04	4.24

Table 2C.8. Effect of addition of neutral salt on demixing.

Volume ratio Top/Bottom phase	Concentration of neutral salt				
30/70	Gravity (0%)	1%	2%	3%	4%
Demixing time (min)	260	50	46	43	41
Rise in temperature °C	-------	8-16	8-16	6-15	8-17

Room temperature: 30°C

* 10/30 PEG/MDX at 10 seconds microwave followed by 10 minutes gravity mode

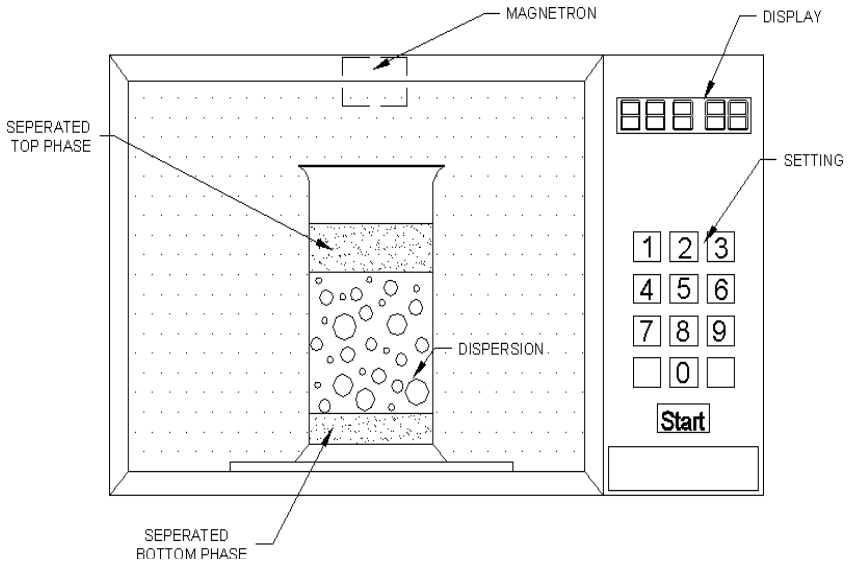
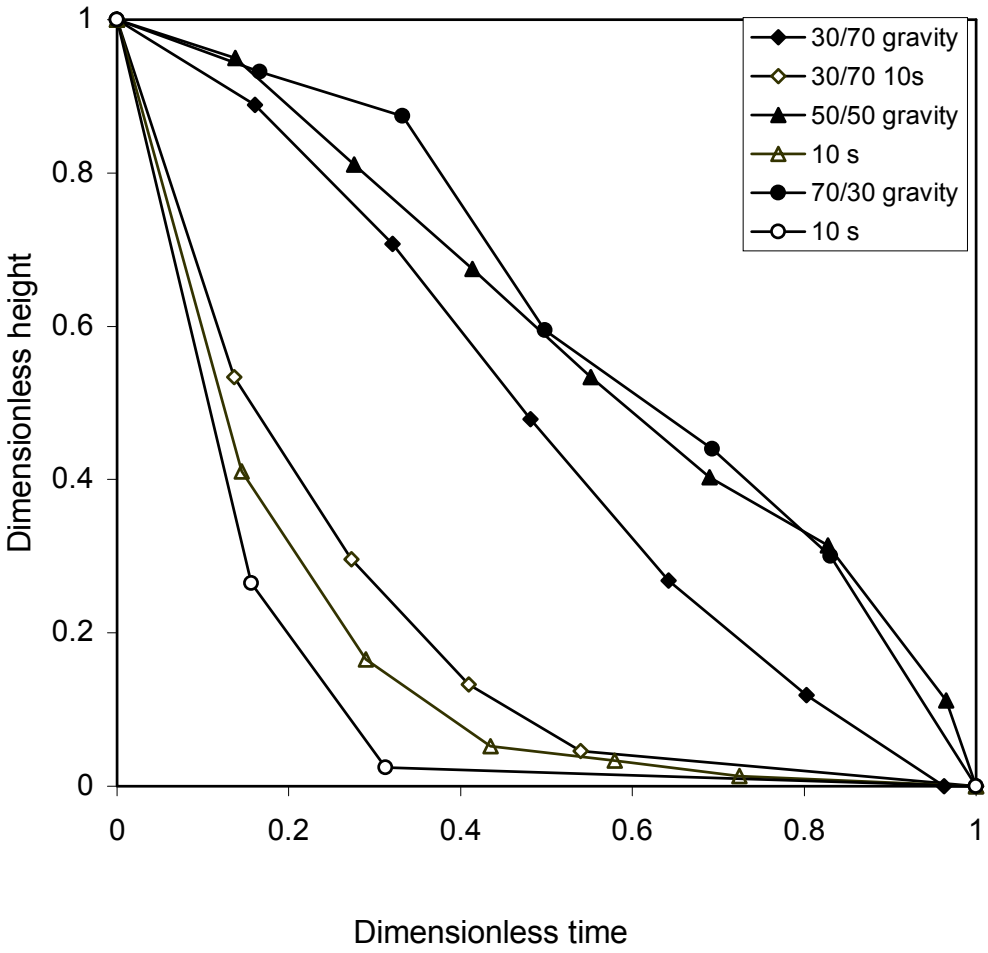


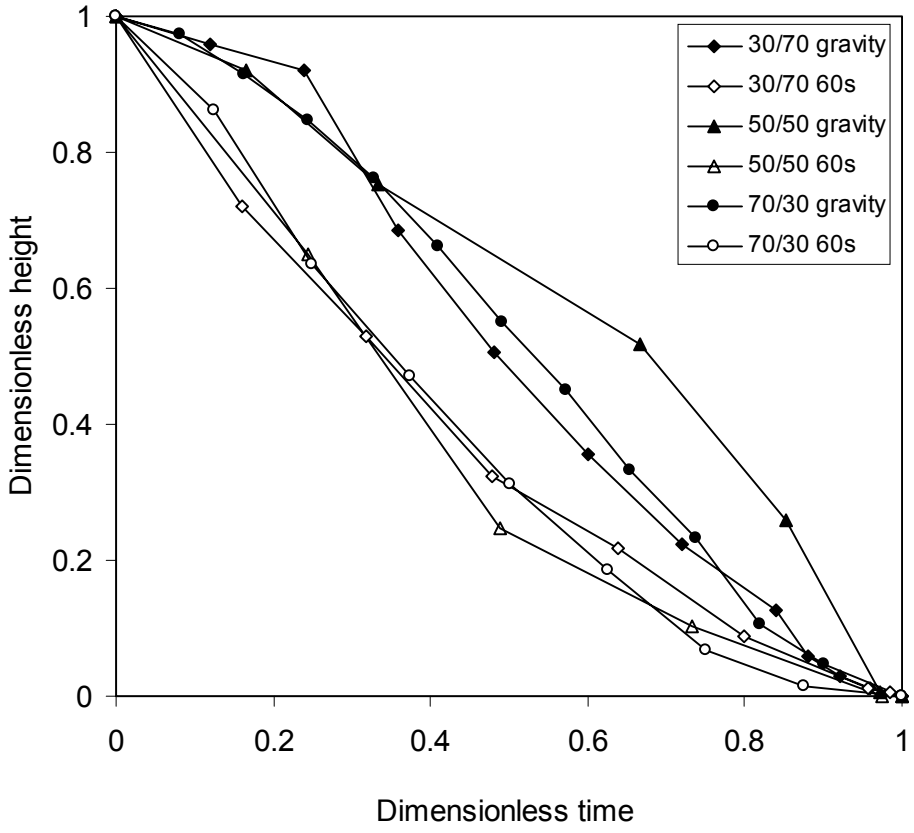
Figure 2C.1 Schematic diagram of microwave field assisted demixing of aqueous two- phase system



Closed symbols - demixing under gravity

Open symbols - demixing under microwave

Figure 2C. 2. Kinetics of microwave field assisted demixing of PEG/potassium phosphate system (15/11)



Closed symbols - demixing under gravity

Open symbols - demixing under microwave

Figure 2C. 3. Kinetics of microwave field assisted demixing of PEG/MDX (10/30)

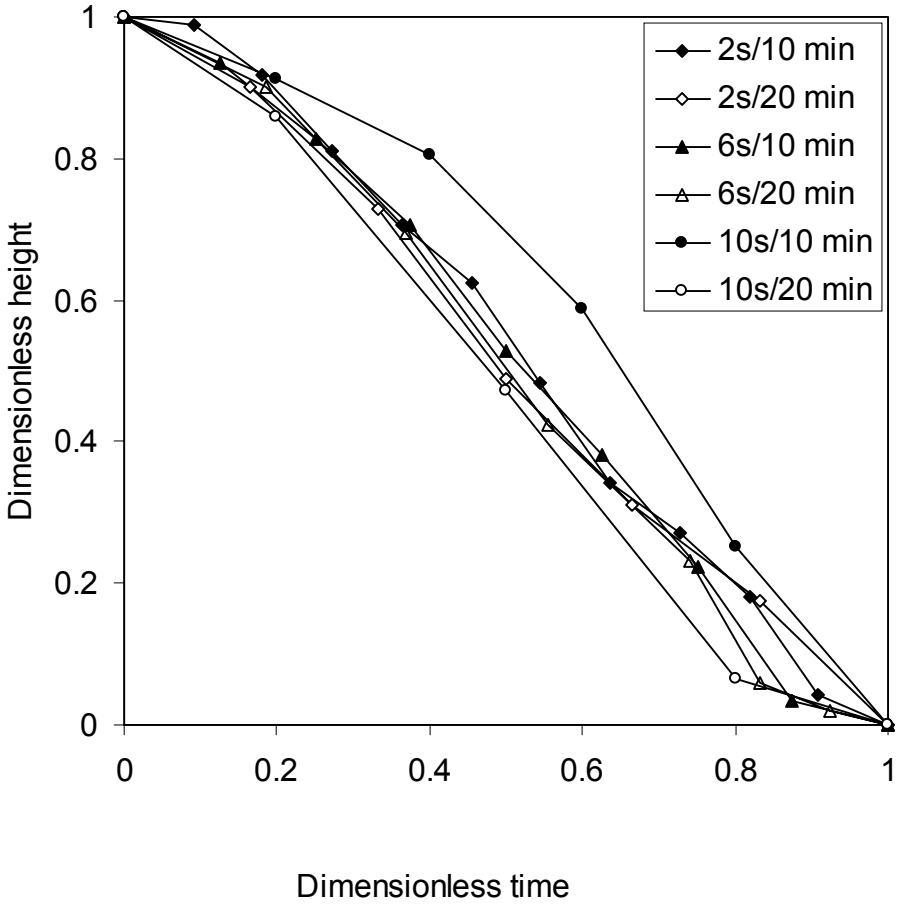


Figure 2C.4. Kinetics of microwave field assisted demixing of PEG/MDX

(Phase volume ratio: 30/70)

Chapter 2D

Electroextraction of betalaines from beet hairy roots

2. D.1. Introduction

In recent years, red color derived from red beet root (*Beta vulgaris*) is gaining importance as an alternative source of red colorant in food and other applications (Psczczola, 1998). The red coloration in beet root is due to red and yellow pigments namely betacyanins and betaxanthins (Von-Elbe, 1979). However, the ratio of betacyanins to betaxanthins content varies in conventionally grown beet root, resulting in non-uniformity of the total betalaines content. In recent years, beet hairy roots (genetically transformed with *Agrobacterum rhizogenes*) are becoming alternate source for natural red color. In comparison with beet root, hairy roots cultures assure uniformity in betalaine color content and can be produced throughout the year under fully controlled conditions (Mukundan *et al.,* 1998). The extraction of betalaines from hairy roots is generally done at low pH, wherein the extraction betalaines is low. Hence, it is desirable to devise simpler and effective methods for the pigment extraction from beet hairy roots. Conventionally, beet pigments have been separated by column chromatography (Aronoff and Aronoff, 1948) and paper electrophoresis on lab scale and these methods pose scale-up problems (Schmidt and Schonleben, 1956).

Attempts have been made to transfer charged biomolecules (proteins) to either one of the phases in aqueous two-phase systems (ATPSs) by the application of electric field (electroextraction). The electric field is applied in perpendicular direction to the phase interface, which

provides the stability against convection facilitating product recovery (Theos and Clarke, 1995).

Lavine and co-workers (Levine and Bier, 1990; Levine *et al.,* 1992) have reported that the electrophoretic mobility of proteins in ATPSs is greatly impeded in one direction. The protein transfer was easily achieved from its non-preferred phase to its preferred phase. However, from preferred to non-preferred phase transfer was not possible. On the contrary, Theos and Clark (1995) have shown that electrophoretic mobility of proteins can be achieved in both directions. Authors separated oppositely charged binary protein mixtures by transferring them into opposite phases of ATPSs by operating electroextraction in between the pI's of two proteins. Electroextraction appears to be a promising technique over the conventional free solution method for the separation of charged proteins at the commercial scale owing to its advantages such as controlling the starting compositions of proteins, limiting convective mixing and facilitating product isolation and recovery (Theos and Clark, 1995).

In the present study, an attempt is made based on selective separation owing to controlled electrokinetic migration of charged species during betalaines extraction from beet hairy roots.

2. D.2. Material and methods

2. D.2.1. Hairy root culture

Beet hairy roots which were grown using red beet variety “Ruby Queen” by infecting with *Agrobacterum rhizogenes* strain LMG-150 as per standard protocols were obtained from Department of plant cell Biotechnology, CFTRI (Taya *et al.,* 1992 and Dilorio *et al.,* 1993). The hairy roots for the present study were grown in Murashige and Skoog’s (MS) liquid medium (Murashige and Skoog, 1962).

2. D.2.2. Analysis

The betalaines pigment concentration was determined spectrophotometrically in Shimadzu-UV spectrophotometer using an extinction value, E (1 cm/l %) of 750 for betaxanthin and E (1cm/l %) of 1120 for betacyanin and expressed as mg per fresh weight (Nilsson, 1970). The amount of betaxanthin and betacyanin were quantified based on respective extinction coefficient as described above.

2. D.2.3. Chemicals

Hydrochloric acid (LR grade) was procured from Loba Chemie, Mumbai, India. Acrylamide and bis-acrylamide, TEMED were purchased from SRL, Mumbai, India. Platinum electrodes and electrical power pack (Model: 8E102-38) were supplied by Bangalore Genie, Bangalore. The extraction medium was prepared using distilled water from Millipore Inc., distillation unit.

2. D.2.4. Electroextraction experiments

A schematic representation of the experimental apparatus is shown in Figure 2D.1. All the extraction experiments were carried out in a water-jacketed electrophoresis column filled with 50 ml of extraction medium (different concentrations of HCL in acidified water). Around 2 g of fresh hairy roots (20 days old) grown in MS medium were taken, washed thoroughly with distill water to remove medium from the hairy roots. The washed hairy roots were placed in extraction medium inside the electrophoresis column, near to cathode as shown in Figure 2D.1. The column is in contact with buffer solution reserviors, which has provision to house side-arm platinum electrodes. These electrodes support and contact the phase dispersion through 15% polyacrylamide gel plugs. The platinum electrodes in the side arms are in turn connected to electrophoresis power pack for electrical supply. The extraction studies were carried out in normal polarity (upper electrode is held positive).

2. D. 3. Results and discussion

In the present study extraction of betalaines pigment from beet hairy roots were carried out using different concentration of extraction medium under controlled conditions (absence of electric field) and in presence of electric field as shown in Table 2D.1(a)-2D.1(e). The applied electric field was in the range of 1-5 V/cm. During the experiments, it was observed that with the application of electric field the betaxanthine pigment was the first to

get extracted from the beet hairy roots, which gradually migrated towards anode and subsequently into the gel plug. Attempts were made to prevent the migration of the pigment into the gel plug by placing a dense polymeric membrane at the entrance of the gel plug with little success. Further, as observed from Table 2D.1 (a)-2D.1 (e) the rate of betalaines extraction increased with time under control conditions. However, under otherwise similar conditions in presence of electric field the rate of betalaines extraction increased initially and decreased later with increase in duration of electric field application. This decrease in betalaines concentration is due to two factors. First, it is the migration of betalaine pigments into the gel plug. Secondly, it is the release of phenolic components from the hairy roots due to continuous application of electric field, which in turn affects the betalaines stability. Another noteworthy observation made in this study is apart from electric field, pH of the extracting medium also plays an important role in influencing the stability of betalaines during extraction. As observed at lower pH of 1.5 (Table 2D.1 (e)), the rate of of betalaines extraction has decreased in absence of electric field due to release of phenolic components along with the pigment into the extraction medium. From the results observed (Table 2D.1(c) and Table 2D.1 (d)) it is evident that, betalaines extraction is optimum in the pH range of 2.0, under the present conditions in presence of electric field without affecting its stability. Inspite of having water circulating jacketed column due to continuous application of electric field there is some

rise in temperature of the extraction medium (5-10 °C) due to movement of charged species. This has also contributed to some extent in influencing the stability betalaines.

2. D.4. Conclusions

A simple method to extract betalaines from beet hairy roots by the application of electric field (low voltage) has been explored in the present study. The initial results obtained are encouraging, however, there is a need for improving the effectiveness for the selective isolation of betaxanthines and betacyanins. In this regard, a better design of electroextraction module needs to be arrived at so as to facilitate selective extraction of betalaines without losing its quality.

Table 2.D.1 (a). Rate of betalaines extraction in 50 μl HCL acidified extraction medium (pH: 2.3)

Applied electric field: 5V/cm

Time, h	% extraction	
	Betacyanin	Betaxanthine
Control		
0	0.25	0.86
1	7.76	18.42
2	8.52	20.69
3	8.67	20.42
Electric field		
0	0.25	0.86
1	1.63	2.67
2	5.8	15.72
3	5.8	21.51

Table 2.D.1 (b). Rate of betalaines extraction in 100 μl HCL acidified extraction medium (pH: 2.15)

Applied electric field: 4V/cm

Time, h	% extraction	
	Betacyanin	Betaxanthine
Control		
0	0.15	0.38
1	7.21	14.69
2	9.01	16.5
3	10.65	19.84
Electric field		
0	0.15	0.38
1	8.21	15.93
2	9.22	17.24
3	9.59	19.83

Table 2.D.1 (c). Rate of betalaines extraction in 125 μl HCL acidified extraction medium (pH: 2.05)

Applied electric field: 3V/cm

Time, h	% extraction	
	Betacyanin	Betaxanthine
Control		
0	0.49	0.16
1	3.88	10.57
2	5.55	15.1
3	12.55	32.1
Electric field		
0	0.11	0.26
1	2.94	7.01
2	10.1	23.8
3	10.4	24.9

Table 2.D.1 (d). Rate of betalaines extraction in 250 μl HCL acidified extraction medium (pH: 2.05)

Applied electric field: 2V/cm

Time, h	% extraction	
	Betacyanin	Betaxanthine
Control		
0	0.26	0.73
1	7.18	21.3
2	7.21	21.9
3	7.34	24.6
Electric field		
0	0.44	1.3
1	7.31	23.5
2	7.35	23.8
3	7.55	21.6

Table 2.D.1 (e). Rate of betalaines extraction in 500 μl HCL acidified extraction medium (pH: 1.53)

Applied electric field: 1V/cm

Time, h	% extraction	
	Betacyanin	Betaxanthine
Control		
0	0.44	0.16
1	8.71	25.03
2	5.44	10.25
3	5.55	10.7
Electric field		
0	0.11	0.33
1	11.66	28.72
2	11.88	29.26
3	6.77	9.31

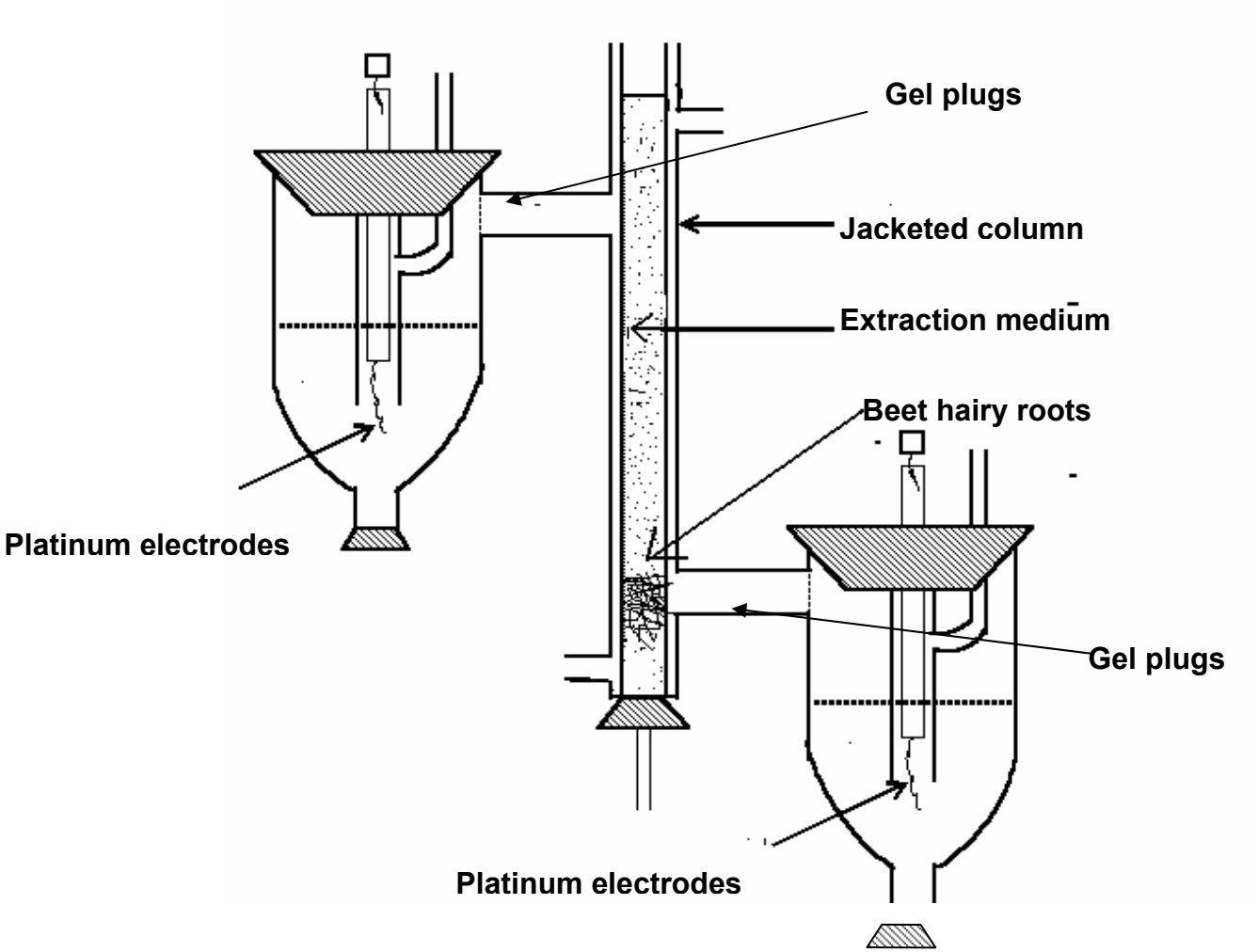


Figure 2D.1. Schematic representation of electroextraction column for betalaines extraction

Chapter 2E

Recovery of phase forming components using microwave field

2E.1. Introduction

As discussed in earlier sections, apart from slow rate of phase demixing, another major hindrance for the industrial adaptation is high cost of phase forming polymers. Extensive studies have been undertaken to recover and recycling of polymers by employing aqueous two-phase extraction comprising of thermoseparating polymers which has solved the problem to a great extent (Johansson *et al.,* 1997). Polyethylene glycol (PEG) is widely studied polymer in most of the extractions involving ATPSs. The advantages which the PEG offers over thermosetting polymers are (i) its low cost, (ii) stabilizing effect in presence of biomolecules and (iii) higher solute solubility in the phase system. However, very few reports are available on PEG recovery. PEG has been recovered by conventional methods such as vacuum evaporation/drying, precipitation, recrystallization (Harris and Yalpani, 1986). These methods are energy intensive and cumbersome due to high water load associated with PEG rich phase. PEG can also be removed by chromatographic adsorption on hydroxypatite or ion-exchange columns (Albertsson, 1986, Kula *et al.,* 1982). These techniques face operational problems on scale-up. More, recently, attempts have been made to study the effect of PEG recycling on protein recovery and its activity (Rito-Palomares and Lyddiatt, 1996; Rito-Palomares *et al.,* 2000; Wu *et al.,* 2001). After partitioning the desired biomolecule to one of the phases (either salt or other polymer phase), to improve the process economics polymer phase is

recycled for subsequent ATPE. However, the polymer phase which is recycled will be having load of impurities/contaminants, which increases upon repeated extractions, thereby affecting the partitioning of the desired biomoelcule during partitioning. So a stage will be reached wherein the polymer from the phase needs to be recovered in solid form or spent phase needs to be disposed completely (the latter posing environmental problems).

Hence, there is a need for faster and efficient/effective recovery process of PEG. During the last few decades applications of microwave field is gaining popularity in food processing industry (Datta, 1990). In recent years, application of microwave is gaining importance also in material processing and chemical synthesis (Ayappa, 1997). Recently, microwave field has been explored to enhance the demixing rates in ATPSs (Nagaraj *et al.,* 2003). When compared to other conventional heating or application of other external fields, microwave field offers advantages of rapid and uniform heating, deep penetration, less process time. Hence in the present in the study an attempt has been made to explore the application of microwave field for the recovery of PEG from spent phases.

2E.2. Materials and methods

2E.2.1. Chemicals

Polyethylene glycol (PEG: MW 6000) was obtained from Loba Chemie (Mumbai, India). Potassium phosphate was procured from Ranbaxy Fine Chemicals Ltd., SAS Nagar, India.

2E.2.2. Methods

Phase system 1 comprising of PEG/potassium phosphate (16.07/10.13) was prepared (Silva *et al.,* 1997). Around 1000 g of the phase system was prepared by mixing the constituents in water for about one hour using a magnetic stirrer. The top and bottom phase were allowed to equilibrate overnight in a separating funnel and later separated. The separated top PEG-rich phase was then used to recover PEG using microwave field.

2E.2.2.1. PEG recovery studies

PEG recovery was carried out by taking around 30g of PEG-rich phase in a separating funnel. The PEG-rich phase was exposed to microwave field at a frequency of 2450 MHz, in a microwave oven (BPL-SANYO, BMC-900T) as shown schematically in Figure 2F.1. The PEG-rich phase was exposed to microwave field till the phase separated into a liquid phase (water) and a solid phase (PEG). The solid phase was taken out after the exposure is over and then dried in hot air oven (TEMPO, model #016-721) to remove the remaining moisture content. The amount of PEG recovered from spent phases was estimated by PEG material balance. All the experiments were carried out in triplicate and average values are reported.

2E.2.2.2. Measurements of physical properties

Phase viscosity and density of individual phases were measured by using specific gravity bottle and Oswald's-U-tube viscometer at temperature of 28±2°C.

2E.2.2.3. Isolation of crude C- phycocyanin

The C-phycocyanin extract was obtained from freshly harvested biomass as per procedure prescribed (Pillai *et al.,* 1996 and Nagaraj *et al.,* 2003). The crude C-phycocyanin solution was then stored in cold storage at a temperature of 4±1°C. The C-phycocyanin solution having an initial concentration of 0.9 mg/ml was subjected to aqueous two-phase extraction (ATPE). The phase system comprising of PEG/potassium phosphate (phase composition of 7.04/14.369; System-II; Table 2E.1) was selected from the phase diagram (Albertsson, 1986). Around 200g of the phase system having above phase composition were prepared from fresh PEG and recovered PEG in C-phycocyanin solution (instead of water) as mentioned earlier. The top and bottom phases were analyzed for the concentration (mg/ml), purity and partition coefficient of C-phycocyanin content.

2E.2.2.4. Analytical Procedures

(a) Determination of C-phyocyanin concentration

The C-phycocyanin concentration was determined using UV-spectrophotometer; model Shimadzu UV1601, Japan, by measuring the

optical density at 280nm for total proteins, 620nm for C-phycocyanin and 650nm for allophycocyanin. The concentration was calculated by the following formula (Tandaeu and Hounard, 1988).

$$Concentraton(mg/ml) = \frac{A_{620} - 0.7(A_{650})}{7.38} \qquad (2E.1)$$

(b) Purity determination

The purity of C-phycocyanin was determined by the ratio of the optical density at 620nm to 280nm.

$$Purity = \frac{A_{620}}{A_{280}} \qquad (2E.2)$$

2E.3. Results and discussion

The phase composition of the phase systems selected for the present study is shown in Table 2E.1. During the experiments, exposure of PEG rich phase to microwave field turned the phase cloudy due to vigorous interactions. At this stage, the microwave field was withdrawn in order to cool the dispersion. Upon cooling two distinct phases (solid phase-PEG and liquid phase-water) were formed. The exposure of the PEG-rich phase to microwave field has resulted in internal heating due to dipole rotation of free water molecules present along with PEG. The resultant internal heating has generated natural convective currents associated with temperature rise. The solubility of PEG in water decreases at higher temperature (Bailey and

Callard, 1959). This decrease in PEG solubility is due to disruption of weak hydrogen bonds by the application of microwave field, which otherwise forms stiff bonding with the polymer (PEG) at lower temperature (Kjellander and Florin, 1981). As the PEG solubility decreased with temperature rise, hydrophobicity of PEG increases, which has resulted in water being driven out water from PEG-rich phase, with the formation of solid (PEG) and liquid (water) phase (Hartounian *et al.,* 1993). Further, by conventional wisdom one anticipates an increase in microwave power should enhance the recovery of PEG recovery. However, this was not found true and the polymer recovery has decreased and there after has remained constant with increase in microwave power (Table 2E.2). This could be attributed to low dielectric properties of the polymers, which has resulted in low power absorption and reaching possible saturation levels in its ability to absorb microwave power at low power mode itself. The physical properties of the PEG-rich and salt-rich phases were practically same for the systems prepared from fresh as well as recovered PEG (Table 2E.3). This indicates that application of microwave field did not affect the physical properties of PEG during the course of recovery.

Further, in order to ensure PEG's suitability for reuse, it was thought desirable to compare the chemical characteristics of the phase systems prepared from fresh and recovered PEG in the presence of a biomolecule. For these partition studies C-phycocyanin (a natural blue colorant and also a

protein solution) was employed as an indirect means. Phase systems having compositions (System II, Table 2E.1) were prepared from recovered and fresh PEG along with salt (K_2HPO4: KH_2PO_4) and C-phycocyanin solution. After phase equilibrium, the top and bottom phases were analyzed and these results are as shown in Table 2E.4. From the analysis, it can be observed that the extent of increase in C-phycocyanin purity and partition coefficient was almost same for both the phase systems. Also, purity of C-phycocyanin during ATPE has increased by about 3 times in a single step. From the above study, it can be concluded that the application of microwave field for the recovery of PEG from spent ATPS has not altered the physical and chemical nature of the polymer. Hence, microwave field can be employed for the faster, efficient/effective recovery of PEG. Already, microwave process have gained acceptance in food industry for various processing operations such as drying, pasteurizing, sterilizations etc (Ayappa, 1997). On similar lines, there is enough potential for the application of microwave field for large scale recovery of PEG from spent phases and can possibly be integrated in the process line for enhanced productivity.

2. E.4. Conclusions

A simple and effective method of recovery of PEG from spent phases by the application of microwave field has been reported for the first time. The separation of water from PEG rich phase is explained based on decrease in PEG solubility with temperature rise and subsequent increase in PEG

hydrophobicity. Application of microwave field has not affected the physical and chemical characteristics during polymer recovery which is evident from the measurement of physical properties of the phases and partition studies carried out in the presence of biomoelcule.

Table 2E.1. Phase composition of PEG/phosphate systems

Phase system (PEG/ K_2HPO_4:KH_2PO_4)	Composition Top/Bottom (w/w%)	Top phase (w/w %)		Bottom phase (w/w%)	
		PEG	phosphate	PEG	phosphate
System I	16.07/10.13	35.68	2.65	2.31	16.47
System II	7.04/14.36	28.0	4.13	0.37	17.66

Table 2E.2. PEG recovery from spent ATPS by the application of microwave field.

Sl no	Power Settings	Out power, W	Duration of exposure, s	Rise phase temperature, °C	% recovery
1	10	175	90	90	88.3
2	30	315	60	95	86.1
3	60	385	35	95	86.0
4	High	800	30	95	86.0

Table 2E.3. Physical properties of phase systems from fresh and recovered PEG

Phase system	Composition Top/Bottom w/w %	Density, kg/m^3		Viscosity, mPas	
		Top	Bottom	Top	Bottom
PEG/phosphate (fresh PEG)	16.07/10.13	1082.5	1149.3	27.0	1.35
PEG/phosphate (recovered PEG)	16.07/10.13	1082.9	1147.7	26.0	1.35
PEG/phosphate (fresh PEG)	7.04/14.36	1088.8	1157.1	32.1	1.51
PEG/phosphate (recovered PEG)	7.04/14.36	1087.5	1157.4	44.9	1.46

Table 2E.4. Purity and partition coefficient of C-phycocyanin using phase systems prepared from fresh and recovered PEG.

Phase composition	Volume, ml		Concentration mg/ml		Purity		Partition coefficient
	Top phase (PEG rich)	Bottom phase (salt rich)	Top phase (PEG rich)	Bottom phase (salt rich)	Top phase (PEG rich)	Bottom phase (salt rich)	
7.04/14.369 (fresh PEG)	29	61	1.49	0.046	3.3	0.402	32.4
7.04/14.369 (recovered PEG)	29	61	1.56	0.055	3.1	0.618	28.4

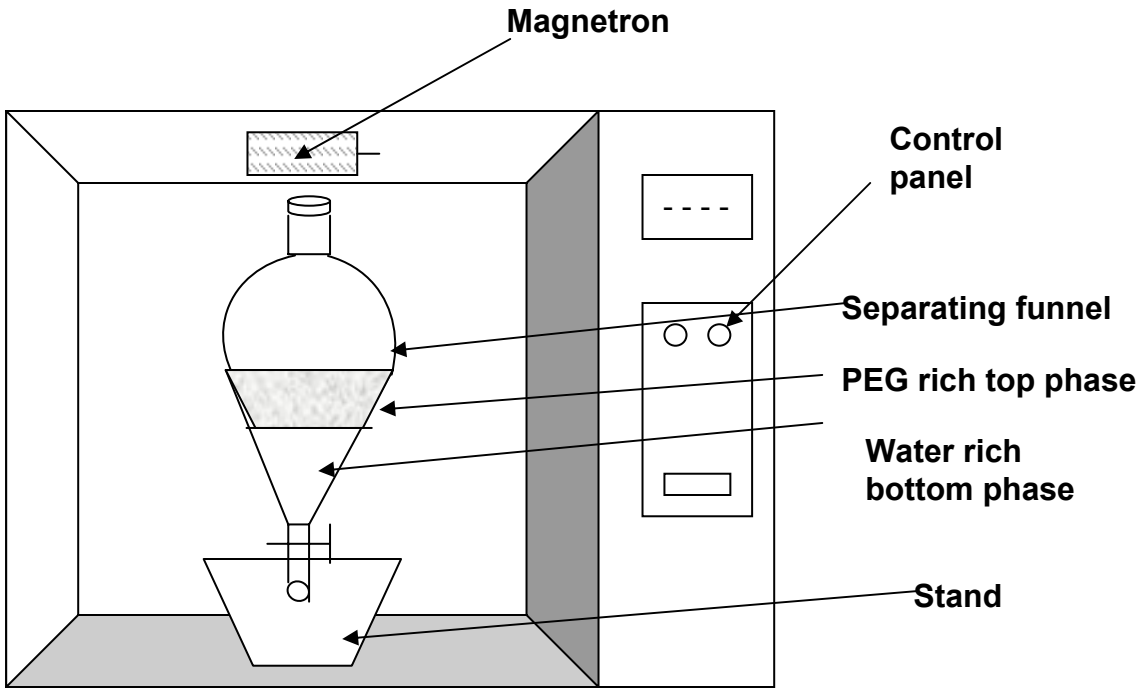


Figure 2E.1. Schematic representation of polymer recovery in the presence of microwave field

Chapter 3

Osmotic Membrane Distillation for the Concentration of Solutions/Liquids

3.1. Introduction

In recent years, proteins/biomolecules, natural food colorants and liquid foods derived from natural sources is gaining importance over their synthetic counterparts in various food, biological and therapeutic applications due to their non-toxic/non-carcinogenic characteristics and high nutritive value (vitamins, essential minerals etc.). Proteins/biomolecules, natural food colorants and liquid foods when extracted from there sources have low solid content, color strength and high water load. Hence, it is desirable to concentrate these biomolecules/liquid foods to improve shelf life, stability and to reduce storage/transportation costs (Thijssen, 1979; Philip, 1984; Petrotos & Lazarides, 2001).

Conventionally, purification and concentration of proteins/biological solutions is achieved by employing combination of different methods such as filtration, homogenization, extraction, centrifugation, precipitation, dialysis, gel filtration, electrophoresis and ion-exchange chromatography (Belter *et al.,* 1988). However, these protocols are cumbersome, since more number of unit operations is involved. Moreover, in some of the processes when chromatography, electrophoresis methods are involved it is difficult and uneconomical to operate on large-scale, unless the product of is high value. In case of liquid foods, traditionally concentration is carried out by evaporation. Application of high temperature during evaporation results in product deterioration (loss of aroma, flavor, nutritive components and color)

resulting in low quality end product (Koseoglu *et al.,* 1990). Moreover, conventional concentration methods are not suitable for the concentration of biological solutions, due to their tendency to denature the biomolecules at higher temperatures (evaporation). Freeze concentration is another method employed for the concentration of liquid foods, which offers advantages over evaporation with respect to minimal loss of aroma, color and nutritive value. However, major drawback of this process is the maximum achievable concentration (only up to 40-45°B) and this technique is not suitable for liquid foods having high pulp content. Moreover, both these processes (evaporation and freeze concentration) are energy intensive, since phase change is involved (Cassano *et al.,* 2003; Despande *et al.,* 1982).

In recent years, membrane technology is gaining importance, since it provides a means of concentrating, fractionating and/or purifying fluids overcoming the drawbacks of the existing processes. Moreover, membrane processes are energy efficient, since they do not involve phase change. These processes normally operate at ambient temperature with minimal product damage thereby retaining the nutritive, color and flavor/aroma components. In view of the above, membrane processes such as microfiltration (MF), ultrafiltration (UF) and reverse osmosis (RO) are being employed for processing of proteins/natural colors and liquid foods.

3.2. Existing membrane processes

Each membrane separation process is characterized by the use of a membrane to accomplish a particular type of separation. The primary role of membrane is to act as a selective barrier. The membrane has the ability to transport one component more readily than the other because of differences in physical and/or chemical properties between the membrane and the permeating components. Transport through the membrane takes place as a result of driving force acting across the membrane, in the form of pressure, concentration, electrical potential or temperature. Also, the nature of the membrane (its structure and material) determines the type of application, ranging from the separation of microscopic particles to the separation of molecules of an identical size or shape (Cheryan, 1986). The existing membrane processes such as microfiltration (MF), ultrafiltration (UF) and reverse osmosis (RO) have been explained briefly in the following sections.

3.2.1. Microfiltration

Microfiltration (MF) is an oldest membrane process in use prior to the industrial application of RO (Glinenius, 1985). The membrane pore size employed for MF process is in the range of 0.05-10μm, having membrane porosity of about 70% and membrane thickness in the range of 10-150 μm. The applied pressure will be in the range of 0.1-2 bar and retains the particles in the micron range. MF finds application in cell harvesting, clarification of fruit juice, waste water treatment, separation of casein and

whey protein from skimmed milk and separation of oil-water emulsions (Grandison and Lewis, 1996).

3.2.2. Ultrafiltration

Ultrafiltration (UF) is a separation process which employs membrane having a pore size in the range of 0.01-0.1μm. UF membranes are made up of polysulfone, polyvinyldene fluoride and cellulose acetate. The applied pressure employed in UF process lies in the range of 1-10 bar. It is mainly employed for the clarification and/or concentration depending on the size of suspended particles in liquid foods, aqueous solutions, suspension, proteins etc. (Cheryan, 1986).

3.2.3. Reverse osmosis

Reverse osmosis (RO) is the membrane process where in a solvent, usually water, is separated from the solution across the semi-permeable membrane. This is accomplished by applying the pressure to the solution in excess of its osmotic pressure (10-100 bar). RO process employs dense membranes having pore size of about 1-10 Å (<2 nm). The porosity of the membrane is about 50%. The separation mechanism in RO is based on solution diffusion across the membrane. Membranes are made up of cellulose triacetate, polyether urea and polyamide. RO finds application in concentration of solutions (liquid foods such as fruit juices, milk), desalination of brackish and seawater (Mulder, 1998).

3.3. Drawbacks of existing membrane processes

The existing membrane processes have drawbacks such as, shear damage to products (especially in case of proteins), membrane fouling, concentration polarization and maximum achievable concentration (only 25-30°B in case of RO) (Girard and Fukomoto, 2000). In order to overcome these drawbacks, alternate/complementary membrane processes needs to be developed for the concentration of thermolabile biomolecules/natural food colors and liquid foods.

3.4. Recent developments in membrane processes

3.4.1. Membrane Distillation

Membrane Distillation (MD) is a relatively new process that is being investigated worldwide as a low cost, energy saving alternative to conventional separation processes such as distillation and RO (Lawson and Lloyd, 1997).

In MD, aqueous feed solution which is at higher temperature is brought in contact with one side (feed side) of hydrophobic microporous membrane. The hydrophobic nature of the membrane prevents penetration of the aqueous solution into pores, resulting in a vapor-liquid interface at each pore entrance as shown in Figure 3.1. The water and solute (if the solute is volatile) evaporates from the liquid-vapor interface on the feed side of the membrane, transfer across the membrane takes place either by diffusion or by convection, and the water/solute condenses or is removed in

the vapor form on the permeate side. MD is operated in different modes and is dependent upon the permeate composition, flux and volatility. The direct contact membrane distillation (DCMD) is best suited for applications such as desalination or the concentration of aqueous solutions, in which water is the major permeate component. Sweep gas membrane distillation (SGMD) or Vacuum membrane distillation (VMD) is employed when volatile organic or a dissolved gas is being removed from an aqueous solution.

The main advantage of MD process is, it requires lower operating temperatures since it is not necessary to heat the process liquid above their boiling temperatures. Feed temperatures in MD typically are in the range of 60-90°C. MD finds application in food industry where concentrated fruit juices can be prepared with better flavor and color retention (Calabro *et al.,* 1994), sterilization of biological fluids (Sakai *et al.,* 1988), removal of trace volatile organic compounds from waste water and concentration of ionic, colloid or other relatively non-volatile aqueous solution (Lawson and Lloyd, 1997).

3.4.2. Direct Osmosis

The concept similar to direct osmosis (DO) was employed by Eastern European farmers for the concentration of fruit juices, wherein a bag filled with juice was immersed in a brine solution (Cussler, 1984). This process could not be exploited commercially due to low flux. After many years, Popper and co-workers employed plate and frame membrane module with flat cellulose acetate sheet membranes to concentrate grape juice using

NaCl as OA (Popper *et al.,* 1964). DO had been employed to achieve maximum concentration (>60°B) of aqueous solutions such as natural food colors/liquid foods after pre-concentration by RO process. (Bolin and Salunke, 1971; Loeb and Bloch, 1973; Rodriguez-Saona *et al.,* 2001). DO process has also been employed for the desalination of sea water (Kravath and Davis, 1975).

DO, which is also known as direct osmosis concentration (DOC), employs a semi-permeable dense hydrophilic membrane which separates two aqueous solutions (feed and OA solution) having different osmotic pressures as shown in Figure 3.2. The driving force in DO process is the difference in osmotic pressure across the membrane (Beaudry, & Lampi, 1990). The transfer of water occurs from the low to high concentrated solution till the osmotic pressures of both the systems become equal. DO, also offers advantages with respect to energy (since there is no phase change) and thermolabile component retention during the concentration of natural food colors/liquid foods and could achieve concentration up to about 45-60°B (Wong and Winger, 1999).

3.4.3. Osmotic Membrane Distillation

From, mid 1960's onwards non-isothermal transport of water through microporous hydrophobic partitions have been studied. The process was called "membrane distillation", (Findley *et al.,* 1969; Sarti *et al.,* 1985). Later

research on osmotic membrane distillation (OMD) was pioneered in late 1980's by Lefebvre of Synix Research Institute Sydney (Lefebvre, 1988), and then on, steady stream of publications have continued to appear (Alves and Coelhoso, 2002; Courel *et al.,* 2000b; Deblay, 1991; Gostoli, 1999; Lefebrve, 1988; Lefebvre, 1992; Mengual *et al.,* 1993; Sheng *et al.,* 1991) accounting various process and membrane related parameters. Further, OMD process has been employed to concentrate various liquid foods such as orange, grape and carrot juices using different osmotic agents (Cassano *et al.,* 2003; Sheng *et al.,* 1991).

OMD uses hydrophobic membrane, thus allowing the components to pass through in the form of vapor. OMD differs from MD in terms of the driving force, wherein, temperature gradient is replaced by vapor pressure gradient. Application of OMD enables to achieve concentration to a very high level at ambient temperature and at atmospheric pressure, with minimal loss of solutes. Hence, OMD plays an important role in processing of thermolabile biomolecules, pharmaceuticals and liquid foods. Further, integration of ATPE with membrane processes such as OMD holds considerable promise for the purification and concentration of heat/shear sensitive biomolecules. Very little information is reported about this process leaving ample scope for research and development as explained below (Sheng *et al.,* 1991; Kunz *et al.,* 1996).

The earlier works in OMD have accounted the resistance offered by only one side boundary layer because they involve only model systems

(water/brine systems) (Mengual *et al.,* 1993; Gostoli, 1999; Courel *et al.,* 2000b; Alves and Coelhoso, 2002). In the present work, during the modeling of mass transfer in OMD, it was thought desirable to account all the three resistances (feed boundary layer/membrane/brine boundary layer) keeping in view the application of OMD for real systems. Thus, a mass transfer resistance-in-series model is developed by considering real system (pineapple juice). The effect of various process parameters such as type, concentration and flow rate of osmotic agent (OA), type and pore size of membrane, temperature with respect to transmembrane flux have been studied for real system. Mass transfer in the boundary layer is estimated as function of dimensionless numbers, whereas, the mass transfer through the membrane is accounted based on either by Knudsen or molecular diffusion. The use of dimensionless numbers enables the quantification of the hydrodynamics on the feed as well as OA boundary layers, which in turn is used to account the contribution of boundary layers to the overall resistance. The model developed based on the overall resistance could predict reasonably well the value of transmembrane flux at all the process parameters studied for real system.

3.5. Process features

OMD is an athermal membrane process which employs microporous hydrophobic membrane to separate the two aqueous solutions (feed and osmotic solution) having different osmotic pressures. The driving force for the

mass (water) transfer is the difference in vapor pressure of the solvent (water) across the membrane. Water evaporates from surface of the solution with the higher vapor pressure (feed), diffuses in the form of vapor through the pores of the membrane and condenses on surface of the solution with lower vapor pressure (osmotic agent, OA) as shown in Figure 3.3. This results in the concentration of the feed and dilution of the OA solution. The evaporation process requires the supply of the latent heat of vaporization at the upstream meniscus. This is provided as sensible heat via conduction or convection from the bulk upstream liquid, or via conduction across the solid phase comprising the membrane. Conversely, at the downstream face of the membrane, condensation of water vapor into the osmotic agent solution occur releasing heat of condensation. The thermal conductance of membrane should be sufficiently high, so that all the energy of vaporization can be supplied by conduction across the membrane at a low temperature gradient. As a consequence, under normal operating conditions, the temperature difference between the liquids on either side of the membrane is quite small (≈2°C). Hence the process is isothermal for all practical purposes.

OMD is also known as osmotic evaporation, membrane evaporation, and isothermal membrane distillation or gas membrane extraction. It can be employed to achieve maximum concentration up to 70°B without product damage. In OMD, the vapor pressure of flavor/fragrance components due to low concentration (relative to that of water) is substantially depressed,

thereby reducing the driving force for transmembrane transport of these solutes. The solubilities of these lipophilic solutes are substantially lower in concentrated saline (OA) solutions than in pure water. As a consequence, the vapor pressure of these solutes, when present even in trace concentration in such solution, is much higher than that over water at the same concentration. Thus, the vapor pressure driving force for vapor phase transfer of these solutes from feed to the strip is far lower. Further, due to the higher molecular weights of these solutes, their diffusive permeabilities through the membrane are lower. The overall result of all these factors contributes to make OMD an attractive complimentary or alternate process for the concentration of liquid foods with high flavor retention. Also, absence of shear (in turn, heat) damage makes it an attractive alternative for the concentration of proteins/natural food colors. Further, OMD can also be employed as a pre-concentration step prior to ATPE, lypholization (freeze drying) of temperature sensitive biological products such as vaccines, hormones, enzymes and natural colors (Hogan *et al.,* 1998).

3.6. Mathematical modeling

3.6.1 Mass transfer aspects

The basic equation, which relates the transmembrane flux (J) to the driving force represented by the difference in vapor pressures at both liquid-vapor interfaces of the membrane (ΔP), is given by

$$J = K\Delta P \qquad (3.1)$$

where '*K*' is the overall mass transfer co-efficient which accounts for all three resistances for water transport as shown in Figure 3.4 and is given by

$$K = \left(\frac{1}{K_f} + \frac{1}{K_m} + \frac{1}{K_{oA}} \right)^{-1} \qquad (3.2)$$

where K_f, K_m and K_{oA} are the mass transfer resistances in feed layer, membrane and osmotic agent layer, respectively.

3.6.2. Mass transfer through the membrane

The resistance for the diffusive transport of water vapor across the microporous hydrophobic membrane is offered by the membrane pore structure as well as air present in the pores. The diffusion of water vapor through this stagnant gas phase (air) of the membrane pore can be described either by Knudsen diffusion or molecular diffusion depending on the pore size (Geankoplis, 1993).

When the mean free path is significant relative to the pore size, the diffusing molecules collide more frequently with the pore wall and the diameter of the pore is important. Such mass transfer is termed as Knudsen diffusion and is given by (Schofield *et al.,* 1987)

$$J_K = \left[1.064 \ \frac{r\varepsilon}{\chi\delta} \left(\frac{M}{RT} \right)^{0.5} \right] (P_1 - P_2) \qquad (3.3)$$

where first term of RHS is the Knudsen diffusion coefficient (K_n) corrected for membrane porosity as well as pore tortuosity and second term accounts for the driving force.

When the membrane pore size is relatively large, the collisions between the diffusing molecules themselves are more frequent, the mode of diffusion is called molecular diffusion. Water flux across the membrane is represented by (Sherwood *et al.,* 1975)

$$J_m = \left[\frac{1}{Y_{ln}}\frac{D\varepsilon}{\chi\delta}\frac{M}{RT}\right](P_1 - P_2) \qquad (3.4)$$

and the molecular diffusion coefficient is expressed as

$$K_m = \frac{1}{Y_{ln}}\frac{D\varepsilon}{\chi\delta}\frac{M}{RT} \qquad (3.5)$$

where 'D' is the Fick's diffusion coefficient and is given by

$$D = \frac{0.001858T^{3/2}\left(1/M_A + 1/M_B\right)^{1/2}}{P\sigma_{AB}{}^2\Omega_D} \qquad (3.6)$$

Both these approaches are useful for predicting the mass transfer through the membrane. However, each of them having its own limitations. The Knudsen equation requires details of membrane pore geometry (such as pore radius, membrane thickness, tortuosity), whereas molecular diffusion is not valid at low partial pressure of the air (as 'Y_{ln}' tends to zero). Hence,

molecular diffusion is clearly undefined, thereby diffusion mechanism approaches Knudsen diffusion (Schofield *et al.,* 1987).

3.6.3. Mass transfer through the boundary layers

The boundary layers are present in the feed and the OA on either sides of the membrane. These layers offer significant resistance to mass transfer, which cannot be ignored, and it depends on the physical properties of the solution (feed and OA) as well as the hydrodynamic conditions of the systems. The liquid mass transfer coefficients in the boundary layers of feed and OA (k_f and k_{oA}) can be estimated by using empirical equation given below, involving only physical properties and hydrodynamic conditions of the solutions.

where,

$$Sh = b_1 Re^{b_2} Sc^{b_3} \qquad (3.7)$$

$$Sh = \frac{k_i d}{D_w}, \quad Re = \frac{ud\rho}{\mu} \quad and \quad Sc = \frac{\mu}{\rho D_w} \qquad (3.8)$$

where 'D_w' is the water diffusion coefficient estimated by the following empirical equation (Wilke and Chang, 1955; Treybal , 1980)

$$Dw = \frac{(117.3\times10^{-18})(\varphi M_B)^{0.5} T}{\mu \vartheta_A^{0.6}} \qquad (3.9)$$

in order to obtain the overall mass transfer coefficient (equation 3.2), the liquid mass transfer coefficients 'k_f' and 'k_{OA}' as well as membrane

coefficient 'K_m' are to be expressed in same units using the following equation (Courel *et al.,* 2000b).

$$K = \frac{k\, C^t\, M_W}{(x_s)_{lm}\gamma\, P^*} \qquad (3.10)$$

where 'γ' is activity co-efficient and 'P*' is the saturation vapor pressure for $CaCl_2.2H_2O$ and NaCl, and were obtained form literature (Patil *et al.,* 1991; Colin *et al.,* 1985)

3.7. Influence of process parameters in OMD

3.7.1. Membrane related parameters

The membranes made out of synthetic polymers such as polytetrafluoroethylene (PTFE), polypropylene (PP) and polyvinylidene difluoride (PVDF), which are hydrophobic in nature can be employed for OMD process, (Kunz *et al.,* 1996). The membrane used in OMD process should be highly porous (60-80%) and as thin as possible (0.1-1μm) since the flux is directly proportional to the porosity and inversely to the membrane thickness (pore length). Furthermore, it should be highly conductive so that energy of vaporization of feed can be supplied by conduction across the membrane at low temperature gradient, thereby making the process practically isothermal.

The membrane having relatively larger pore size at the surface showed higher organic volatiles retention per unit water removal than those

with smaller openings (Barbe *et al.,* 1998). Accordingly, pores with larger diameters at the membrane surface allow greater intrusion of the feed and OA streams, which in turn leads to the formation of an extended boundary layer. This extended layer offers extra resistance through which the volatile components must diffuse. In order to obtain the product of desirable quality (retaining all the volatiles, flavor/fragrance components), it is essential to employ membranes with larger surface pore diameters. Further, the effect of membrane pore size on transmembrane flux was studied (Mengual *et al.,* 1993) and not much variation in flux was observed when the pore size range was 0.05-0.5 μm. Recently, Brodard *et al* (2003) have employed ceramic (inorganic) membranes made up of alumina having pore sizes of 0.2 μm and 0.8 μm. In the study they observed, the mechanism of water transport to be independent of pore size and follow molecular diffusion.

The hydrophobicity of the membrane is a decisive parameter which will influence the viability of OMD process. However, quantifying this parameter on a porous material is not easy, as it is not supported by any theory. The method of estimating contact angle by accounting the surface energy of smooth dense material does not apply for porous membranes. The pressure variable can be included in the wettability definition via the liquid entry pressure represented by Laplace equation.

$$\Delta P_{entry} = \frac{-2B\,\gamma_L Cos\,\theta}{\gamma_{max}} \qquad (3.11)$$

where 'ΔP_{entry}' is the liquid entry pressure, 'B' is geometric factor, 'γ_L' is liquid surface tension, 'θ' is liquid-solid contact angle and 'γ_{max}' is largest pore radius. Once pressure drop across vapor-liquid interface '$\Delta P_{interface}$' exceeds penetration pressure 'ΔP_{entry}', the liquid can penetrate into the membrane pores and membrane is termed 'wetted'. Hence, wettability of OMD membranes can be better defined by a surface tension combined with operating pressure conditions rather than by contact angle measurements. (Courel *et al.,* 2001).

3.7.2. Process related parameters

The rate of water (solvent) transport increases with increase in vapor pressure gradient across the membrane. In order to maintain the required driving force (vapor pressure difference), generally salts of high water solubility and low equivalent weights such as NaCl, $CaCl_2$, $MgCl_2$, $MgSO_4$, K_2HPO_4, KH_2PO_4 are preferred as OA's in OMD process. It may be noted that equivalent weights of salts increase in the order of NaCl>$CaCl_2$> K_2HPO_4 as do their water solubilities. The effect of OA concentration on transmembrane flux was studied in considerable detail for a model system, water as feed (Alves and Coelhoso, 2002; Courel *et al.,*2000a; Mengual *et al.,* 1993).

The effect of OA flow rate on transmembrane flux was reported by various research groups for model as well as real systems (Courel *et al.,* 2000a, b). In all these cases, transmembrane flux increased with an increase in OA flow rate, which can be attributed to reduction in concentration polarization layer.

The effect of temperature on transmembrane flux has been studied (Alves and Coelhoso, 2002; Courel *et al.,* 2000a; Gostoli, 1999; Mengual *et al.,* 1993). The transmembrane flux has increased with increase in temperature.

3.8. Methodology

3.8.1. Materials

3.8.1.1. Membranes

Hydrophobic polypropylene (PP) membrane of pore sizes 0.05 μm and 0.2 μm manufactured by Accurel, Enka, Germany (obtained from NCL, Pune) were used in the study. The pore size ranges as well as the type of the membrane was selected based on earlier work (Narayan *et al.,* 2002). The details of the membrane characteristics used in the present study (PP - 0.05 μm and 0.2 μm) are given in Table 3.1.

3.8.1.2. Chemicals

All chemicals and reagents used in the study were of laboratory reagent (LR) grade and procured from local suppliers listed as in Table 3.2.

For all the experiments conducted, distilled water from the distillation unit (Millipore, Inc.,) was used.

3.8.1.3. System selection

C-phycocyanin, a natural food colorant derived from blue-green algae finds its use in coloring of many food products such as fermented milk products, ice creams, chewing gum, soft drinks, alcoholic drinks, desserts, sweet cake decoration, milk shakes, cosmetics (used in eyeliners, lipsticks), and also in pharmaceutical applications (as phycofluor probes in immunodiagnostics, prevention or inhibition of cancer; Pillai *et al.,* 1996). The C-phycocyanin solution, obtained from *Spirulina platensis*, is crude and dilute, which requires purification and concentration. After employing initial purification steps for the removal of impurities such as chlorophyll, cell debris etc., concentration of C-phycocyanin solution is to be undertaken by athermal concentration process, since C-phycocyanin (also a protein) is shear/heat sensitive (Glazer *et al.,* 1994). Hence, in the present study OMD process has been employed for the concentration of C-phycocyanin solution, to achieve higher concentration levels, since this process operates at ambient temperature and at atmospheric pressure without causing heat/shear damage to the product. Also, application of OMD process for the concentration of C-phycocyanin solution will significantly reduce the water load on subsequent processing steps (such as ATPE, freeze-drying etc.)

Pineapple is grown abundantly in tropical and sub-tropical regions. In, India, pineapple fruits are grown mainly in southern region. Since, the fruits are seasonal the fruit offer good potential to be processed as fruit juice. The fruit is rich in vitamin C and minerals apart from citric acid, malic acid, sugars, fatty acids and pectins. Pineapple thrives well in dry arid regions with temperature ranging from 25 to 42°C. (Nagy *et al.,* 1993). The juices prepared from these fruits have fairly good flavor and taste. Very less information is available on the processing of C - phycocyanin and pineapple juice by membrane processes especially by OMD process. Hence, both these solutes were selected as systems for the present study. Keeping in view of the commercial potential of OMD process involving real systems, the effect of various parameters on transmembrane flux employing pineapple juice as feed has been carried out in flat membrane module. Osmotic agents (OA's) namely NaCl, $CaCl_2.2H_2O$ were selected, based on their order of equivalent weights and their water solubilites. Further, feasibility studies were carried out to concentrate these solutes employing OMD process in order to achieve maximum concentration without product damage.

3.8.2. Methods

3.8.2.1. Fruit juice preparation

The fresh pineapple (*Annanus comasus*) fruits were purchased from local market and juice was extracted (after peeling) using Food Processor (Singer # FP-450).

3.8.2.2. Enzyme preparation for fruit juice clarification

Lyophilized pectinase (*Aspergillus niger*, B.No: T: 826160) powder having an activity of 5-20 units per mg protein (Lowry *et al.,* 1951) was obtained from SRL Ltd, Mumbai, India. The pectinase solution was prepared by dissolving pectinase powder (0.1 % v/w) in 100 ml of 0.1M sodium acetate buffer (Kester and Pisser, 1990). The resulting enzyme solution was used for the clarification of fruit juice. For large-scale trials (Section 4C) Biopectinase enzyme (activity of 5000 PG units/ml;950 PME units/ml) procured from Biocon India Ltd., Bangalore, India was used (0.1%v/w) after incubating the enzyme with around 200 ml of fruit juice maintained at an temperature of 40°C for a period of one hour. The activity of the enzyme was estimated in terms of polygalacturonase (PG) activity expressed as PG unit/ml and Polymethylesterase (PME) activity expressed as (U/g) as per methodology prescribed by Ranganna, 1986.

3.8.2.3. Experimental Procedure

OMD experiments were performed using a specially fabricated flat membrane module having a membrane area of 0.011544 m^2, as shown schematically in Figure 3.5. The module consists of Polyester mesh, Viton gasket and hydrophobic microporous membrane supported in between two stainless steel (SS316) frames. Feed solution comprising of fruit juice (pineapple juice) and osmotic agent solution (OA) were circulated on either side of the membrane in co-current mode using peristaltic pumps. OMD

experiments were performed using 0.05 and 0.2 μm polypropylene membrane with Sodium chloride (NaCl) and calcium chloride dihydrate ($CaCl_2$. $2H_2O$) as OA's. The flux was calculated, by measuring the increase in volume of OA once every hour. All the experiments were performed for a period of 5 hours and the corresponding average fluxes were reported. Further, experiments were performed as mentioned above to study the effect of various parameters such as type, concentration and flow rate of OA, type and pore size of the membrane and temperature with respect to transmembrane flux across the membrane during OMD process. Feed flow rate was maintained constant (100 ml/min) during the entire study. All the OMD experiments unless otherwise mentioned were carried out at the temperature of 28±2 ºC.

3.8.2.4. Confirmatory Tests

The following confirmatory tests were carried out to confirm the absence of (if any) OA towards the feed side (fruit juice side) during OMD studies (Vogel, 1980)

3.8.2.4.1. Test for Orthophosphate

The test was conducted to confirm the transfer of orthophosphate into the feed solution. Known amount of sample was taken in a test tube, to which freshly prepared copper sulphate solution was added, appearance of pale blue precipitate confirms the presence of orthophosphate.

3.8.2.4.2. Dilute Sulphuric Acid Test for Calcium

Addition of dilute sulphuric acid to the sample containing Ca^{2+}, will result in the formation of white precipitate of calcium sulphate.

$$Ca^{2+} + SO_4^{2-} \longrightarrow CaSO_4$$

3.8.2.4.3. Silver Nitrate Solution Test for Chloride

When silver nitrate solution is added to the solution containing chloride then white milky precipitate of silver chloride, which is insoluble in water and in dilute nitric acid but soluble in potassium cyanide solution, will form.

$$Cl^- + Ag^+ \longrightarrow AgCl$$

During OMD experiments, confirmatory tests for phosphate, calcium, chloride transfer across the membrane into the feed side were performed. The tests confirmed that there was no transfer of OA on to the feed side across the membrane.

3.8.3. Analysis of chemical composition in liquid food

3.8.3.1. pH determination

Around 20 ml of juice was taken and its pH was determined at the room temperature of $27\pm2\ ^0C$ in Control dynamics (Model: APX175 E/C) pH meter.

3.8.3.2. Titrable acidity

After determination of the pH, a known amount fruit juice was titrated against 0.1N Sodium hydroxide using phenolphthalein as an indicator until the end point was pale pink. Results expressed as percentage Total acid

$$\%\,Total\ acid = \frac{[(Titre\ value)(normality\ of\ alkali)(volume\ of\ madeup)(eq.wt\ ofacid)x100]}{(Volume\ of\ sample\ taken)(weight\ of\ sample\ taken)x100}$$

3.8.3.3. Juice concentration

Juice concentration in terms of soluble solids was measured using Erma's Handheld refractometer at 27±2°C. Results were reported as degrees Brix (°B).

3.8.3.4. Ascorbic acid determination

Amount of ascorbic acid present in the fruit juice was determined by titrating known amount of fruit juice and metaphosphoric acid-acetic acid mixture against 2,6-dichlorophenolindophenol dye solution. The results are expressed as mg of ascorbic acid per 100 ml of fruit juice (James, 1995).

3.8.4. Protocol for Membrane Cleaning and Storage as specified by the manufacturer

Step1. The membrane was removed from the module and was rinsed thoroughly with distilled water.

Step 2. Then the membrane was rinsed in hot 4% NaOH solution (50°C) for about 20 min.

Step 3. The membrane was washed twice with hot water (50°C) for about 30 min.

Step 4. Again the membrane was washed in cold water until all the alkali was removed from it which was confirmed by checking pH.

Step 5. Finally, the membrane was stored in 1-2 % formaldehyde solution under cold conditions.

Step 6. Prior to use, the membrane was washed with abundant distilled water.

3.9. Effect of various process parameters on transmembrane flux

In the present study for real systems, the effect of various process parameters such as type, concentration and flow rate of OA, type and pore size of membrane, temperature and on transmembrane flux across the membrane are discussed in the following sections.

3.9.1. Effect of concentration of the osmotic agent

The concentration of OA solutions was varied over 2-6 m (sodium chloride), and 2-14 m (calcium chloride dihydrate). During the experiments, osmotic solution flow rate was maintained constant (100ml/min). The values of transmembrane flux observed at different concentrations of osmotic solution are shown in Figures 3.6–3.9. In both the cases (NaCl and $CaCl_2$. $2H_2O$) transmembrane flux has linearly increased with an increase in OA

concentration. This increase is mainly due to higher vapor pressure difference across the membrane, which results in an increase in the driving force for water transport through the membrane. The theoretical fluxes were predicted by accounting the individual mass transfer resistances for water transport through the boundary layers (both feed and OA side) and also through the membrane based on molecular or Knudsen diffusion.

3.9.2. Effect of flow rate of the osmotic agent

Experiments were performed at varying flow rates (25 – 100 ml/min) by maintaining OA solution at its saturation level ($CaCl_2.2H_2O$ – 14m ; NaCl - 6m). The transmembrane flux has gradually increased with an increase in flow rate as shown in Figure 3.10. This can be attributed to reduction in concentration polarization layer (due to the reduction in hydrodynamic boundary layer thickness). This confirms the detrimental effect of concentration polarization in reducing the driving force across the membrane and its decrease with an increase in flow rate.

3.9.3. Effect of membrane pore size

To analyze the effect of pore size on transmembrane flux, experiments were performed using hydrophobic polypropylene membranes of 0.05 and 0.2μm pore size. The osmotic solutions namely $CaCl_2.2H_2O$ (2 - 14 m) and NaCl (2 - 6m) were circulated at constant flow rate of 100 ml/min, employing pineapple juice as feed. The results are shown in Figure 3.11 and it can be observed that the transmembrane flux remained almost constant with an increase in OA concentration for both the pore sizes. In other words,

pore size did not show much effect on transmembrane flux in the range studied. However, from the theoretical calculations it was observed that mode of diffusion mechanism (and in turn the transmembrane flux) depends on the membrane pore size employed, which has been explained later in the discussion.

3.9.5. Effect of osmotic agent on transmembrane flux

Two OA's namely NaCl and $CaCl_2.2H_2O$ were employed during OMD process. Transmembrane flux was higher in case of $CaCl_2 .2H_2O$ at all the concentrations when compared to NaCl as shown in Table 3.3. This is mainly due to the synergistic effect of flow rate and higher osmotic activity (ratio of its water solubility to its equivalent weight) in case of $CaCl_2.2H_2O$, which has resulted in higher vapor pressure gradient across the membrane.

3.9.6. Effect of temperature

Experiments were carried out by varying feed (pineapple juice) temperature in the range of 30 – 38 °C employing a constant temperature water bath. This study will be useful for the concentration of liquids that are not temperature sensitive. For these experiments both feed and osmotic solutions ($CaCl_2.2H_2O$; 14 m) were circulated at constant flow rate of 100 ml/min. From the results it was observed that the transmembrane flux across the membrane has increased significantly with an increase in temperature as shown in Figure 3.12. It is known that the mass transfer coefficient in transport processes shows Arrhenius dependency on temperature. Similar

behavior was observed in the present study, Further, rise in feed temperature results in an additional driving force that works synergistically with driving force generated due to the concentration gradient.

3.9.7. Model validation

To validate the model in case of real systems, theoretical fluxes were estimated by accounting the individual mass transfer coefficient for boundary layers (feed and OA) as well as for membrane as shown in Table 3.4. In present study, (real systems) it was thought desirable to account the mass transfer resistance through both the boundary layers (feed as well as OA side). In order to estimate the water transport through these boundary layers empirical correlation comprising of dimensionless numbers (equation 3.7) was used. The membrane module employed in the present work is flat and the flow is laminar (N_{Re} values ranging from 18-430) with low mass transfer rates. The values of the constants in equation (3.7) are considered as b_1 =0.664, b_2 =0.5 and b_3 =0.33 (Geankoplis, 1993). The correlation employed was able to predict the mass transfer coefficients for both boundary layers at the prevailing hydrodynamic conditions (Flat module, laminar flow). It is clear that pore size plays an important role in influencing the type of diffusion for water transport through the membrane. Mass transport of water through membrane has been estimated based on mode of diffusion mechanism in the pores either by Knudsen or molecular diffusion (equations 3.3 and equation 3.4). It was observed that, the Knudsen to be the mode of diffusion when

membrane pore size is 0.05 μm, whereas the molecular diffusion was the mode of diffusion when pore size is 0.2 μm. It may be noted that in the literature, the mechanism of mass transfer in the membrane could not be clearly pointed to be either Knudsen or molecular (Courel *et al.,* 2000b; Gostoli *et al.,* 1999; Schofield *et al.,* 1987). This may be mainly due to the fact that the membranes employed by those researchers are composite type where mechanism will be different in the active layer when compared to the support layer. Since the membranes used in the present work are without support, the mechanism could be identified.

Theoretical values of the transmembrane flux could be estimated after calculating the overall mass transfer resistance (membrane plus boundary layers). The model could predict the transmembrane flux and also effect of different process parameters on transmembrane flux quite satisfactorily for the real systems, namely pineapple and sweet lime juices. However, there are still deviations from experimental values (Table 3.5), which could be attributed to the uneven pore distribution of the membrane, porosity, thickness and tortuosity, apart from the complex hydrodynamic nature of the viscous boundary layers In order to minimize these deviations, there is need for precision analysis of the membrane structure.

3.9.7. Conclusions

The influence of various process parameters such as type, concentration and flow rate of OA, type and pore size of membrane,

temperature with respect to transmembrane flux were studied for real systems. Mass transfer-in-series resistance model was proposed fro mass transfer in OMD. The model could predict the transmembrane flux values and also the effect of the parameters. The feed and OA boundary layer resistances could not be ignored even though they were low. The observed deviations of the predicted values from the experimental values of the transmembrane flux could be attributed to uneven pore distribution, geometry of the membrane and complex hydrodynamic nature of the boundary layer (feed and OA). Membrane pore size did not show any significant effect on flux in the range studied.

3.10. Acoustic enhancement of transmembrane flux

3.10.1. Introduction

Application of acoustic field is gaining more attention as an alternative to chemical or thermal means in various aspects of food processing. The ability of ultrasound to enhance chemical reactions (sono-chemistry) and the physical effect (sono-processing) are well exploited in chemical and other allied industries (McClements, 1997).

Membrane based processes, namely microfiltration (MF), ultrafiltration (UF) and reverse osmosis (RO) are being employed for the concentration of aqueous solutions. However, membrane based processes suffer from the common draw back of low transmembrane flux, making these

processes too slow, especially when the process stream is to be concentrated by removing the solvent (water) from it. The problem is much more severe if the process stream is a viscous solution of polysaccharides such as dextran, maltodextrin or viscous fruit juice (like liquified mango pulp) etc.

Research efforts have been focused to address this problem for quite some time, which can be summarized as 1) Pretreatment of feed solution to increase the transmembrane flux either by enzymatic or UF (Barbe *et al.,* 1998) 2) Modifying membrane material (McDonough *et al.,* 1989) and 3) Membrane cleaning and altering operating conditions (Durham and Nguyen, 1993). Out of these, membrane cleaning by different means such as hydraulic, mechanical, chemical is most widely practiced. Mechanical/chemical cleaning tends to damage the membrane while hydraulic cleaning interferes with the process.

External field assisted membrane processes are relatively recent development. Pulsed electrokinetic cleaning of MF membranes has been reported (Bowen and Sabuni, 1992). Acoustically assisted diffusion through membranes has been gaining interest in recent times. However, most of the work reported is for biological membranes (Julian and Zenter, 1990; Edwards and Laugen, 1994) and only one report is available on solute diffusion through polymeric membrane (Lanart and Auslaudes, 1980).

The aim of the present work is to explore the application of acoustic field in the enhancement of transmembrane flux on lab-scale.

3.10.2. Methods

3.10.2.1. Sugar cane juice preparation

Fresh sugarcane juice (17°Brix) was obtained from the local market and vacuum filtered before experimentation.

3.10.2.2. Experimental procedure

The experimental set up is shown in Figure 3.13. Experiments were conducted using a flat membrane test cell (Amicon cell No. 8050). The hydrophobic polypropylene membranes (pore size 0.05 and 0.2 μm) were cut to the desired size (circular discs, effective diameter 4.5 x 10^{-2} m), placed over the porous support and then fixed to the cell. Around 30 ml of osmotic solution of known concentration was taken in the membrane cell while, whereas in the feed reservoir, 30 ml of distilled water/sugarcane juice is taken and connected to the cell by means of a Teflon tube. Experiments in presence of acoustic field (1.2 MHz) were carried out by placing the membrane cell over an ultrasonic transducer (Model # HM-460, Holmer Products Corp, Milford, MA). For all the experiments, both membrane cell and feed reservoir are placed at the same level in order to avoid the hydrostatic pressure difference across the membrane. Increase in height of OA solution in cell side was noted (which occurs as pure water diffuses across the membrane into the cell) every one hour and the corresponding

flux was calculated. All the experiments were performed for duration of 5 hours and the corresponding average fluxes were estimated. After each experiment, membranes were thoroughly cleaned as per manufacturer's instructions.

3.10.3. Results and discussion

The results of the experiments with acoustic field and control runs (without acoustic field) are given in Table 3.6. The experiments performed with 5M NaCl/pure water, 5M $CaCl_2$/pure water systems, showed an increase in flux in the range of 35 – 98%, when compared with control runs. In the case of 5M NaCl/sugarcane juice and 5M $CaCl_2$/sugarcane juice, the rise was in the range 22 to 205%. The increase in flux is due to the fact that acoustic field induces mild circulation currents, which disturbs the hydrodynamic boundary layer of OA solution thereby reducing the effect of concentration polarization phenomena. It may be noted that the effect of the acoustic field in MHz range is entirely different from the acoustic field of kHz range.

It is interesting to note that the effect of acoustic field is more in case of salt/sugarcane juice system when compared with salt/pure water system. This phenomenon could be explained as follows. In case of salt/ water system the concentration polarization layer is only on one side of the membrane (OA solution side) while in the salt/sugarcane juice system it was on both sides. As a result, the transmembrane flux during control runs

(without acoustic field) itself is low in case of salt/sugarcane juice system when compared to salt/water system. Hence, this effect of acoustic field is more prominent in case of the former.

3.10.4. Conclusions

Application of acoustic field has enhanced the transmembrane flux for model systems as well as real systems. Further, studies need to be undertaken for the enhancement of transmembrane flux using real systems in presence of acoustic field at a larger scale.

Nomenclature

D = diffusion coefficient, m^2 -s^{-1}

J = flux, l-m^{-2} h^{-1}

k = mass transfer coefficient in boundary layer, m-s^{-1}

K = mass transfer coefficient (feed and OA), kg-m^{-2} h^{-1} Pa^{-1}

K = overall mass transfer co-efficient, kg-m^{-2} h^{-1} Pa^{-1}

L= length of the membrane, m

M = molecular weights of constituents, kg – mol^{-1}

P = vapor pressure, kPa

ΔP = vapor pressure difference, K Pa

P* = saturated vapor pressure, kPa

R = gas constant, kJ - mol^{-1} – K^{-1}

r = membrane pore radius, m

T = temperature, K

u = velocity of the fluid, m -s^{-1}

x_s = osmotic agent molar agent

Y_{ln} = mole fraction of air (log-mean),[-]

Greek letters

μ = viscosity of the fluid , Pa –s

ρ = density of the fluid, kg – m^{-3}

δ = membrane thickness, m

ε = porosity, [-]

χ = tortuosity factor, [-]

ε_{AB} , σ_{AB} = Lennard – Jones force constants for the binary

Ω_D = collision integral, Å

Subscripts:

1-feed side

2-OA side

f -feed

k- knudsen diffusion,

M- molecular diffusion

o - osmotic agent

w – water

Table 3.1. Relevant characteristics of the membranes used in the OMD experiments

Membrane	Accurel 2E-PP	Accurel 0.5E-PP
Pore size	0.2 μm	0.05 μm
Bubble point (N_2 against IPA) minimum	1.05 bar	2.1 bar
Transmembrane flow at 25°C (IPA)	4.0 ml/cm^2.min.bar	2.5 ml/cm^2.min.bar
Transmembrane flow at 25°C (nitrogen)	1.5 l/cm^2.min.bar	1.0 l/cm^2.min.bar
Standard Width	300 ± 10 mm	300 ± 10 mm
Thickness	130 – 170 μm	75 – 110 μm
Burst pressure	> 1.1 bar	>0.9 bar
Tensile strength (longitudinal)	575 CN / 15 mm	550 CN / 15mm
Tensile strength (transversal)	650 CN / 15 mm	550 CN / 15 mm
Extractables	0.1%	0.1%

(Source: Technical information obtained from manufacturers Accurel, Enka, Germany)

Table 3.2. List of chemicals and reagents used and name of their supplier

Sl no	Name of chemicals	Name of manufacturer
1	Sodium chloride	Ranbaxy Ltd., Punjab, India
2	Calcium chloride dehydrate	Ranbaxy Ltd., Punjab, India
3	Copper sulphate	Ranbaxy Ltd., Punjab, India
4	Sodium benzoate	Ranbaxy Ltd., Punjab, India
5	Silver nitrate	Ranbaxy Ltd., Punjab, India
6	Formaldehyde solution	Ranbaxy Ltd., Punjab, India
7	Phenolphthalein	Ranbaxy Ltd., Punjab, India
8	di-potassium hydrogen phosphate	S.d. Fine Chemicals, Mumbai, India
9	Sulphuric acid	Merck Ltd, Mumbai, India
10	2,6-dichlorophenolindophenol	Himedia Lab. Ltd, Mumbai, India
11	Ascorbic acid	SRL Ltd., Mumbail, India.
12	Metaphosphoric acid	Loba Chemie, Mumbai, India
13	Sodium Hydroxide	Qualigens Fine Chemicals, Mumbai, India

Table 3.3. Experimental flux values at different concentration of OA solutions

Systems	Experimental flux for 0.2 μm ($kg\text{-}m^{-2}\ h^{-1}$)		
	$CaCl_2.\ 2H_2O$		NaCl*
	14 m	6m	
Pineapple	1.963	1.326	0.621
Sweet lime	1.910	0.988	0.781

* at maximum concentration of 6m

Table 3.4. Mass transfer coefficients values for real system

Osmotic Agent	Mass transfer coefficients (kg-m^{-2} h^{-1} Pa^{-1})			
	K_{feed}	$K_{membrane}$		K_{OA}
		Knudsen X 10^3	Molecular X 10^3	
	Pineapple juice			
$CaCl_2$. $2H_2O$ (0.05μm)	0.036	1.072	2.894	0.246
$CaCl_2$. $2H_2O$ (0.2μm)	0.036	2.572	1.737	0.310
NaCl (0.05μm)	0.039	1.072	2.894	0.033
NaCl (0.2μm)	0.039	2.572	1.737	0.033

Table 3.5. Values of experimental and theoretical flux at different OA concentration

Systems	Experimental flux, kg/m^2h		Theoretical flux , kg/m^2h			
			Molecular diffusion		Knudsen diffusion	
	$CaCl_2$	NaCl	$CaCl_2$	NaCl	$CaCl_2$	NaCl
Pine apple (0.05 μm)	0.6-1.6	0.3-0.64	1.1-6.6	0.52-1.82	0.4-2.5	0.2-0.75
Pine apple (0.2 μm)	0.5-1.75	0.3-0.62	0.69-4.0	0.3-1.17	1.01-5.8	0.47-1.6

Table 3.6. Comparison of fluxes in presence and in absence of acoustic field

System	Avg. flux for PP (0.05μm)		% increase in flux (l/m^2h)	Avg. flux for PP (0.2μm)		% increase in flux (l/m^2h)
	Control(0rpm) (l/m^2h)	Acoustics (l/m^2h)		Control(0rpm) (l/m^2h)	Acoustics (l/m^2h)	
5M NaCl/ pure water	0.52	0.81	58.9	0.41	0.81	97.6
5.3M $CaCl_2.2H_2O$/ pure water	0.69	0.93	34.8	0.69	1.04	50.7
5M NaCl/ sugarcane juice	0.42	0.51	21.4	0.24	0.73	204.2
5.3M $CaCl_2.2H_2O$/ sugarcane juice	0.42	0.81	92.9	0.32	0.94	193.8

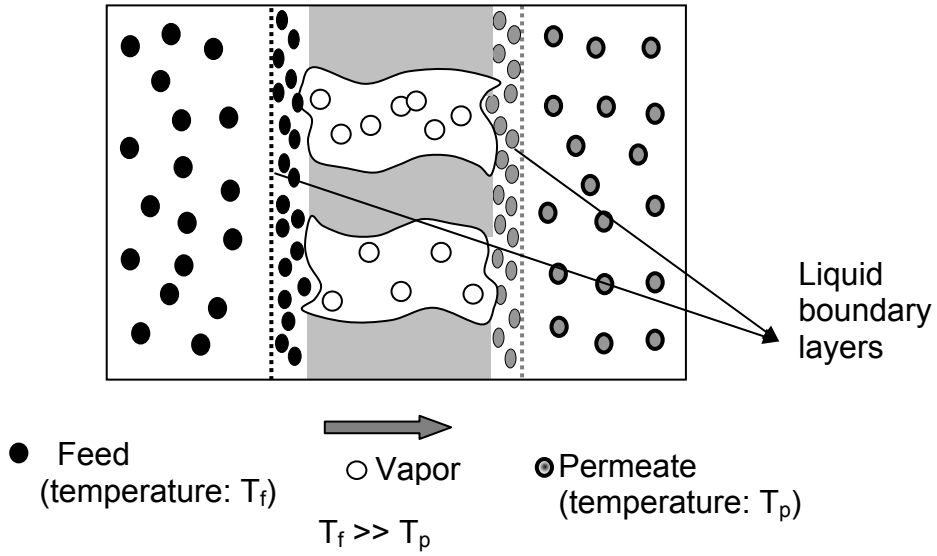


Figure 3.1. Principle of the Membrane distillation (MD) process

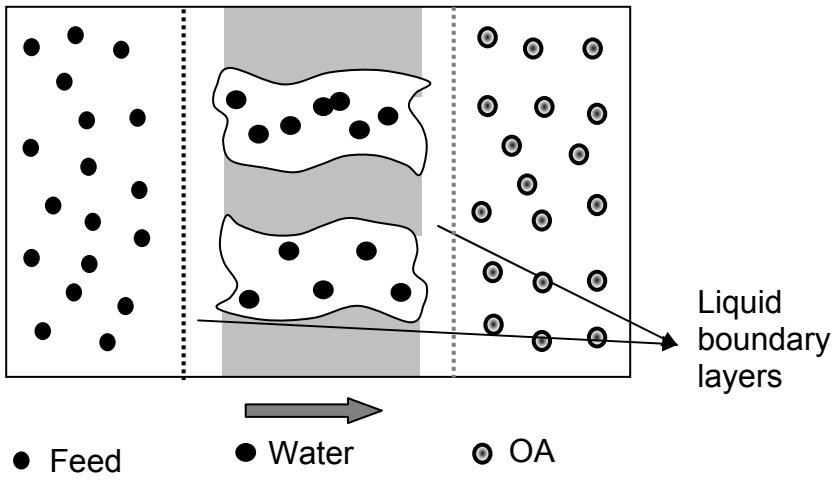


Figure 3.2. Principle of direct osmosis (DO) process

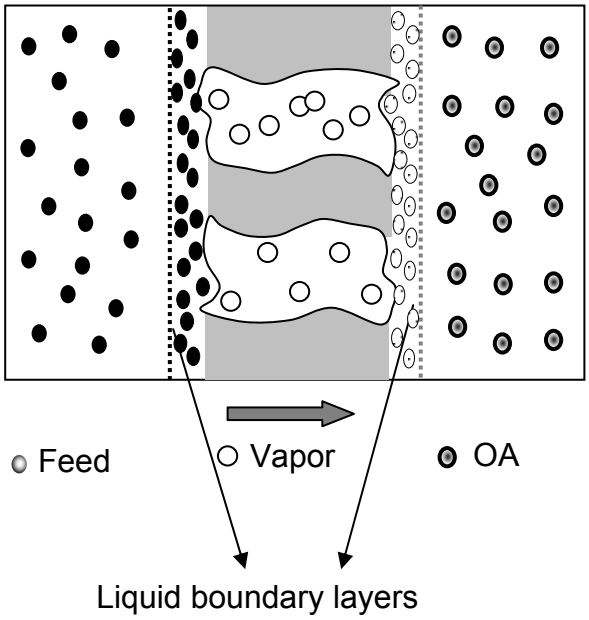


Figure 3.3. Schematic representation of OMD process

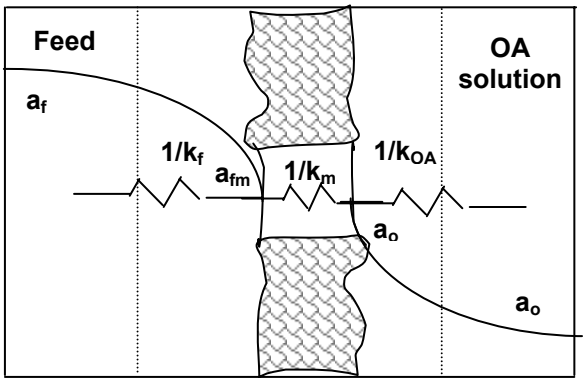
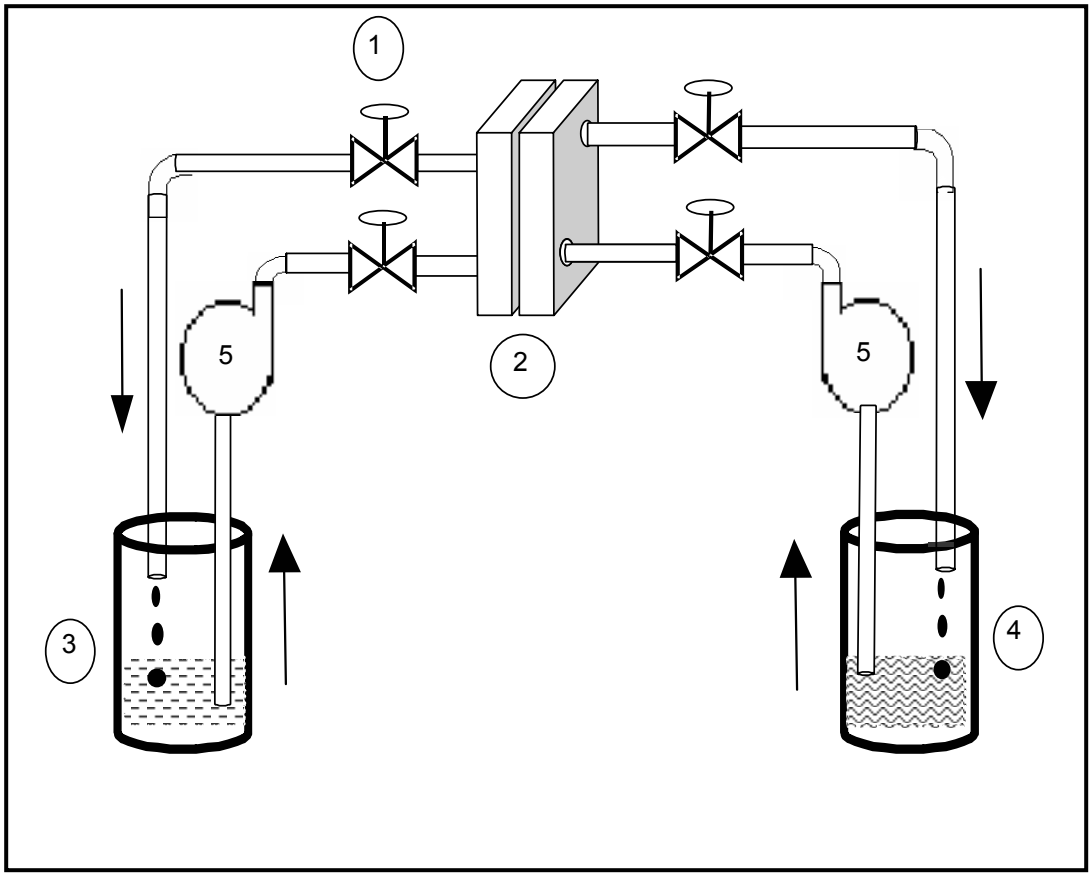


Figure 3.4. Water activity profile and mass transfer resistances in OMD



1. Ball valve
2. Flat membrane module
3. Feed reservoir
4. Osmotic agent reservoir
5. Peristaltic pump

Figure 3.5. Schematic representation of OMD process

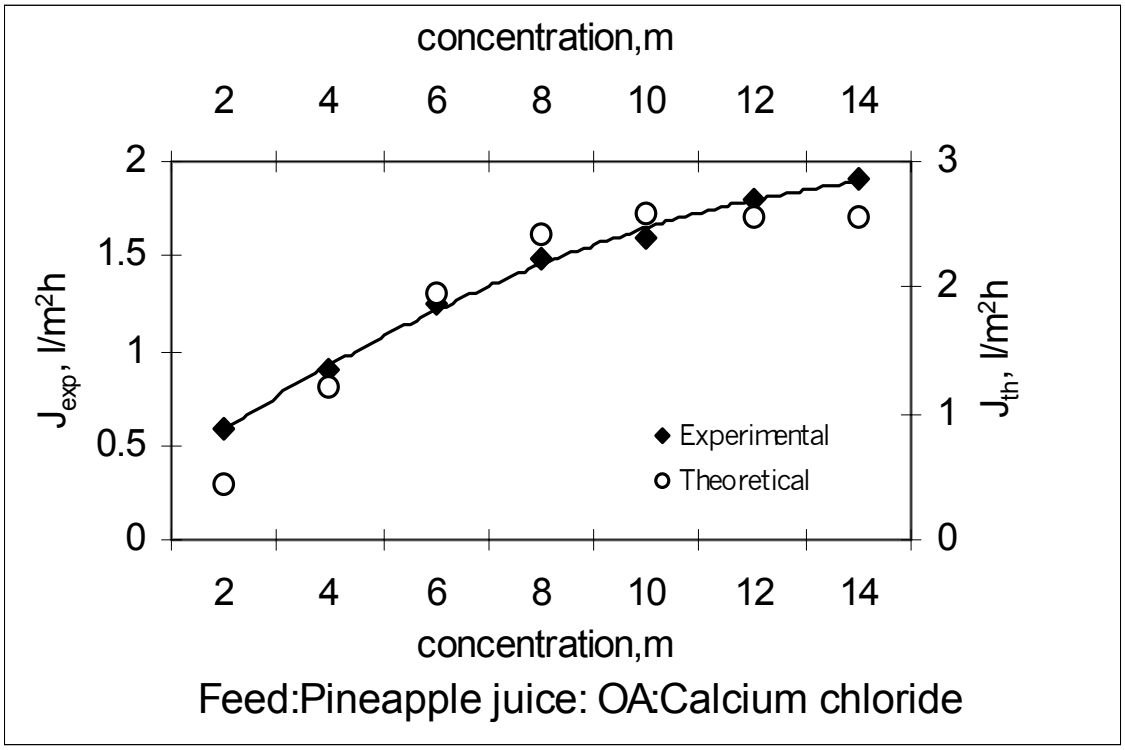


Figure 3.6. Effect of OA concentration on flux when membrane pore size is 0.05 μm

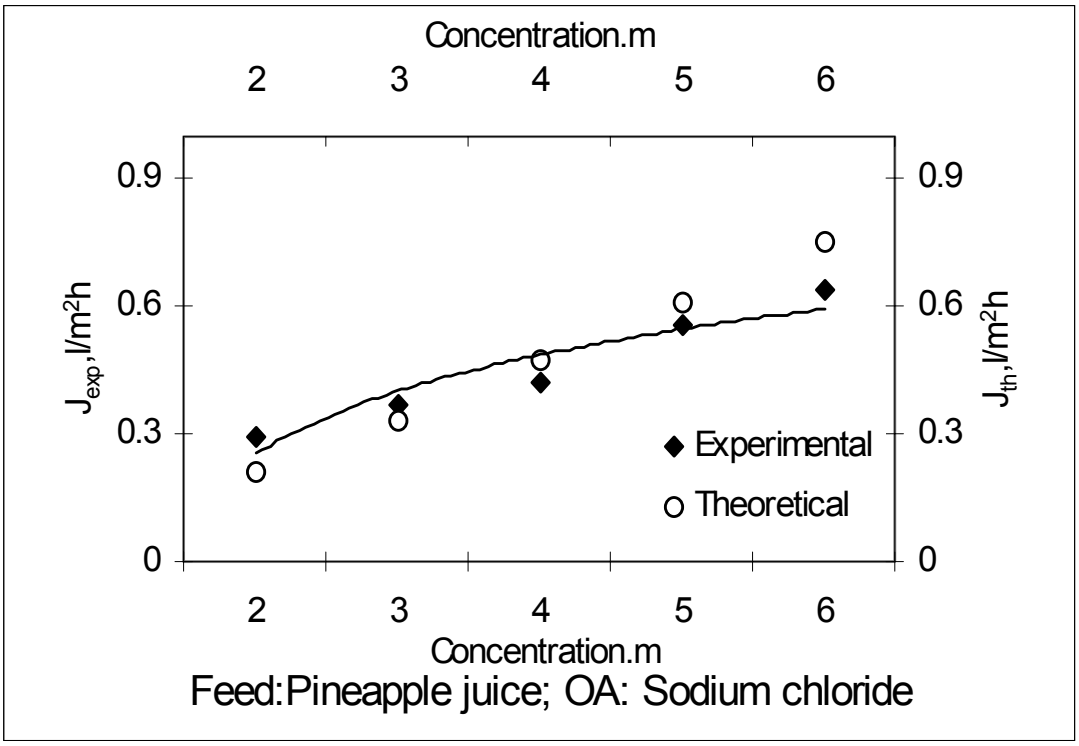


Figure 3.7. Effect of OA concentration on flux when membrane pore size is 0.05 μm

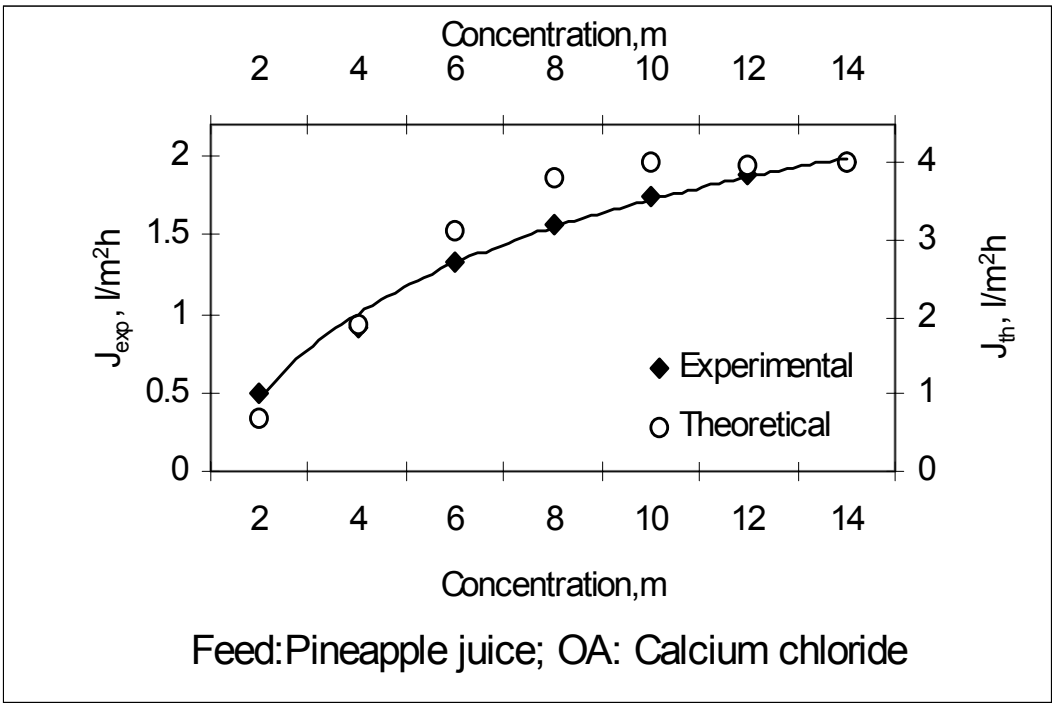


Figure 3.8. Effect of OA concentration on flux when membrane pore size is 0.2 μm

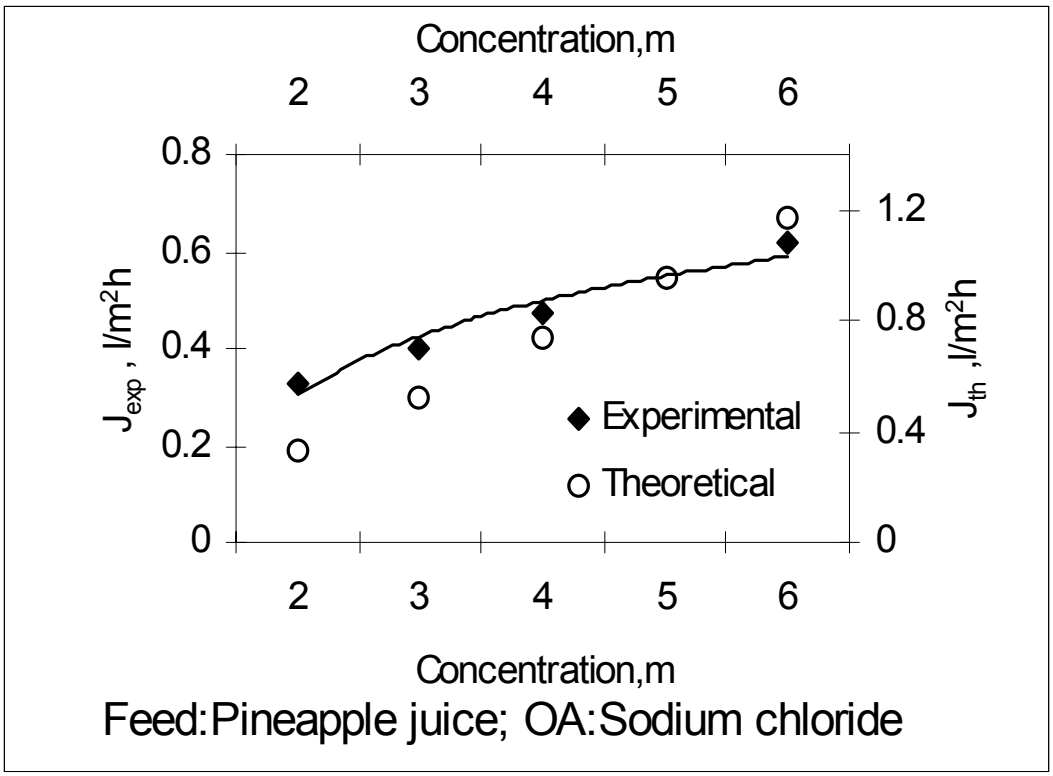


Figure 3.9. Effect of OA concentration on flux when membrane pore size is 0.2 μm

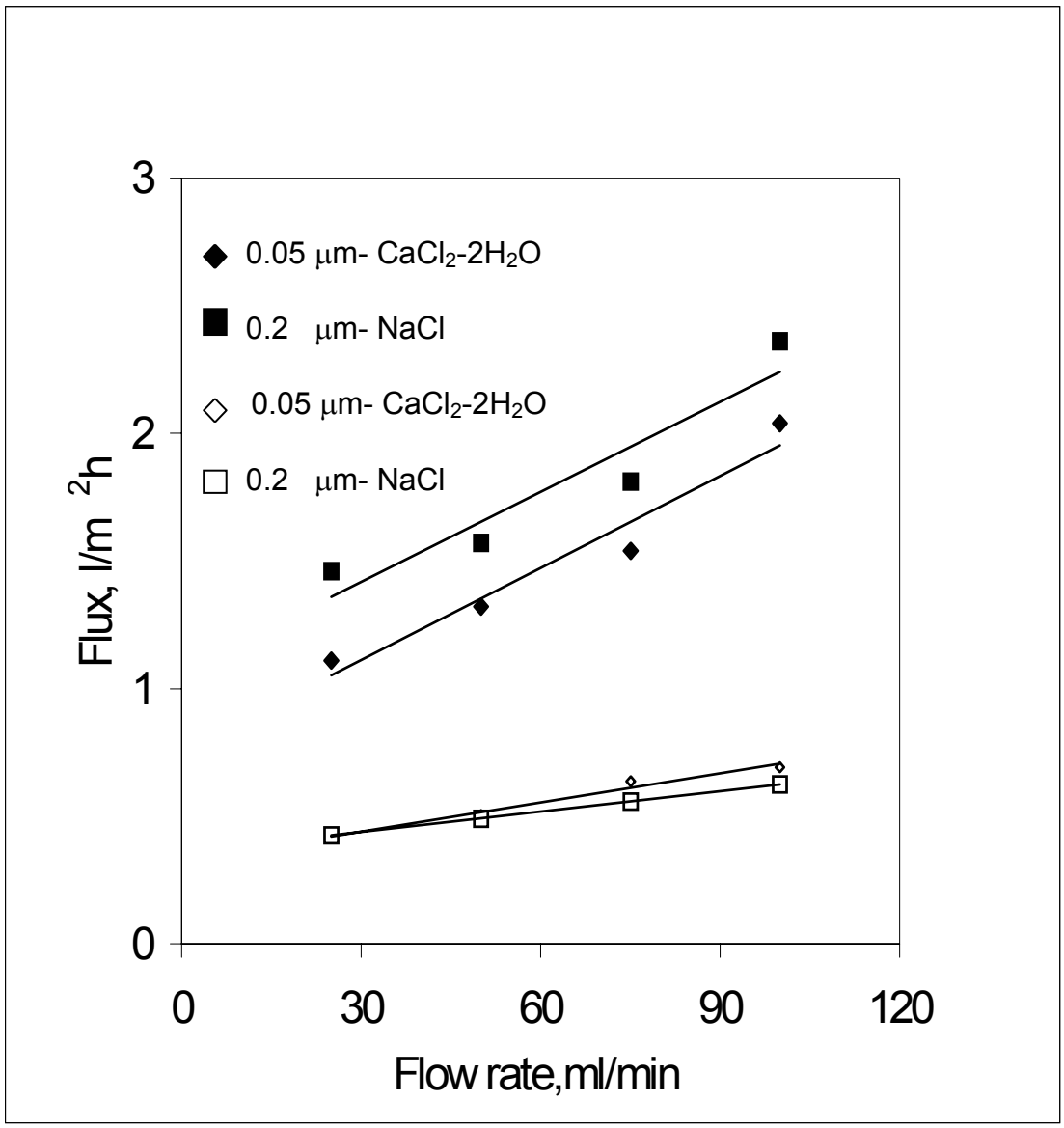


Figure 3.10. Effect of flow rate on transmembrane flux

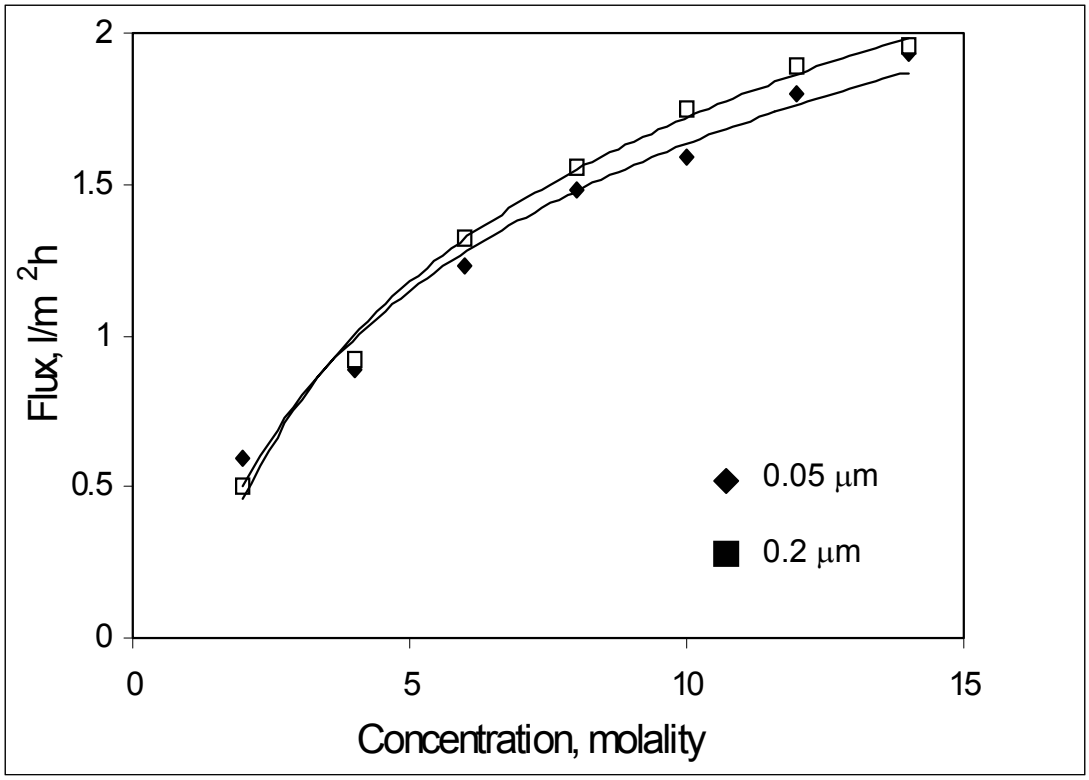


Figure 3.11. Effect of membrane pore size of transmembrane flux

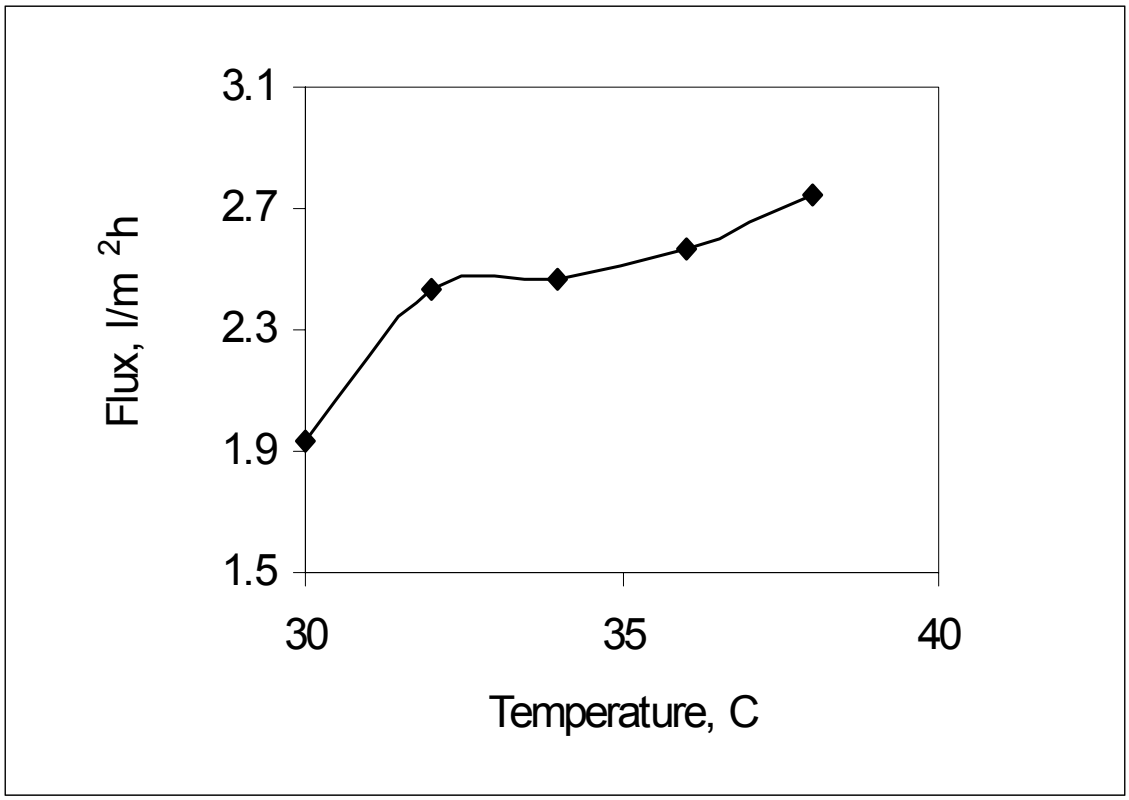


Figure 3.12. Effect of temperature on transmembrane flux

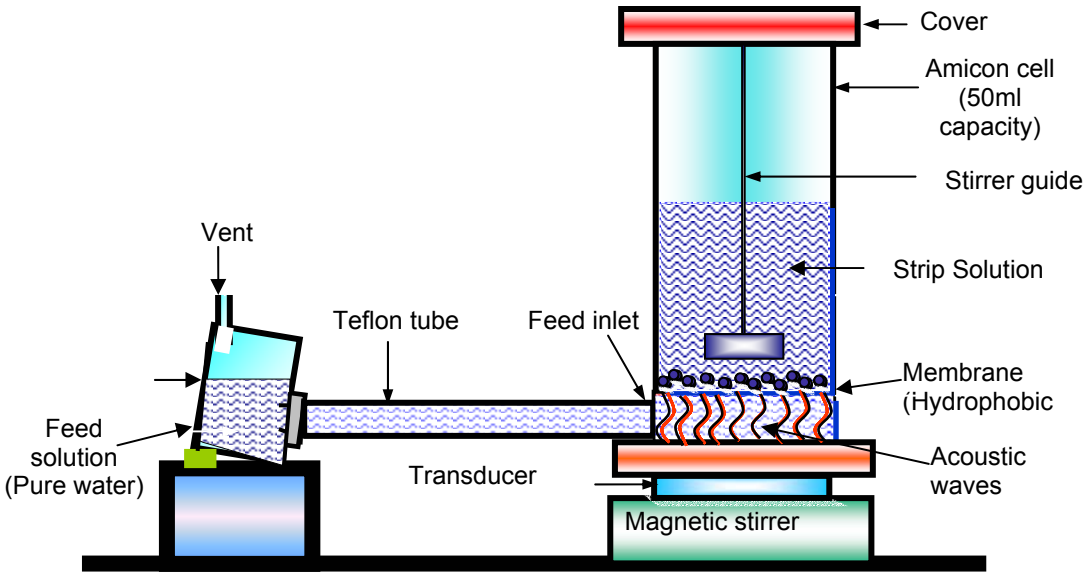


Figure 3.13. Conceptual diagram of acoustic field assisted OMD

Chapter 4
Applications of Osmotic Membrane Distillation

Section 4A

Purification and concentration of C-phycocyanin

4A.1. Introduction

Spirulina platensis which is commonly known as blue green algae/cyanobactrium belongs to family cyanophyceae. This algae is the source for phycobiliproteins apart from being rich source of protein (>60 %w/w), vitamins (vitamin A which is precursor β-carotene), cyanacobalamins (β-group vitamin), vitamin E (tocopherol) and polyunsaturated fatty acids (predominantly γ-linoleic acid). Phycobiliproteins are assembled into particles named phycobilisomes, which are attached in regular arrays to the external surface of the thylakoid membrane and act as major light harvesting pigments in cyanobacteria/red algae. Phycobilisomes consists of allophycocyanin cores surrounded by C-phycocyanin on the periphery, which is the major constituent (Gray and Gantt 1975; Glazer, 1994). C-phycocyanin finds its applications in coloring of many food products such as fermented milk products, ice creams, chewing gum, soft drinks, alcoholic drinks, desserts, sweet cake decoration, milk shakes, cosmetics (used in eyeliners, lipsticks), and also in pharmaceutical applications as phycofluor probes in immunodiagnostics, prevention or inhibition of cancer (Dainippon ink and chemicals, 1985; Kronik and Grossman, 1983). The classical purification procedure of C-phycocyanin consists of following methods such as filtration, sonication, milling, homogenization, extraction, centrifugation, precipitation, dialysis, gel filtration and ion-exchange chromatography (Kageyama *et al.,* 1994; Herrera *et al.,* 1989). However, this protocol has disadvantages of

having many unit operations and some of the techniques such as chromatography, gel filtration is difficult to scale-up. Another disadvantage of these methods is that even after centrifugation, C-phycocyanin solution will not be free from suspended impurities. In view of the above, there is a need for simpler and efficient methods involving a few unit operations for the processing of C-phycocyanin solution. Also, C-phycocyanin solution, obtained after the initial processing will be dilute, which needs to be concentrated for food/pharmaceutical applications. For the concentration of C-phycocyanin solution, it is desirable to have a process which operates under mild operating conditions, since C-phycocyanin (which is also protein) is heat/shear sensitive (Gantt, 1981). Recently, attempts have been made to clarify and concentrate C-phycocyanin employing membrane processes such as ultrafiltration, reverse osmosis, nanofiltration (Jaouen *et al.,* 1999). All these processes exhibited good pigment recovery (100%) and could achieve a concentration by a factor of 7. However, since these processes operate under high pressure there is possibility of shear damage in case of C-phycocynanin. Therefore in the present study, an attempt has been made to separate and purify C-phycocyanin from freshly harvested biomass involving minimum number of unit operations and also to concentrate to higher levels without product damage by employing osmotic membrane distillation (OMD). The main advantage of OMD process is it operates at ambient temperature and pressure without causing any heat/shear damage to the product.

Further, pre-concentration of C-phycocyanin extract by OMD will significantly reduce the amount of water load on the subsequent processing steps (such as ATPE, freeze-drying etc.).

4A.2. Analytical Procedures

4A.2.1. Determination of C-phyocyanin concentration

The C-phycocyanin concentration was determined using UV-spectrophotometer (model Shimadzu UV1601, Japan), by measuring the optical density at 280nm for total proteins, 620nm for C-phycocyanin and 650nm for allophycocyanin. The concentration was calculated by the formula (Tandaeu and Hounard, 1988).

$$Concentration\,(mg/ml) = \frac{A_{620} - 0.7(A_{650})}{7.38} \qquad (4A.1)$$

4A.2.2. Purity determination

The purity of C-phycocyanin was determined by the ratio of the optical density at 620nm to 280nm.

$$Purity = \frac{A_{620}}{A_{280}} \qquad (4A.2)$$

4A.3. Separation and Purification of C-phycocyanin

The blue green algae *Spirulina platensis* was grown in outdoor open raceway pond (70 m^3) using CFTRI medium at Department of Plant Cell Biotechnology, CFTRI. The processing of spirulina biomass was carried out

involving a few unit operations as shown in Figure 4A.1. The Spirulina was harvested for its biomass in a conveyor type filter. The biomass thus obtained was homogenized in a homogenizer at a pressure of 150 kg/m^2. Further, C-phycocyanin solution was centrifuged in a disc centrifuge (Westfalia Separator, Germany) to remove the impurities such as chlorophyll and cell debris. The C-phycocyanin extract thus obtained was stored in cold storage at a temperature of 4±1°C and was used for further studies.

4A. 4. Concentration of phycocynanin solution by OMD process

In the present study, the best process operating conditions as observed in Section-3.9 of Chapter 3 were selected. Apart from calcium chloride dihydrate ($CaCl_2.2H_2O$; 14m) another OA, dihydrogen potassium phosphate (K_2HPO_4; 14m) were employed. The flow rates of OA and feed were maintained at 100 ml/min. The dilute C-phycocyanin solution concentration was carried out by OMD process in a flat membrane module using hydrophobic polypropylene membrane (0.05 μm) as shown in Figure 4A.2. Test for calcium and phosphate leakage on to the feed side across the membrane were carried out per the procedure mentioned in section-3.8 of Chapter 3 at regular time intervals. The increase in C-phycocyanin concentration by OMD was measured spectrophotometrically every hour.

4A.5. Results and Discussion

The C-phycocyanin extract obtained after the processing of freshly harvested biomass was analyzed spectrophotometrically for its initial concentration and purity using equations 4A.1 and 4A.2 and found to be 0.72 mg/ml and 1.0 respectively. The dilute C-phycocyanin solution was then concentrated by OMD process employing two OA's namely K_2HPO_4 and $CaCl_2.2H_20$. The concentration of C-phycocyanin increased by about 127% and 220% respectively when K_2HPO_4 and $CaCl_2.2H_2O$, were employed as OA's as shown in Figure 4A.3. Under otherwise similar conditions, $CaCl_2.2H_2O$ induced higher transmembrane flux as shown in Table 4A.1. This is due to the better osmotic activity in case of $CaCl_2.2H_20$ when compared to K_2HPO_4. Also, under otherwise similar conditions, when $CaCl_2.2H_2O$ was employed as OA, the concentration rate was higher when compared to K_2HPO_4 as OA. During the C-phycocyanin concentration by OMD process irrespective of OA, there was no product damage (purity remained constant; confirmed by spectral analysis) as represented in Figure 4A.4 (a) - Figure 4A.4 (g) and Figure 4A.5 (a) - Figure 4A.5 (e). When the volume of the C-phycocyanin solution reduced below $1/5^{th}$ of its original volume, the concentrate was freeze dried (Heterodyne freeze drier: Model Fd-3; vacuum -50 bar) to powder form. The freeze drying of the product will enable to increase stability and ease of storage of the product.

4A.6. Conclusions

A simple and efficient method with a few number of unit operations has been employed for the initial separation and purification of C-phycocyanin from its biomass. This was followed by the concentration of C-phycocyanin solution with minimal product damage by OMD process. Also, the water load will be reduced significantly on subsequent processing steps such as ATPE, freeze drying. The possible integration of ATPE with OMD can be of considerable promise in enhancing the overall productivity of the process during the purification and concentration of C-phycocyanin and other thermolabile biomoelcules.

Table 4A.1. Effect of OA type on transmembrane flux during C-phycocyanin solution concentration by OMD

Time, h	OA: K_2HPO_4; PP- 0.05μm Flux, l/m^2 h	OA: $CaCl_2$ $2H_20$; PP- 0.05μm Flux, l/m^2 h
0	---	---
2	2.25	3.2
4	1.66	2.66
6	1.38	2.39
8	1.32	2.3
10	1.94	---
12	1.31	---
14	1.05	---

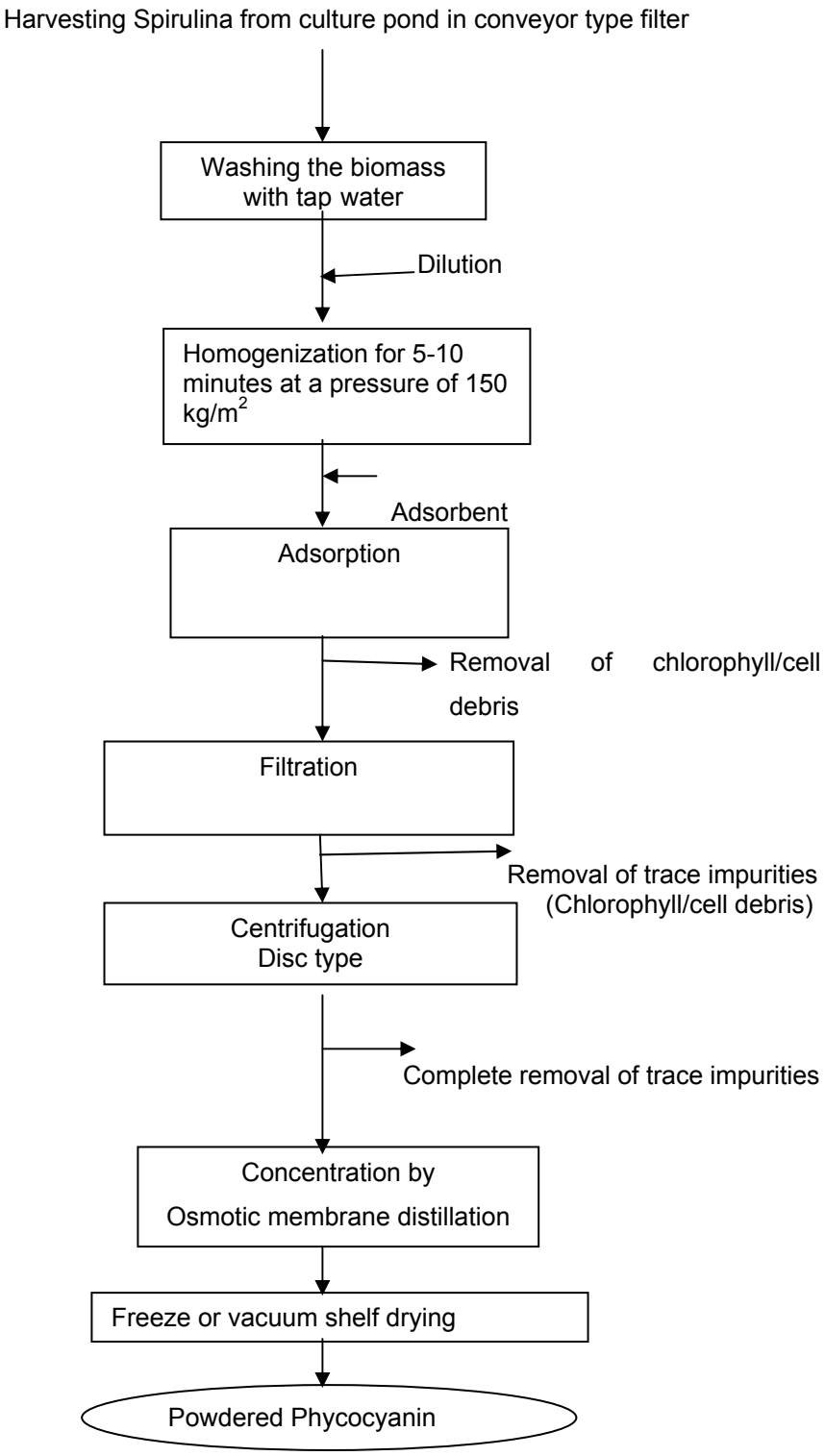


Figure 4A.1. Schematic representation of the C-phycocyanin processing

Figure 4A.2. Flat membrane cell employed for OMD – concentration of C-phycocyanin

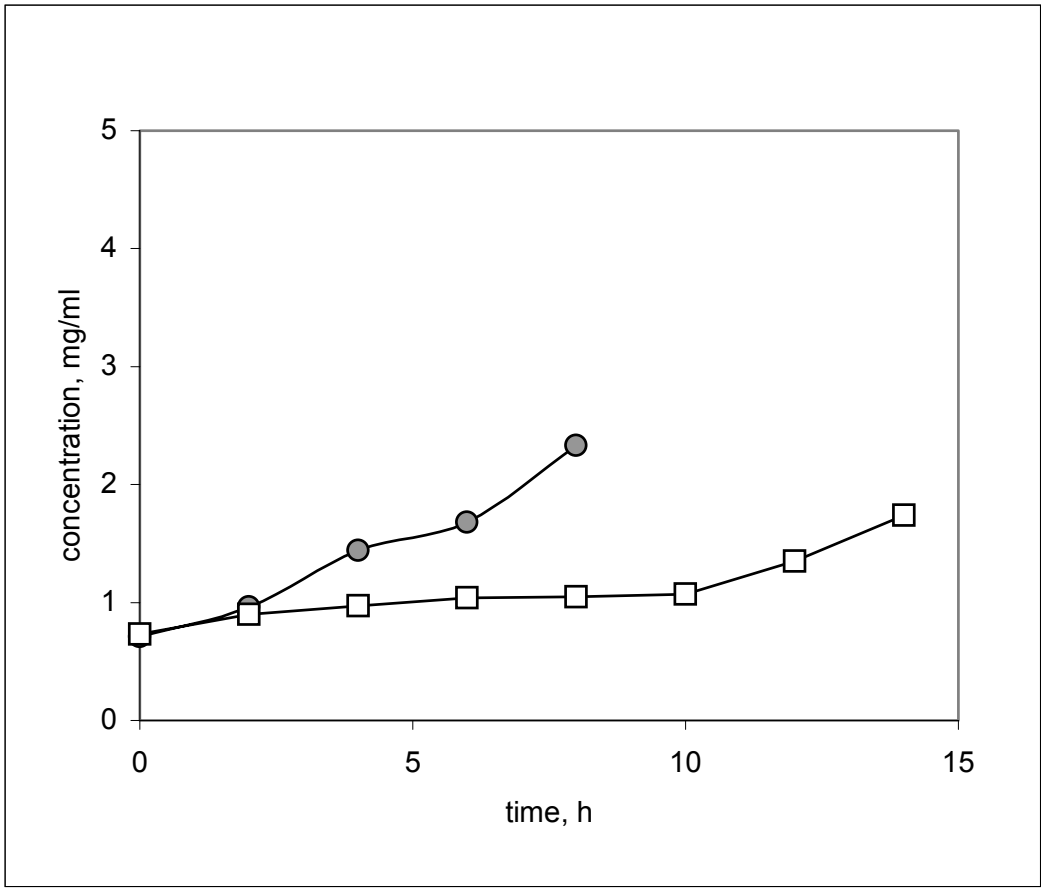


Figure 4A.3. Effect of C-phycocyanin solution concentration with time during OMD process

Figure 4A.4 (a) – zero hours

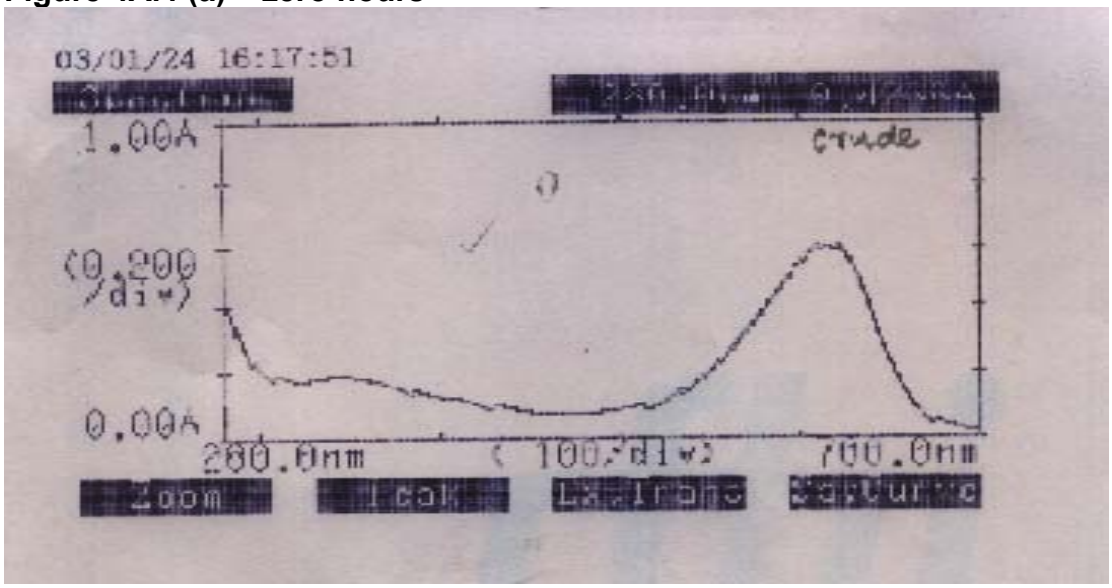


Figure 4A.4 (b) – 2 hours

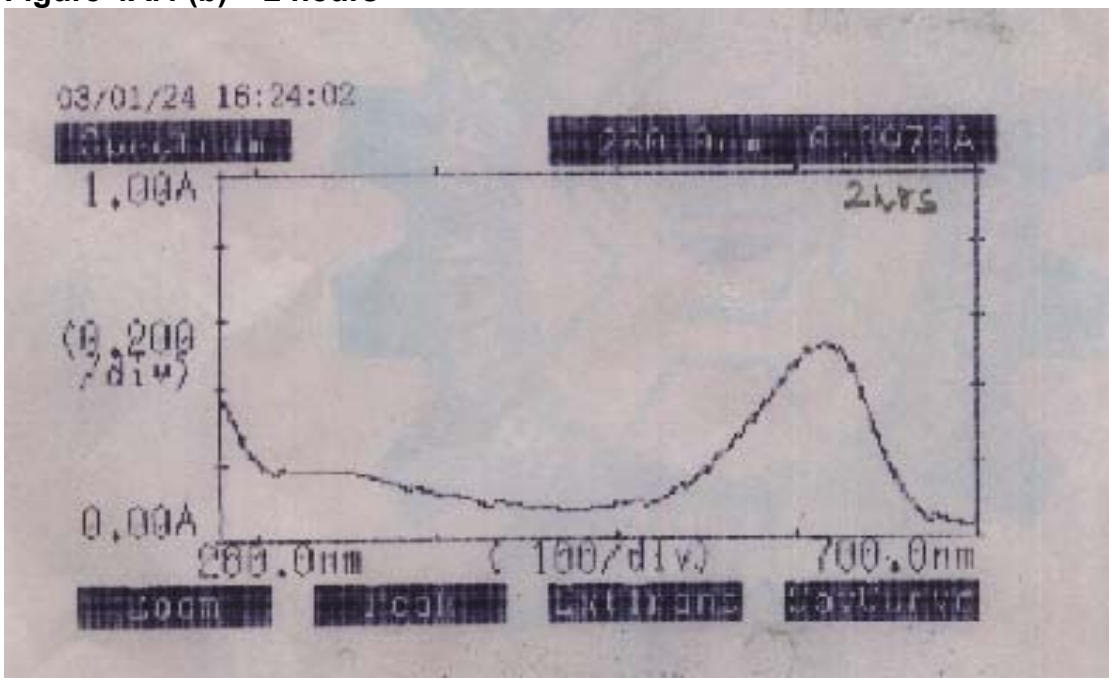


Figure 4A.4. Spectral profile (wavelength vs optical density) of C-phycocyanin concentration by OMD at different time intervals when K_2HPO_4 employed as OA

`**Figure 4A.4 (c) – 4 hours**

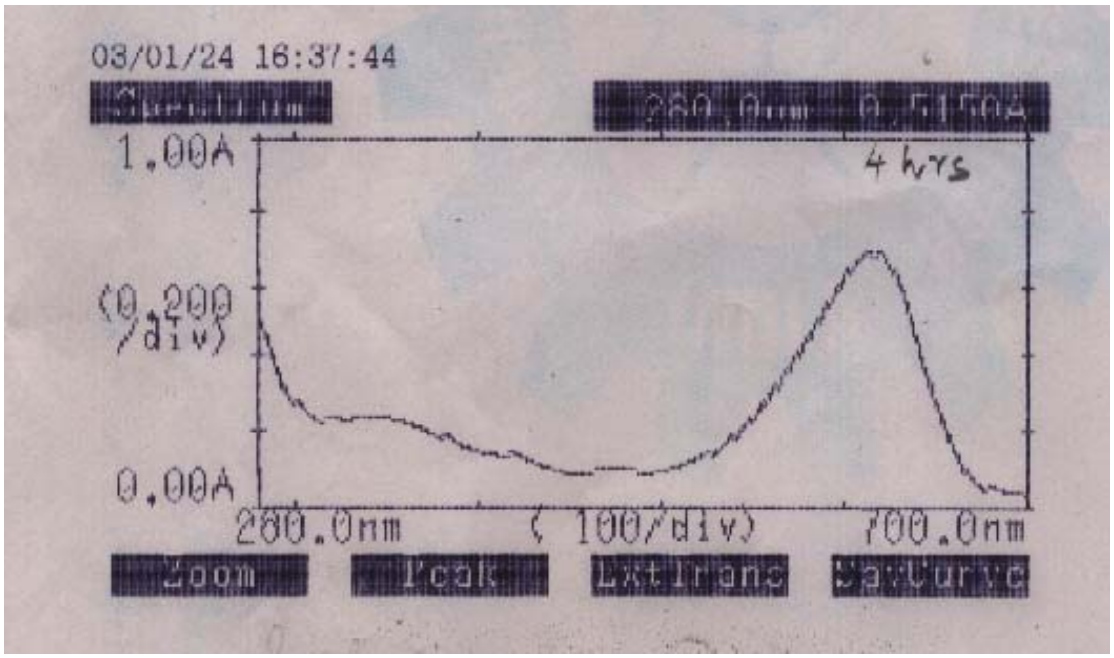


Figure 4A.4 (d) – 6 hours

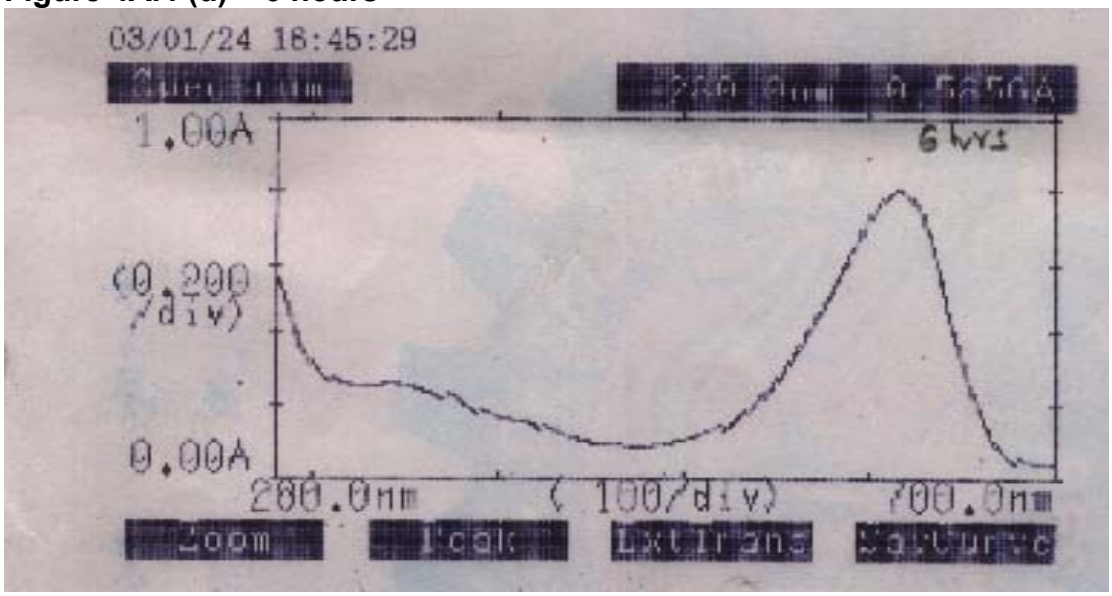


Figure 4A.4. Spectral profile (wavelength vs optical density) of C- phycocyanin concentration by OMD at different time intervals when K_2HPO_4 employed as OA

Figure 4A.4 (e) – 8 hours

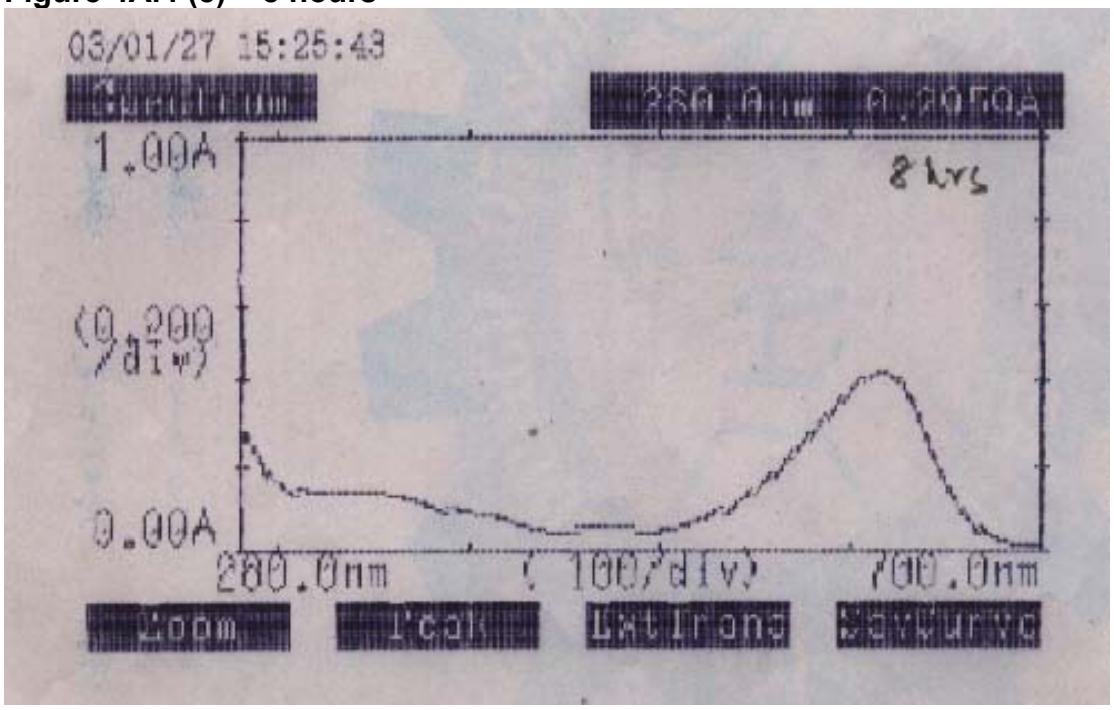


Figure 4A.4 (f) – 10 hours

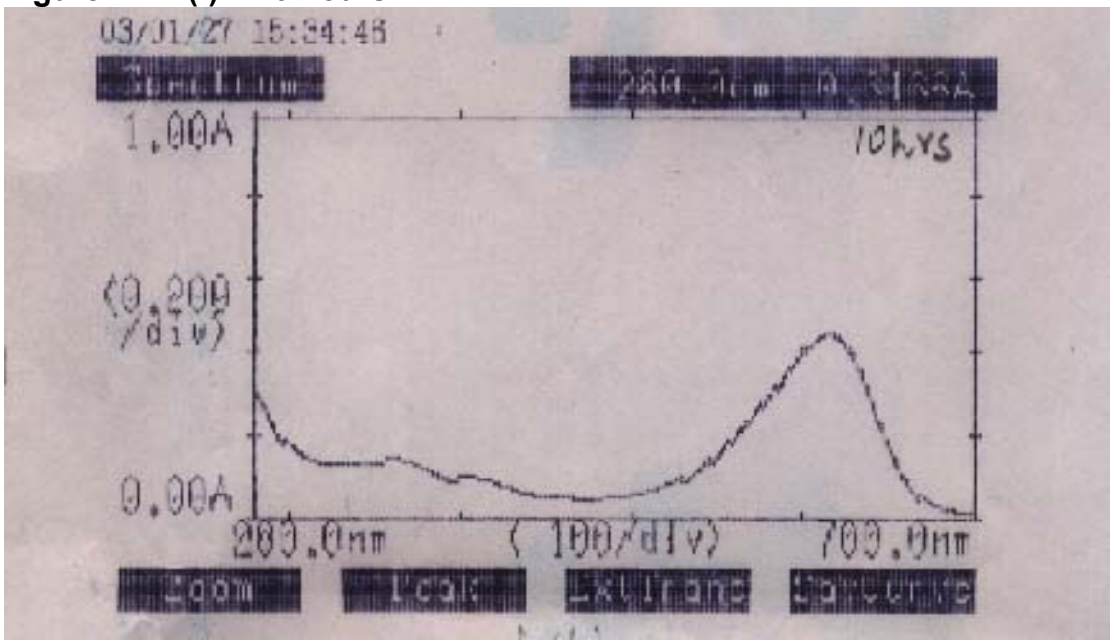


Figure 4A.4. Spectral profile (wavelength vs optical density) of C-phycocyanin concentration by OMD at different time intervals when K_2HPO_4 employed as OA

Figure 4A.4 (g) – 12 hours

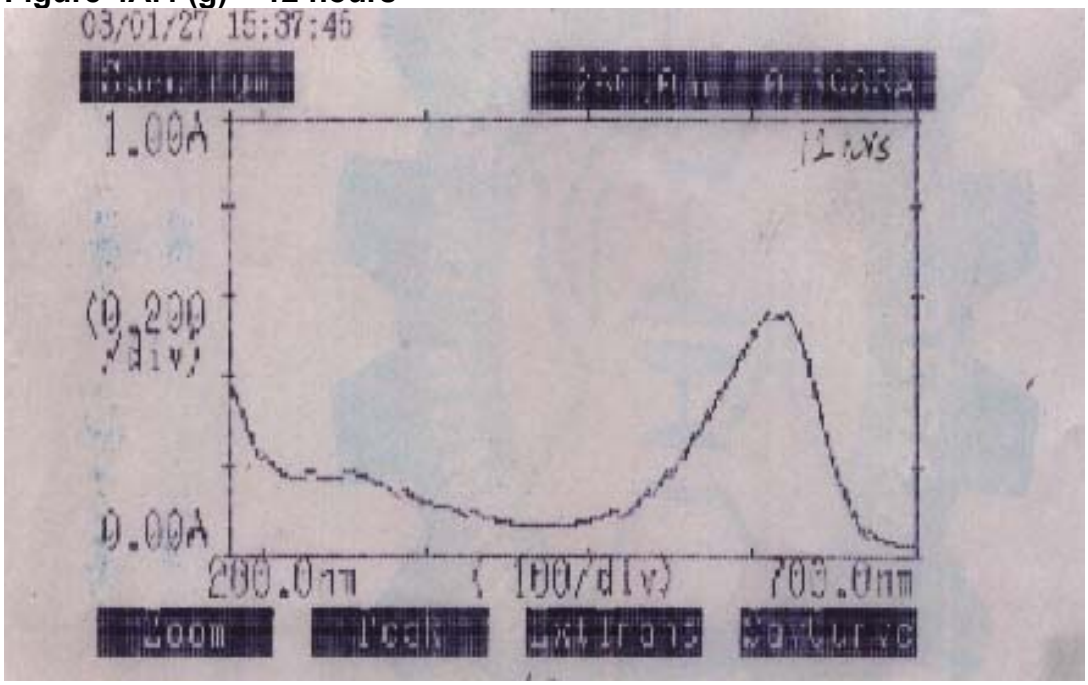


Figure 4A.4 (h) – 14 hours

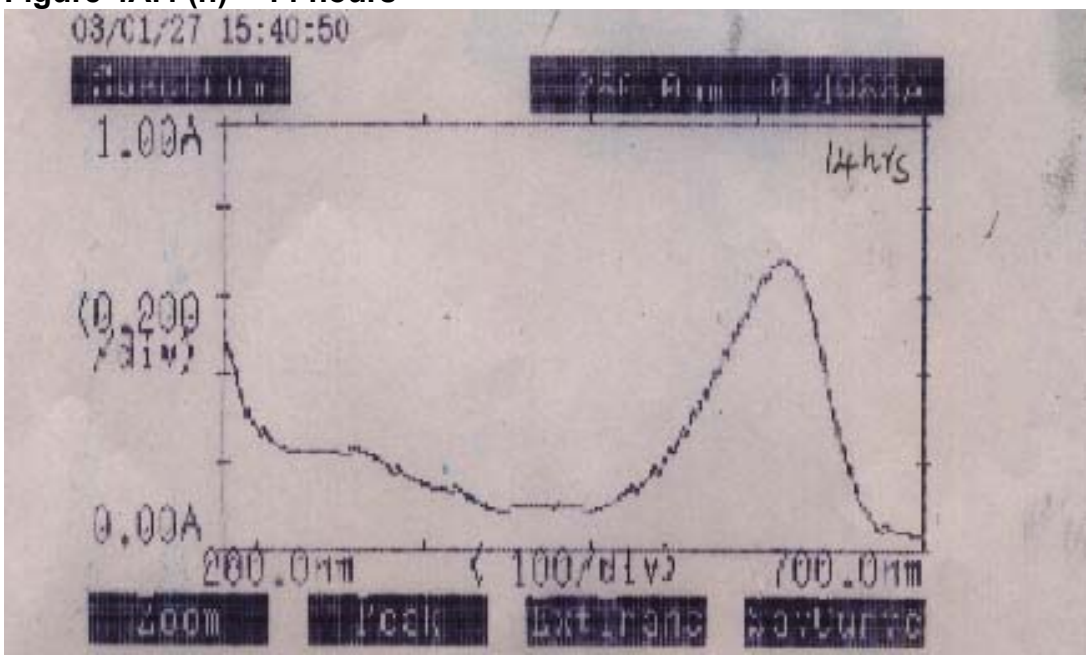


Figure 4A.4. Spectral profile (wavelength vs optical density) of C-phycocyanin concentration by OMD at different time intervals when K_2HPO_4 employed as OA

Figure 4A.5 (a) – zero hours

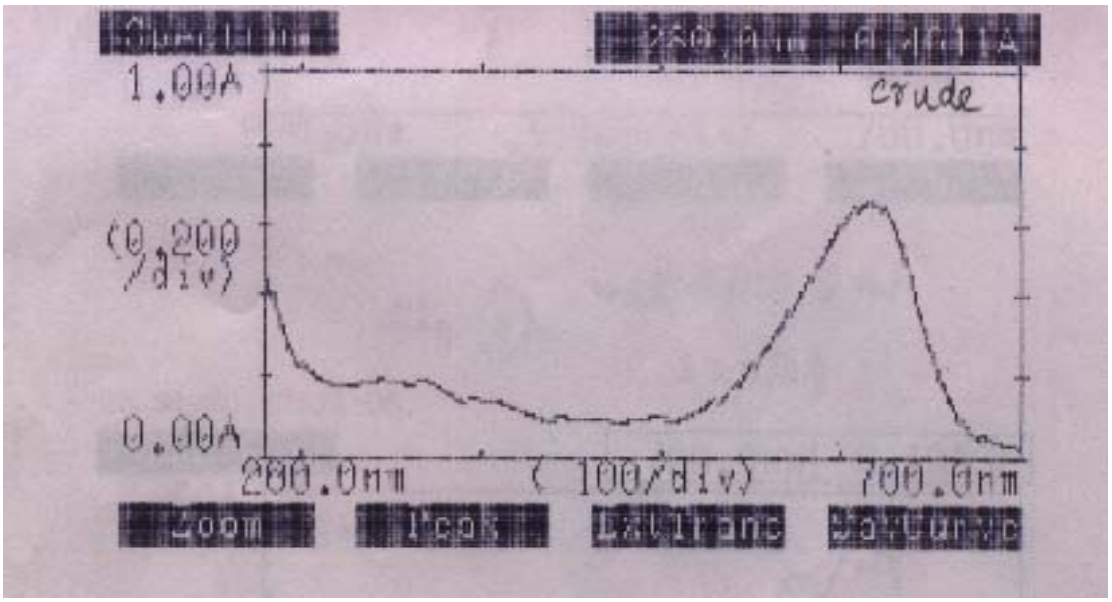


Figure 4A.5 (b) –2 hours

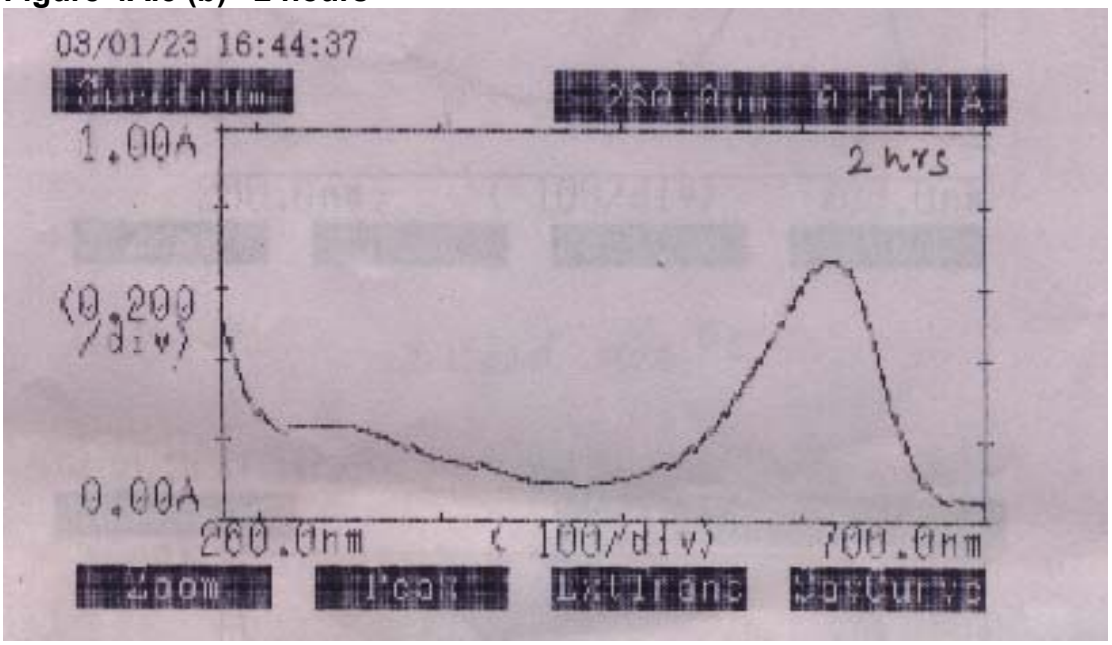


Figure 4A.5. Spectral profile (wavelength vs optical density) of C-phycocyanin concentration by OMD at different time intervals when $CaCl_2.2H_2O$ employed as OA

Figure 4A. 5 (c) – 4 hours

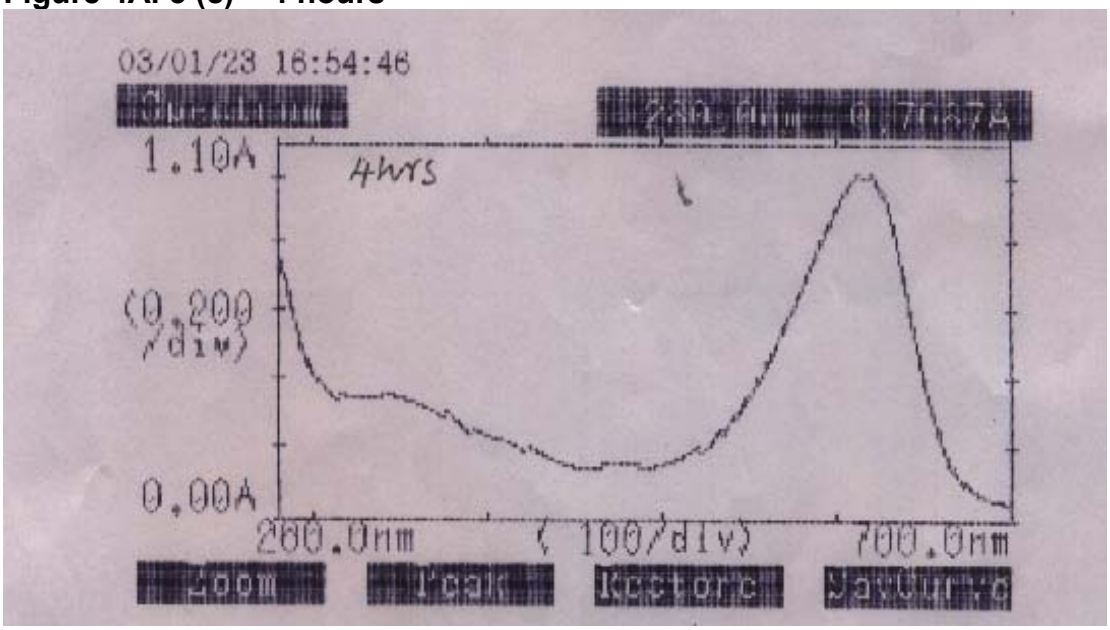


Figure 4A.5 (d) – 6 hours

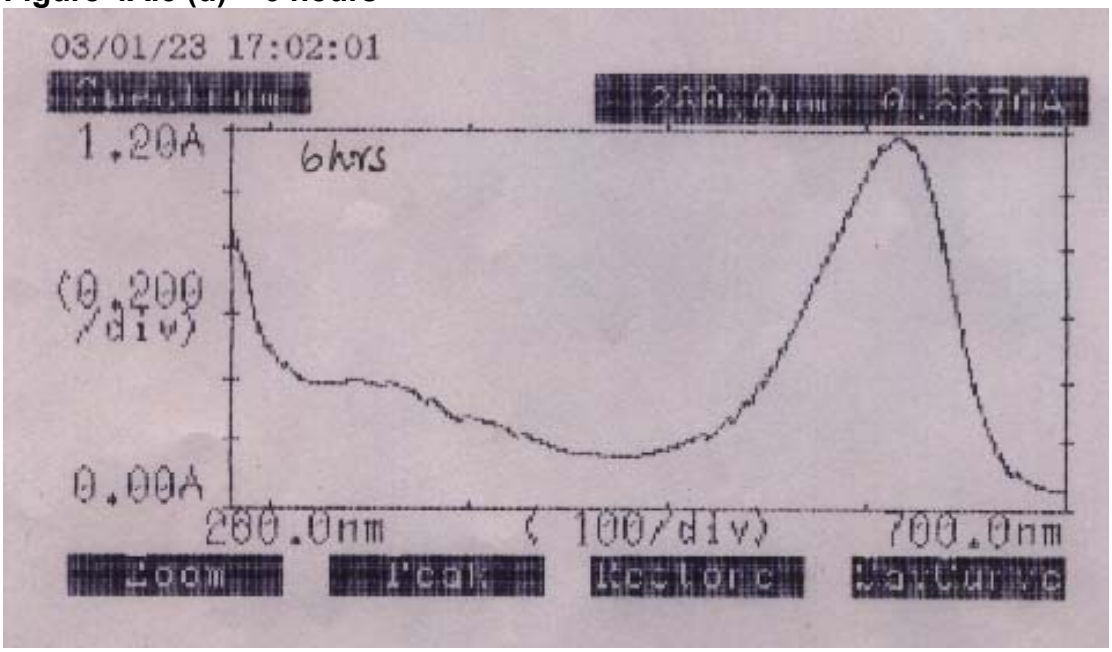


Figure 4A.5. Spectral profile of (wavelength vs optical density) C-phycocyanin concentration by OMD at different time intervals when $CaCl_2.2H_2O$ employed as OA

Figure 4A.5 (e) – 8 hours

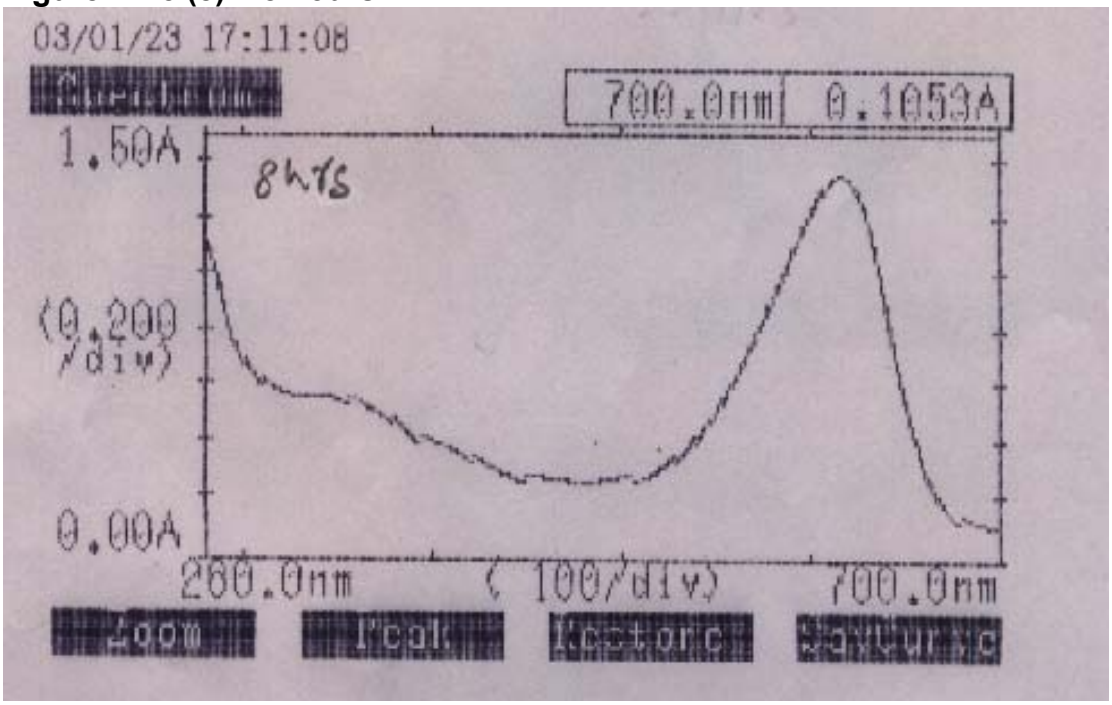


Figure 4A.5. Spectral profile (wavelength vs optical density) of C-phycocyanin concentration by OMD at different time intervals when $CaCl_2.2H_2O$ is used as OA

Section 4B

Concentration of Pineapple Juice

4B.1. Introduction

Pineapple is called as “The King of Fruits” and is known botanically by the name *Ananas comosus* L.Merril, which is one of the most popular among non-citrus tropical and subtropical fruits (Bartolome *et al.,* 1995). It is seasonal and needs to be processed suitably so as to extend its shelf life thereby making its availability throughout the years (Nagy *et al.,* 1993). Pineapple fruit has refreshing sugar-acid balance, attractive flavor and aroma. Nevertheless, like all fruit juices, color, aroma and flavor of pineapple juice are extremely sensitive to change during conventional thermal concentration. Hence, superior quality pineapple fruit juice having all the original organoleptic properties is desired. The pineapple juice concentrate apart from being a refreshing drink, also finds application during the production of juice blends, liquor and carbonated soft drinks. In this regard, membrane processes such as microfiltration (MF)/ultrafiltration (UF) and reverse osmosis (RO) provides a means of processing the liquid foods such as pineapple juice by retaining all the original organoleptic properties. Moreover, membrane processes are energy efficient since no phase change is involved (Cheryan, 1986). For fruit juice processing, MF/UF can be employed for clarification and RO for concentration. However, the existing membrane processes suffer from drawbacks such as membrane fouling, concentration polarization and maximum achievable concentration (only about 25°B in case of RO). Hence, there is a need to develop

alternate/complementary membrane based concentration process. Osmotic membrane distillation (OMD) has such potential, since it facilitates the concentration of liquid foods/solutions under mild operating conditions without product damage. Attempts have been made to concentrate various fruit/vegetable juices such as, orange, passion and tomato juices by OMD process (Durham and Nguyen, 1994; Shaw *et al.,* 2001) After performing the initial studies involving effect of various operating parameters on transmembrane flux during OMD process (Section 3.9, Chapter 3), further studies has been undertaken for the concentration of pineapple juice. The concentrated pineapple juice obtained from OMD process has been evaluated for its sensory qualities.

4B.2. Materials and methods

4B.2.1. Fruit juice preparation

Fresh pineapple fruits (Queen Variety) were purchased from local market. The extraction of juice from pineapple was carried out in a table top juice extractor (Lexus juice extractor: Model JMG1842). The extracted juice was clarified using pectinase enzyme (0.1%v/w; prepared as per procedure in section 3.8, Chapter 3) and was filtered using muslin cloth to remove suspended solids, fiber, coarse pulp and pieces of peel. The clarified juice was stored in cold storage at a temperature in the range of 4±1°C.

4B.2.2. Concentration of juice by OMD process

Around 250 ml of clarified pineapple juice having initial concentration 14°B was subjected to concentration by OMD process. Flat membrane module comprising of hydrophobic polypropylene membrane (pore size of 0.05μm) with a membrane area 0.115 m^2 was employed as shown in Figure 4B.1. Feed (pineapple juice) and OA ($CaCl_2.2H_2O$; 14m) were circulated from their respective reservoirs at the flow rate of 100 ml/min in co-current mode. After every 5-hours of operation, dilute OA solution was replaced with a fresh OA solution in order to maintain effective driving force across the membrane.

4B.3. Sensory analysis

The pineapple juice concentrates samples obtained by OMD process was analyzed for its sensory qualities at Department of Sensory Science, CFTRI. The panel comprising of 12-15 members, participated in the sensory evaluation of these samples. All the samples were evaluated after bringing the juices to ready-to serve (RTS levels; 14°B) by diluting it with water. The panelists evaluated all the samples simultaneously for sensory attributes such as pineapple aroma, sweetness color, flavor and overall quality of the product. The data obtained was then interpreted according to Kramer's Rank Sum Test (Kramer et al., 1970).

4B.4. Results and Discussion

The present study confirms the feasibility of achieving maximum concentration (>60°B) of a given liquid/solution (pineapple juice) by OMD process as shown in Figure 4B.2. With increase in concentration of pineapple juice there was significant change in physical properties of the juice as shown in Table 4B.1. The change in OA once every 5 hours helped in maintaining effective driving force with transmembrane flux remaining practically constant in the range of 2.0- 2.5 l/m^2h as represented in Table 4B.2. During OMD experiments, confirmatory test for possible calcium leakage, across the membrane on to the feed side was carried out as per the procedure mentioned in (Section 3.8; Chapter 3) at regular time intervals. The test confirmed that there was no OA leakage on to the feed side.

Rank sum of the each sample based on sensory attributes was calculated using Kramer's rank sum method. Statistical analysis showed that there was no significant difference between the pineapple aroma, sweetness and overall quality of the concentrate and control sample. This clearly establishes the capability of OMD in retaining the flavor/aroma without product/quality damage.

4B.5. Conclusions

Pineapple juice has been concentrated by OMD process to higher levels (>60°B). The sensory analysis of the concentrated juice clearly indicated that there was no significant change in the overall quality when

compared to control sample. From the studies undertaken, the feasibility of OMD process has been established during the concentration of solutions/liquid foods to higher concentration levels with minimal product damage.

Table 4B.1. Physical properties of pineapple juice

Type of Juice	Density, kg/m^3	Viscosity, mPas
Pineapple juice before OMD	1060.12	1.4
Pineapple juice after OMD	1294.83	90.0

Table 4B.2. Values of transmembrane flux during the concentration of pineapple juice by OMD

Membrane : Polypropylene (0.05μm)
Osmotic agent : Calcium chloride dihydrate
Feed : Pineapple juice

Time, h	Flux, l/m^2 h
0	-
2	2.55
4	2.47
6	2.39
8	2.68
10	2.62
12	2.60
14	2.51
16	2.31
18	2.17
20	2.08
22	2.02

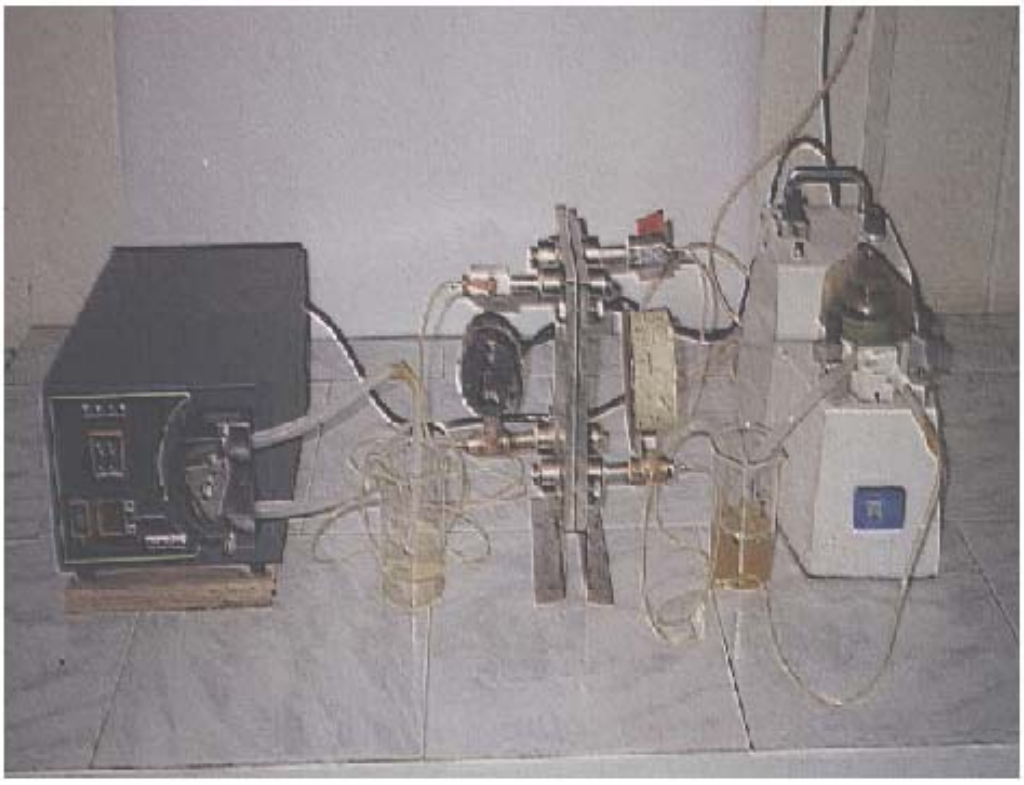


Figure 4B.1. Flat membrane cell employed for OMD – concentration of pineapple juice

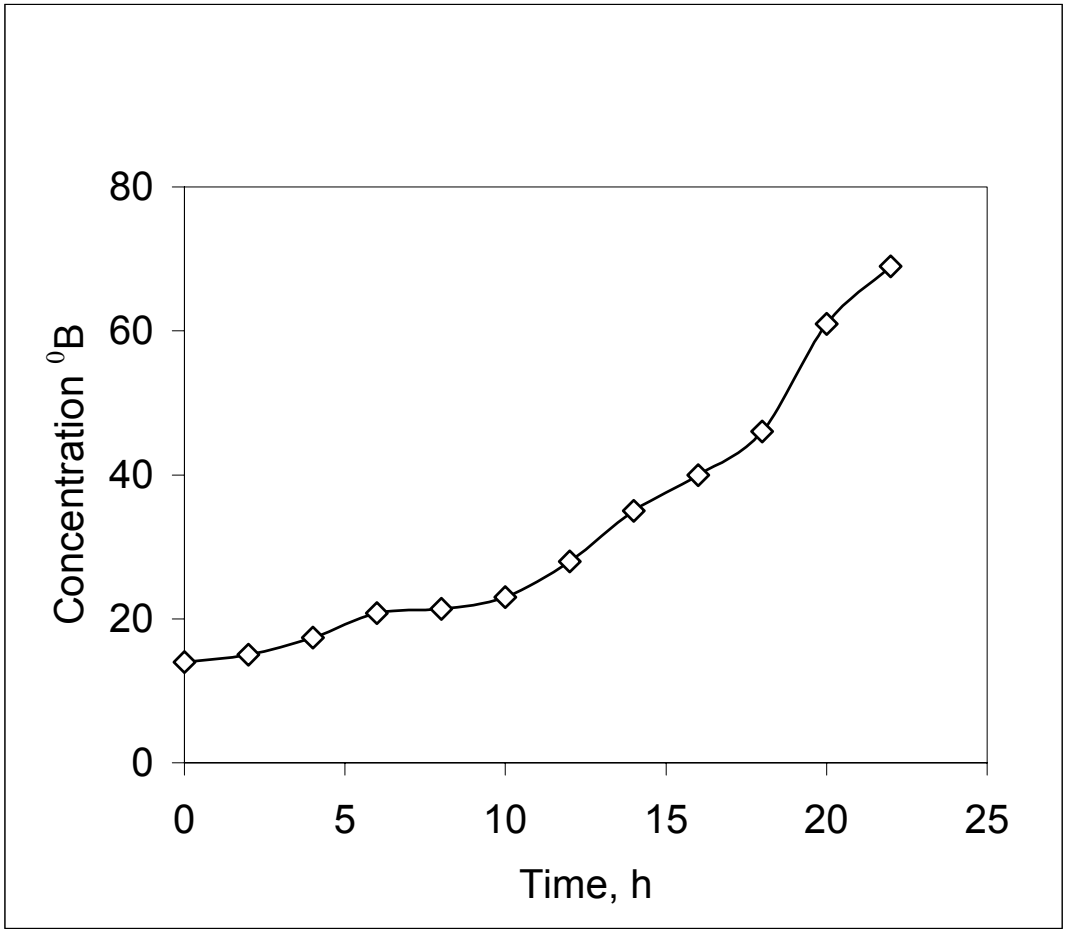


Figure 4B.2. Increase in pineapple juice concentration during OMD process

Section 4C

Scale-up Studies

4C.1. Introduction

Liquid foods such as fruit juices are to be concentrated since most of the fruits are seasonal, perishable and also for economic reasons (reduced transport/storage costs). Conventional thermal concentration methods (such as evaporative concentration) result in loss of sensory and nutritional value of the fruit juice concentrate, thereby resulting in low quality end product. Also, these methods are energy intensive, since phase change is involved (Petrotos and Lazarides 2001).

Hence, efforts have been directed towards the development of membrane based processes such as microfiltration (MF), ultrafiltration (UF) and reverse osmosis (RO) that work more satisfactorily with much lesser product damage and higher retention of the original organoleptic properties of fruit juices (Gostoli, 1998). Nevertheless, these methods also have drawbacks such as maximum achievable concentration (up to 25°B), concentration polarization and membrane fouling. Concentration of liquid foods by osmotic membrane distillation (OMD) is capable of achieving maximum concentration (>60°B) without product damage (Kunz *et al.,* 1996). However, like any other membrane processes OMD suffers from low transmembrane flux. Hence, OMD process becomes inherently uneconomical when operated as a single step process on a larger scale. Therefore, hybrid process by integrating microfiltration (MF)/ultrafiltration (UF) for clarification and with reverse osmosis (RO) for the concentration of

liquid foods will enhance the overall productivity of processing liquid foods and biomolecules.

Attempts have been made by various researchers to process liquid foods by integrating different membrane processes on large scale. Johnson and co-workers (2000) employed UF along with OMD for the processing of grape juice. Feasibility of using OMD in combination with MF has been undertaken during the concentration of orange and passion fruit juices (Shaw *et al.,* 2001; Vallient *et al.,* 2001). In all these processes though the studies have been carried out on large scale, there is still considerable amount of water load prior to OMD process since no pre-concentration step (such as RO process) is involved. This in turn affects the overall productivity of the process. Also, it is always advantageous to undertake studies on larger and in continuous mode so as to understand and overcome the problems that could be encountered during the process. These trouble shooting methods will be of immense help for the successful implementation of the process on to industrial scale.

In the current work, studies have been undertaken to examine the possibility of large-scale processing of pineapple juice by integrating different membrane processing steps involving pre-clarification of the juice by UF, pre-concentration by RO followed by final concentration by OMD. The concentrated pineapple juice obtained by this process was evaluated for

sensory and nutritional values in comparison with that of pineapple juice concentrate obtained from OMD alone.

4C.2. Materials and methods

4C.2.1. Preparation of pineapple juice

Around 200 kgs of fresh *Ananas commasus L. Merril* (Queen variety) was purchased from local market in Mysore, India. Pineapple fruits were decrowned, hand peeled, sliced and cut into small pieces before juice extraction in a screw type juice extractor as shown Figure 4C.1.

4C.2.2. Enzymatic clarification of pineapple juice

The extracted pineapple juice was clarified using Bio-pectinase enzyme (section 3.8; Chapter 3) and was allowed to stand overnight. Later, the juice was filtered to remove the fiber and suspended solids. The juice was then stored in cold storage at a temperature in the range of 4±1°C.

4C.2.3. Preparation of concentrated juices

After pre-clarification of pineapple juice by pectinase enzyme, juice was further clarified by UF process in a tubular membrane module (Figure 4C.2:PCI Membrane Systems Ltd., Hampshire, UK; having provision to operate both UF/RO processes). Polymeric membrane (FP100-PVDF; MWCO -1,00,000) having a total membrane area of 5.4 m^2 was employed. During the clarification the transmembrane flux of the juice was in the range of 1-8 l/m^2h. The processing of the juice was done at 27±2°C at a

transmembrane pressure of 15 bar. The reduction in the volume of the juice after UF was about 1.1 fold. The clarified pineapple juice was then pre-concentrated by RO process in the PCI tubular membrane using AFC99 polyamide membrane (99% rejection). The RO process was operated at transmembrane pressure of 60 bar having permeate flux in the range of 1-10 l/m^2h. The concentration of pineapple juice after RO process was 26°B with final mass reduction by about 2.0 fold. After pre-concentration of the juice, final concentration of the juice was carried out by OMD process in specially fabricated flat membrane module (Figure 4C.3) employing hydrophobic polypropylene (PP) membrane of pore size 0.2 μm, and area, 0.1154 m^2. Both OA ($CaCl_2.2H_2O$; 14m) and feed were circulated in co-current mode at the flow rate of 100 ml/min. The concentration of pineapple juice was carried out till the concentration of the juice reached > 62 °B. The volume of juice reduced by a factor of 1/5 th of its original volume.

4C.3. Sensory analysis

The pineapple juice concentrates obtained were evaluated by a trained panel for their sensory qualities at Department of Sensory Science, CFTRI.

The four samples of pineapple juice concentrate namely

A – UF clarified pineapple juice – 14°B

B – RO concentrated juice – 26°B

C – OMD concentrated juice - >62°B

D – Concentration by OMD process alone- >62°B

E - Control juice sample – 14°B

were analyzed for their sensory attributes using Quantitative Descriptive Analysis (QDA; Stone *et al.,* 1974). The individual attribute's intensity such as pleasantness, aroma, body cooked aroma, sweetness, sourness, bitterness, stale and ferment of the juice was quantified on 15 cm scale. The panel comprised of 12-15 members who participated in the sensory evaluation of these samples. All the samples were evaluated after bringing the juices to RTS levels (14°B) by diluting it with water. The panelists evaluated all the samples simultaneously for sensory attributes.

4C.4. Results and discussion

The pineapple juice concentration by hybrid process involving UF, RO and OMD was carried out on large scale are shown in Figure 4C.1- 4C.3 and the samples of the concentrates are shown in Figure 4C.4. The quality of pineapple juice obtained after each processing step was analyzed for its chemical composition and its nutritional value as shown in Table 4C.1. As observed, the concentration of the juice increased from 14 to 26°B during pre-concentration by RO process and finally increased up to around 62°B after concentration by OMD process. From nutritional evaluation (Table 4C.1), it can be observed that the composition of ascorbic acid was lower

when compared to control sample. Also, sensory evaluation of these samples termed the intensity of pineapple's characteristic aroma and pleasantness lighter when compared to control sample, represented in Figure 4C.5. This is due to the retention of ascorbic acid and aroma components during clarification of pineapple juice by UF process. The same juice concentrate could regain its original characteristic pineapple aroma and pleasantness when the juice was reconstituted with 50% UF retentate (confirmed by sensory evaluation). This clearly confirms the retention of juice aroma/flavor and nutritive components during UF process. However, this was not the case with pineapple juice concentrated by OMD process alone. The nutritive analysis of the OMD concentrate juice sample showed higher ascorbic acid content, confirming that there was no product damage (Table 4C.1). Hence, it can be concluded that hybrid process involving UF, RO and OMD have yielded pineapple juice without product damage. However, in order to obtain the product quality close to its original characteristics of the fruit proper selection of the membranes having minimal retention of aroma/nutritional components is required. Finally, this study consisting of hybrid process has shown considerable promise towards enhancing overall productivity on al larger scale.

4C.5. Conclusions

The processing of pineapple juice has been demonstrated on large scale by employing hybrid process involving UF, RO and OMD. The quality

of the product has been evaluated for its sensory and nutritional aspects. Also, it can be inferred that the integration of OMD process with other processes such as ATPE, freeze drying during the processing of solutions/liquids on large scale can significantly enhance the process productivity.

Table 4C.1. Chemical and nutritional evaluation of pineapple juice concentrate

Type of juice	control	UF clarified juice	RO concentrated juice	OMD concentrated juice	Juice concentrated by OMD alone
pH	3.7	3.8	3.9	3.8	3.8
Titrable acidity (mg)	812.0	672.0	1358.0	3920.0	2218.0
%TSS, °B	14.0	14.0	26.0	62.0	62.0
Ascorbic acid (mg/1000ml)	125.0	98.0	161.4	212.3	452.8

Figure 4C.1 Screw type juice extractor

Figure 4C.2 Ultrafiltration/Reverse osmosis tubular unit

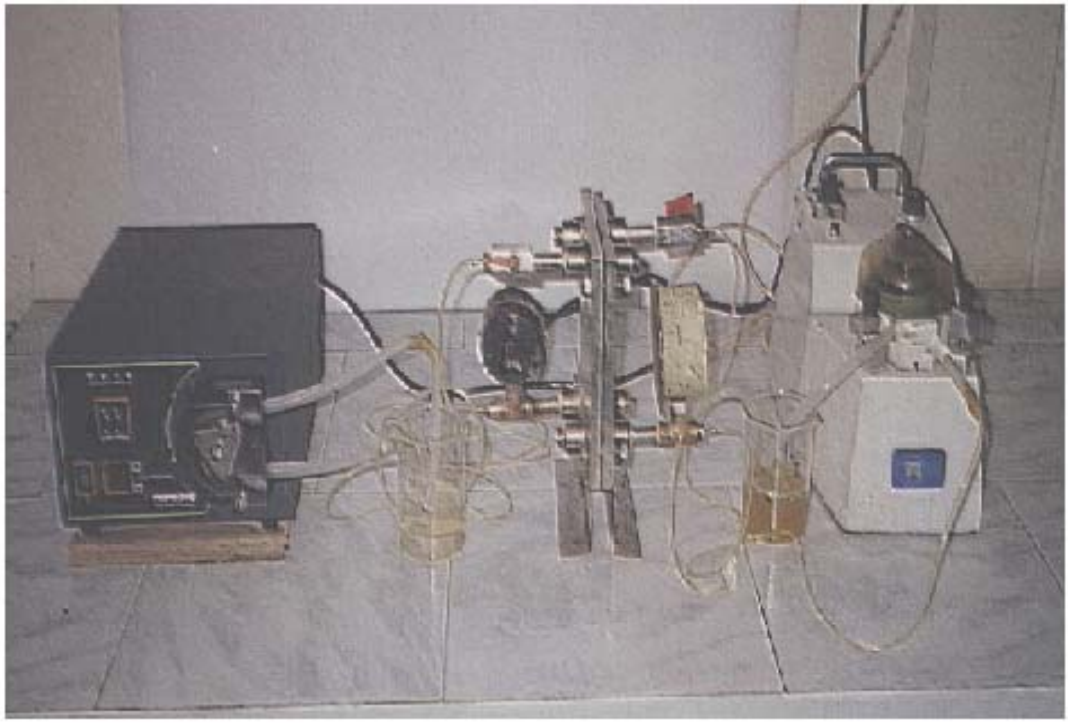


Figure 4C.3. Flat membrane cell employed for OMD - for concentrating pineapple juice

Pineapple juice - control

Figure 4C.4. Pineapple juice samples

A - UF Clarified juice, B - RO Concentrated juice, C - OMD Concentrated juice

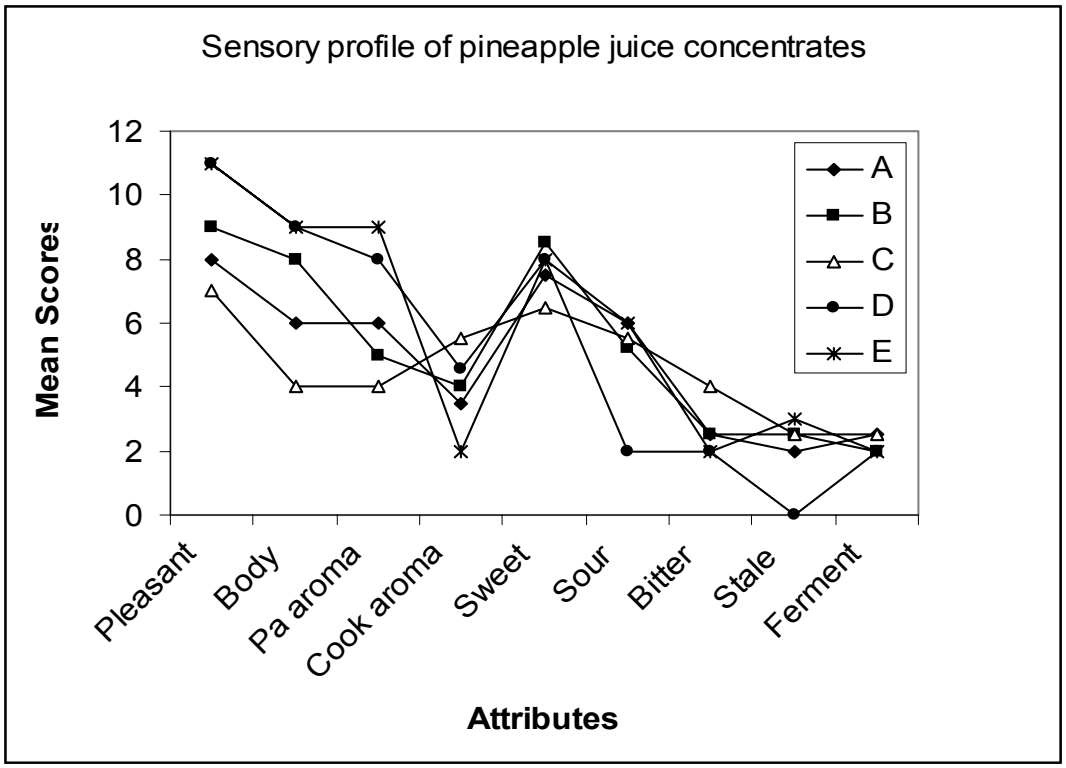


Figure 4C.5. Sensory profile of pineapple juice concentrates

Chapter 5

Other Applications, Constraints and Suggestions for Future Work

5.1. Other applications

Most of the work in osmotic membrane distillation (OMD) has been carried out at lab scale to concentrate numerous liquid foods such as fruit juices, vegetable juices and other aqueous solutions. Only a few reports are available at pilot scale.

In the present study, feasibility of OMD process has been demonstrated on large scale for the first time. For this purpose a facility has been established to carry out studies on large scale under controlled conditions.

OMD can be employed as pre-concentration step prior to relatively cost intensive processes such as lypholization (freeze drying), in case of thermally sensitive products such as enzymes/proteins, natural food colors and biological materials. Main advantage of OMD process is that it enables to concentrate aqueous solutions like fruit juices (>60°B) without product damage. OMD also finds application during de-alcolization of fermented beverages (wine or beer). The use of OMD helps in the selective removal of ethanol from these alcoholic beverages without adversely affecting their taste, odor or mouth feel. Ethanol recovered from the stripping solutions can be further used as a potential blending stock in the manufacture of fortified alcoholic beverages (Hogen *et al.,* 1998).

OMD like any other membrane process has low flux. However, the strength lies in its ability to yield a concentrate having superior product

quality. In order to overcome the drawback and to improve the process economics, it has been proposed to have hybrid process involving clarification by microfiltration/ultrafiltration (MF/UF), pre-concentration by RO and final concentration by OMD.

Many researchers have carried out concentration of fruit juices (orange, passion, grape) involving MF/UF followed by OMD process on a pilot scale level. The feasibility of integrating OMD process with MF has been demonstrated during the concentration of fruit juice to an intermediate concentration (>30°B) with high flavor retention (Bailey *et al.,* 2000; Shaw *et al.,* 2001)

Further, the integrated membrane process has been explored for the clarification and the concentration of citrus (orange and lemon) and vegetable juice (carrot), using UF for clarification at pilot scale level. This was followed by concentration of juices by employing RO and OMD process in lab-scale unit. The concentrated juices obtained were of high product quality (Cassano *et al.,* 2003).

More recently, three-stage hybrid membrane process for the concentration of ethanol-water extracts of the *Echinacea* plant (which is used as immunostimulant) has been investigated. This resulted in a highly concentrated product suitable to market the product in capsule form. (Johnson *et al.,* 2002).

Work is underway in our laboratory to integrate OMD with RO for the concentration of tender coconut water. The tender coconut water, having an initial concentration of about 5°B, was concentrated by RO process (PCI membrane module) to a concentration of about 20-25°B. This RO retentate was concentrated further by OMD process up to about 56°B. The sensory analysis indicated that this process could be successfully employed for the concentration of heat sensitive products having delicate flavors such as tender coconut water (Rastogi *et al.,* 2003).

OMD process has been employed successfully to concentrate Hydroxy-citric acid (HCA) derived from *Garcinia pendaculata*. The acid content increased up to about 38% from an initial acid content of 9% without product damage during the concentration by OMD process. (Anandharamakrishnan *et al.,* 2004).

According to the reports available, hybrid process consisting of UF and RO followed by OMD has been successfully demonstrated on a pilot scale for the concentration of fruit and vegetable juices in Mildura, Melbourne, Australia. This OMD pilot plant has been designed and fabricated by Zenon Environmental (Burlington, Ont.)

Partitioning studies in ATPE of C-phycocyanin (protein/natural blue colorant) and separation of sugar from betalaines during the downstream processing of betalaines (another natural color) employing ATPE are also in progress.

Possible integration of aqueous two-phase extraction (ATPE) with membrane processes such as OMD is being explored for the purification and concentration of food colors (especially when there are proteins). The use of ATPE will enable desired products (enzyme/protein), partition to one of the phases, and the impurities to the other phase, thus purifying while reducing the volume of the process stream to be handled. OMD process can also be used as pre-concentration step prior to subsequent purification steps such as electrophoresis, chromatography etc.

ATPE appears to be a promising technique for efficient downstream processing of biomolecules due to its wide range of applications. Some applications of ATPE have been demonstrated on large/pilot scale. However, major constraint still remain with regard to the availability of information in the literature on engineering aspects of ATPE (involving mass transfer and hydrodynamics) are scant or remains proprietary and only few reports are available. In addition, the efficient methods for recovery and recycling of phase forming components are important for the development of environmentally benign aqueous two-phase extraction technique.

In case of OMD, apart from relatively low transmembrane flux, another major constraint for the wide commercial application of OMD process is dependent on the effective management of spent OA solution. Though, membrane fouling seems to be of minor importance, periodic membrane cleaning is essential in general. Several effective cleaning agents and

methods are employed. However, studies have shown that repetitive cleaning and fouling can affect the membrane durability and thereby reducing its life cycle, an important aspect to be considered in regard of industrial application.

5.2. Suggestions for future work

Acoustic field assisted demixing resulted in significant enhancement in demixing rates in aqueous polymer-salt two phase systems (Chapter 2, Section 2A). In near future, the major parameters such as effect of acoustic intensity, acoustic frequency on demixing rates (which could not be studied due to the limitations of the equipment) needs to be studied. All these factors need to be considered while designing and fabricating demixing contactors/acoustic bioreactors for large-scale/industrial applications.

Faster demixing rates in the presence of electric field was observed even in case of polymer-salt aqueous two phase systems (Chapter 2; Section 2B). Detailed studies need to be undertaken to study the effect of electric field on phase volume ratio and phase composition in case of different aqueous polymer-polymer and other polymer-salt two-phase systems.

Microwave field assisted demixing has been reported (Chapter 2; Section2C). The process is simple, efficient and appears to be scalable and can be made continuous. Detailed studies to understand the effect of phase

composition, molecular weight of the polymer in presence of microwave field needs to be undertaken.

The encouraging results obtained during initial electroextraction of betalaines (Chapter 2; Section 2D) can be employed further to carry out the detailed and systematic studies for the selective separation of betaxanthin and betacyanins from betalaines, isoenzymes from plant peroxidase and other charged biomoelcules. However, there is a need to arrive at an electroextraction module with better design, better control for the selective partitioning of biomolecules.

Microwave field assisted PEG recovery from spent phases has been discussed (Chapter 2; Section 2E). This method is simple, faster and efficient when compared to conventional methods of polymer recovery. Further, studies are needed on large scale and exploration of possible integration of the polymer recovery in the process stream needs to be investigated to improve the process economics.

Attention needs to be focused on the development of new phase systems for ATPE. These phase systems should be of low cost, ease availability, non-toxic, biocompatible and biodegradable, so as to improve the versatility and ecofriendliness of ATPE.

Reverse Micellar Extraction (RME) is another attractive and well established method for the liquid-liquid extraction and purification of

biomolecules (Luisi and Magid, 1986). Another method employed for the extraction and purification proteins is three-phase partitioning (TPP) (Dennison and Lovrien, 1997). Hence, comparative studies for the extraction and purification of biomolecules using ATPE, RME and TPP would be of immense use for the selection of most suitable method. Work in this regard is already in progress.

Mass transfer-in-series resistance model which could predict the transmembrane flux and also the effect of the parameters was proposed (Chapter 3). Another major constraint of OMD process is relatively low flux. Attempts have been made to enhance the transmembrane flux by the application of acoustic field in lab-scale membrane cell (Chapter 3; Section 3.10). Some more studies are essential on large scale involving real systems to study the effect of various parameters on flux. Also, efforts are needed to enhance the transmembrane flux by the application acoustic field on a large scale using real systems.

The successful application of OMD process has been demonstrated for the concentration of natural food color/protein and liquid food without product damage (Chapter 4; Section 4A and Section 4B). This is followed by the large-scale studies for the processing of pineapple juice involving UF, RO followed by OMD (Chapter 4; Section 4C). Still detailed studies involving process and membrane parameters are required for hybrid processes when operated on a large scale/pilot scale.

To make OMD commercially viable option there is a need for the development of membranes with improved diffusional characteristics, new materials (polymeric, ceramic, zeolite etc.), selectivity, better pore geometry, stability and membranes with longer life cycles at affordable costs. Another major constraint for the wide commercial application of OMD process is the management of spent OA solution. An integrated effective and environmentally benign reconcentration technique comprising of solar/thermal evaporation and RO needs to be developed.

The large diversity of plant/marine sources constitutes major source for different types of biomolecules such as natural colors, proteins/enzymes etc. having wide applications. Hence, efforts are required to carry out detailed studies in order to develop simple, efficient and economic methods for the downstream processing of these biomolecules and possible process integration of ATPE with OMD. Keeping in view of the scientific and industrial potential of ATPE/OMD, even if some of these aspects are addressed in greater depth by future researchers the objective of this thesis can be considered fulfilled, since it contributes to both these processes (ATPE/OMD) in gaining wide application in food and allied industry in the years to follow.

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