

**Thermodynamic Modeling for Liquid-Liquid Extraction
of PEG-MgSO₄ system in MATLAB**

by

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Dissertation submitted in partial fulfillment of
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CERTIFICATION OF APPROVAL

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Approved by,



(Dr.Murni Melati)

UNIVERSITI TEKNOLOGI PETRONAS

TRONOH, PERAK

January 2009

CERTIFICATION OF ORIGINALITY

This is to certify that I am responsible for the work submitted in this project, that the original work is my own except as specified in the references and acknowledgements, and that the original work contained herein have not been undertaken or done by unspecified sources or persons.



LOGESWARAN A/L MURUGAN

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ABSTRACT

This project focuses on the application of protein extraction in aqueous salt system. The objective is to simulate a system which contains polymer and salt which will subsequently be used to extract protein. This system is called as Aqueous Two-Phase Extraction System (ATPES). ATPES is a liquid-liquid extraction system where the extraction takes place when it reaches liquid-liquid equilibrium (LLE). This system consists of water, polymer and salt. Both polymer and aqueous is allowed to attain equilibrium where it forms two clear phase. The formation of two phase depends on parameters such as pH value of system, temperature and concentration level. The model used in this thermodynamic approach is Flory-Huggins model [1,2]. Flory-Huggins model is a mathematical model of the thermodynamics of polymer solutions which takes account of the great dissimilarity in molecular sizes in adapting the usual expression for the entropy of mixing [1,2]. This project is a simulation-based project using MATLAB. This software enables the author to find possible combination of ATPES and to optimize protein recovery. First of all, the author needs to learn and master using MATLAB software. Then some literature reviews need to be performed to select the right ATPES. At the end of the project the results of this project will be compared with experimental result which will be obtained through literature review.

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CHAPTER 1: INTRODUCTION

1.1 Background Studies

Proteins are large organic compounds made of amino acids arranged in linear chain. High value proteins are essential for organisms because they participate in most process within cells. Due to vital role of protein in organism, the needs of protein product market evolves dynamically. There are several techniques to extract these proteins. The best technique is chosen based on cost, quality and time of production [3,10]. Most of the time, protein extraction is very expensive where it can be as high as 60% of total production cost.

Chromatography is one of the method which can be used in protein separation. It involves passing a mixture dissolved in a "mobile phase" through a stationary phase, which separates the analyte to be measured from other molecules in the mixture and allows it to be isolated [7]. There are many types chromatography but all these methods require very high maintenance for the column and rapid turn-over of packaging material. So this method is less suitable [3].

Membrane protein separation is one of the method which is widely being used in both academic and industrial process engineering. A pressure driving force and a semi permeable membrane are used to affect a separation of components in a solution or colloidal dispersion [9].

Furthermore since solid-liquid separation by centrifugation or filtration results in some technical difficulties, for example filter fouling and viscous slurries [26], therefore, there is an ongoing need for new, fast, cost-effective, ecofriendly simple separation techniques [25]. Thus, for separation of biomolecules, aqueous two phase

systems (ATPES) offer an attractive alternative that meets the above-mentioned requirements as well as the criteria for industrially compatible procedures.

ATPES first discovered by Beijerinck and Albertsson were first used for extraction and separation purposes [3]. Aqueous two-phase system was developed in Sweden during mid-1950s for the separation of macromolecules, and cells and organelles [21]. These systems were initially applied to the separation of plant organelles and viruses. Since then, attention has been directed towards widening its application scenario. During the last two decades, lot of work has been done to develop feasible separation processes using aqueous two phase systems for various biological materials, and proteins and recombinant proteins [27].

1.2 Aqueous two-phase extraction system (ATPES)

Aqueous two-phase extraction systems are made by combining two water soluble polymers or a polymer and a salt in water, above a “critical condition.” These systems can be two polymer solutions or a polymer and a salt solution and it separate into two immiscible liquid phases, one of them enriched in one polymer and the other enriched in the other polymer or salt. Soluble or particulate separands, added to the system, partition themselves between the two phases. ATPES has become recognized as a nondenaturing and nondegrading technique for the separation of a number of biological entities such as protein, enzymes, viruses, cells, and cell organelles [7].

Initially, the LLE behavior of ATPES can be determined by finding the phase composition at which the components’ chemical potentials are equal [31,32,33,34] but Johansson et. al. reported that a thermodynamically equivalent approach of calculating the phase composition in ATPES by minimizing the Gibbs energy of mixing using Flory Huggins theory[35,36]. Minimizing Gibbs energy approach is used in this project.

When polymer-polymer solution or polymer-salt solution are added together in water, the molecules in the system interact with one another, forming new bonds and rearrange themselves in the system until the system attains lowest Gibbs energy. These system

forms two immiscible liquid phases. This is due to the interaction between two components is repulsive, leaving the system at energetically most favorable state when the components are separated. Hence, the phase separation occurs when the interactions between the phase-forming components are unfavorable enough to overcome the loss in entropy of demixing.

According to second law of thermodynamics, two components will mix to form a solution, if the Gibbs free energy of mixing, ΔG^{mix} is negative:

$$\Delta G_{\text{mix}} = \Delta H_{\text{mix}} - T \Delta S_{\text{mix}} < 0 \quad (1.1)$$

Where ΔH^{mix} and ΔS^{mix} are the enthalpy and entropy of mixing, respectively; T is the temperature. ΔH^{mix} is the enthalpy due to interaction with other molecules and $T \Delta S^{\text{m}}$ is the entropy due to arrangement or movement of molecules. The phase separation is highly dependent on enthalpic domination by the polymers due to their high molecular weight [3,20,21,22].

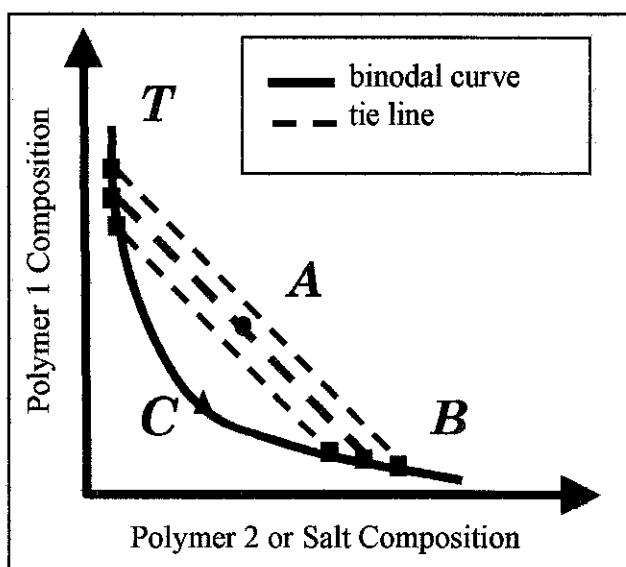


Figure 1.1 Phase diagram for ATPES

Each two-phase aqueous system can be characterized by a phase diagram, an example of which is shown figure 1.1. At concentration of polymer 1 and polymer 2 or salt below the curve, there is only one liquid phase. On the curve, there are two liquids phases, and tie line connect the composition of the phases that are in equilibrium. As

observed in figure 1.1, the phase enriched in polymer 1 can contain almost no polymer 2 or salt. Critical point, C is the threshold composition where beyond this point immiscible two phase formation occurs. A is the initial composition upon mixing. Once equilibrium reaches, T as top phase and B as bottom formed. T and B are the top and bottom phase composition. Tie line is obtained by connecting all three points. The most common systems use dextran and PEG or PEG and potassium phosphate [7]. The PEG rich phase is less dense than either dextran rich or salt rich phase and thus PEG will be the top phase when separation takes place at equilibrium. This is due to when the two phase-forming components are added together, the molecules in the system interact with each other, forming new bonds and rearrange themselves in the system to attain lowest Gibbs energy where subsequently it reaches equilibrium.

When a system reaches equilibrium, the energy content is at the lowest possible state. This applies to an ATPES, where the equilibrium state attained would enable it to partition into two distinguishable phases, namely the top and the bottom phase, with respective components in each phase achieving the following:

1. Minimize in the difference of Gibbs energy of mixing
2. Equal chemical potentials
3. Equal fugacity

A suitable modeling approach has to be adopted to calculate the composition of each component in each phase at equilibrium. Flory-Huggins theory is able to capture and calculate for the minimization of the Gibbs energy of mixing level of the components at equilibrium in the ATPES. This modeling approach, based on Gibbs free energy is chosen because:

1. It is simple with only a few parameters required.
2. It provides good mathematical insights into the phase formation phenomena.
3. It enables qualitative prediction or correlation for measured phase compositions, and partitioning behaviors.

The most important factor for phase separation is the chemical nature of both polymer and salt. The partitioning behavior of a biomolecule depends on the composition of the

two phases in equilibrium. When the composition of the two phases in equilibrium changes, the partition coefficient changes accordingly – for instance, usually the partitioning becomes more one sided when the length of the tie-line increases [28].

In ATPES, the phase separation is due to small repulsive interactions between the two types of monomers in the solution. The total interaction between the two polymers is large (high energy) because each one is composed of several monomers [29]. Hence, for an ATPES with the composition lying in the two phase possible region, the immiscible polymers prefer to separate from each other, arriving to an energetically more favorable lower energy state, thus achieving equilibrium.

$$K_i = \frac{C_i^{top-phase}}{C_i^{bottom-phase}} \quad (1.2)$$

K_i is the partition coefficient for component i which can be water, salt or polymer. C_i is the concentration of component i in top and bottom phase. If $K > 1$, then it means the top phase concentration is higher for water, salt or polymer while vice versa for $K < 1$.

1.3 ATPES as protein separation technique

ATPES comprises a liquid-liquid extraction technique that exploits phase separation phenomenon to partition a desired protein away from cell debris or other substances [3]. Since these phase components in ATPES are inert towards biological materials, these can therefore be employed for partitioning of biomolecules, and cell organelles and whole cells as well.

The advantages of ATPES as protein separation technique are:-

- a) Both phases of the system are of aqueous nature.
- b) Rapid mass transfer and mixing until equilibrium requires little energy input.
- c) Technique facilitates the processing of solid-containing streams.
- d) Polymers stabilize the proteins.
- e) Separation can be made selective.
- f) Scale-up from small laboratory experiments is easy and reliable.
- g) Continuous operation is possible.
- h) Technique is cost effective

In general for ATPES, contaminants such as cells and cell debris partition to the bottom phase or interface. For proteins, the partition is affected by many parameters such as protein molecular weight, protein charge and surface properties, polymer molecular weight, phase composition and tie line length, salt effects and affinity ligands attached to polymers [7]. These parameters are difficult to separate and analyze individually, which means prior prediction of partition coefficient is very difficult.

1.4 Modeling of LLE Behaviour in ATPES

When polymer-polymer solution or polymer-salt solutions are brought together in water, the molecules in the system interact with one another, forming new bonds and rearrange themselves in the system and it reaches LLE. At LLE the system forms two immiscible liquid phases which also has the following characteristic:

1. Lowest Gibbs energy difference
2. Equal chemical potential
3. Equal fugacity

The LLE can be calculated from the three characteristic stated above. In this project the preferred modeling approach chosen is by calculating minimization of Gibbs energy level of mixing component at equilibrium.

Other approaches in modeling ATPES are Wilson, NRTL and UNiVersal-QUAsi-Chemical (UNIQUAC). These models are also known as local compositional models. Hiel and Prausnitz[30] first introduced an equation for the excess Gibbs energy based on a non-random distribution of molecules into polymer solution. In liquid models it is considered that the molecules are arranged randomly while in local compositional model consider that there are forces of attraction between the molecules which cause the non-random arrangement of molecules.

The fundamental modeling approach used is by using Flory-Huggins theory. The reason this theory been chosen is because of its simplicity and limited number of parameters. Furthermore, it provides good mathematical insight into the phase formation phenomena [26,37,38] and enables qualitative prediction or correlation for measured phase composition and partition behavior [26,35,39].

Flory [1] and Huggins[40] suggested the Flory-Huggins theory based on lattice model. Lattice models are a form of Coarse Graining, where only the most important molecular details of a system are retained in a statistical mechanical model. This is a powerful approach to create statistical mechanical models where meaningful predictions can be made for complex materials [41].

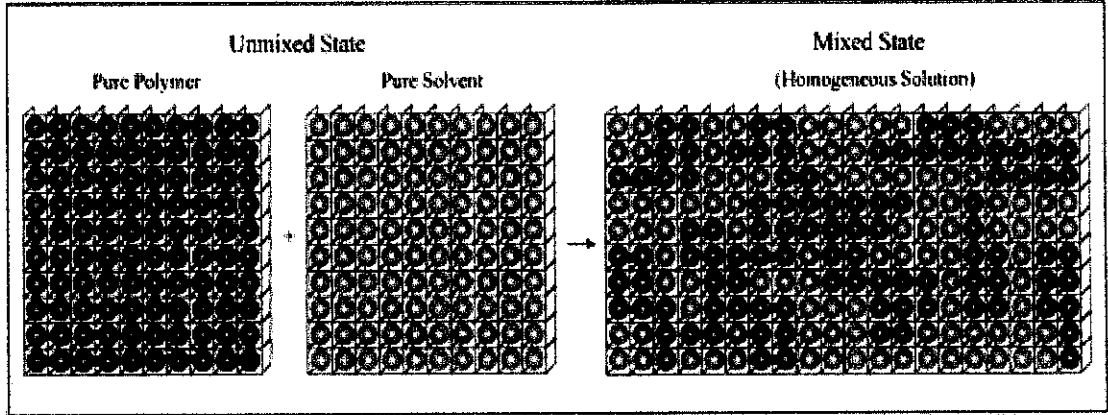


Figure 1.2: Lattice model

The figure above shows that the lattice model in a ATPES. Each box represents a lattice site, each circle represents a polymer segment in a chain and empty lattice sites are filled with solvent molecules. The fraction of the lattice occupied by polymer segments is given by volume fractions ϕ_i with $i = 1, 2, \dots, m$ where m is the number of polymers in the solution and the solvent volume fraction is given by $(1 - \sum_i^m \phi_i)$. There are few assumption made in this model where volumes of one solvent molecule and one segment of the polymer chain are equal to one lattice site. It is also assumed that the no volume change upon mixing, molecules are indistinguishable and molecules mixed randomly.

Based on the lattice theory, Flory[1] and Huggins[40] expressed minimization of Gibbs energy by using the equation given below:

$$\overline{\Delta G^k} = \sum_i \sum_{j=i} \omega_{ij} \phi_i^k \phi_j^k + RT \sum_i \frac{\phi_i^k}{r_i} \ln \phi_i^k \quad (1.3)$$

where:

k = top phase or bottom phase,

ϕ_i^k = volume fraction of component i in phase k ,

r_i = degree of polymerization of component i , and

ω_{ij} = effective pair-wise interchange energy between component i and j (Positive value indicates endothermic net attraction, whereas negative values indicates enthalpic repulsion),

T = absolute temperature, and

R = the universal gas constant.

As shown in equation, the term on the left hand site represents energy of mixing while the right hand site term shows that the net enthalpy change associated with the formation of interactions between the polymer segments and solvent, at the expense of breaking the interactions between the same components in the pure state [3].

The total Gibbs energy of mixing for system k , ΔG_{mix}^k or ΔG^k is given by the product $N^k \cdot \overline{\Delta G}$. The total Gibbs energy of mixing for two phase system is calculated by the following equation:

$$\overline{\Delta G}^{\text{two phase}} = N^{\text{top phase}} \cdot \overline{\Delta G}^{\text{top phase}} + N^{\text{bottom phase}} \cdot \overline{\Delta G}^{\text{bottom phase}} \quad (1.4)$$

N^k represents the total moles of lattice in phase k and is calculated using euquation (1.5)

$$N^k = \sum_i^m n_i^k \cdot r_i \quad (1.5)$$

Where N^k represents the moles of component i in phase k . The volume fraction of component i in phase k is calculated using equation (1.6)

$$\phi_i^k = \frac{n_k^i \cdot r_i}{N^k} \quad (1.6)$$

The chemical potential of each component in the phase is equal at equilibrium. Similarly the changes in chemical potential on mixing are equal as well,

$$\Delta\mu_i^{\text{top-phase}} = \Delta\mu_i^{\text{bottom-phase}} \quad (1.7)$$

One of the theoretical treatments of protein partitioning in aqueous two phase systems is based on the Flory-Huggins theory [17]. Flory-Huggins theory is a mathematical model of the thermodynamics of polymer solutions which takes account of the great dissimilarity in molecular sizes in adapting the usual expression for the entropy of mixing. The result is an equation for the Gibbs free energy change ΔG_m for mixing a polymer with a solvent. Although it makes simplifying assumptions, it generates useful results for interpreting experiments. The thermodynamic equation for the Gibbs free energy change accompanying mixing at constant temperature and (external) pressure is

$$\Delta G_m = \Delta H_m - T\Delta S_m \quad (1.8)$$

The objective is to find explicit formulas for ΔH_m and ΔS_m , the enthalpy and entropy increments associated with the mixing process.

Based on Equation (1.7), the chemical potential change on mixing for each component in the phase can be expressed as:

$$\Delta\mu_i^k = N^k \left(-r_i \sum_{j=1(j \neq i)}^{m-1} \sum_{l=j+1(l \neq i)}^m \omega_{j,l} \phi_j^k \phi_l^k + r_i \sum_{j=1(j \neq i)}^m \omega_{i,j} \phi_j^k (1 - \phi_i^k) \right) + N^k RT \left(\ln \phi_i^k + 1 - r_i \sum_{j=1}^m \frac{\phi_j^k}{r_j} \right) \quad (1.9)$$

The LLE compositions are the $\phi_i^{\text{top-phase}}$ and $\phi_i^{\text{bottom-phase}}$ that give the minimum $\Delta G^{\text{two-phase}}$ or the maximum $(\Delta G^{\text{one-phase}} - \Delta G^{\text{two-phase}})$, or equivalent chemical potential changes on mixing between the phases $(\Delta\mu^{\text{top-phase}} = \Delta\mu^{\text{bottom-phase}})$.

The parameters ω_{ij} and r_i which is in equation (1.3) and (1.9) respectively can vary can vary by orders of magnitude and by nature (positive or negative) between the components. If the value is a positive value, it shows that the force between the molecules is attractive while the negative value shows it's a repulsive force.

The changes in Gibbs energy on mixing of the paired phases $\overline{\Delta G}^{\text{top phase}}$ and $\overline{\Delta G}^{\text{bottom phase}}$ and the total energy changes by using the rule rule

$$\overline{\Delta G}^{\text{two-phase}} = \alpha \cdot \overline{\Delta G}^{\text{top phase}} + (1 - \alpha) \overline{\Delta G}^{\text{bottom phase}} \quad (1.10)$$

where $\alpha = \text{sign} \left(\frac{\overline{\Delta G}^{\text{top-phase}}}{\overline{\Delta G}^{\text{bottom-phase}}} \right) \left| \frac{\overline{\Delta G}^{\text{one-phase}} - \overline{\Delta G}^{\text{bottom-phase}}}{\overline{\Delta G}^{\text{top-phase}} - \overline{\Delta G}^{\text{bottom-phase}}} \right|$. Equation (1.10) defines the

total Gibbs energy of the two-phases system as the linear combination of the Gibbs energy of the phases, and the parameter α is the relative Gibbs energy between the mixture and the two phases. The numerator in this equation is the difference between the Gibbs energy on mixing in the top phase and the Gibbs energy on mixing in the mixture. The denominator is the difference between the Gibbs energy of mixing in the top phase and that in the bottom phase.

Then the difference in Gibbs energy of mixing between the one-phase and two-phase systems can be calculated for all possible combinations of top and bottom phase compositions generated. The equilibrium compositions for the ATPES are the combination of top and bottom phase compositions giving the most negative value of $(\overline{\Delta G}^{\text{two phase}} - \overline{\Delta G}^{\text{one phase}})$ and the smallest value of $\left\| \Delta\mu_i^{\text{top-phase}} - \Delta\mu_i^{\text{bottom-phase}} \right\|$, with the conditions that $\overline{\Delta G}^{\text{two-phase}} < \overline{\Delta G}^{\text{one-phase}}$ and the top and bottom compositions are not equal. The final conditions eliminate trivial solutions that fulfill $\Delta\mu_i^{\text{top-phase}} = \Delta\mu_i^{\text{bottom-phase}}$.

As an alternative, developed a non-linear programming (NLP) formulation is developed to minimize the Gibbs energy of mixing difference, $(\overline{\Delta G}^{\text{two phase}} - \overline{\Delta G}^{\text{one phase}})$. In this approach, the m components is accounted simultaneously by using a vector of design variables with the size of $1 \times m$. Mole fractions of the components in the bottom phase, f_i is the design variable and the total moles in the system is pre-specified as N . The relationship between f_i and N is defined by Equation (1.11) and (1.12).

$$N = N^{\text{top-phase}} + N^{\text{bottom-phase}} \quad (1.11)$$

$$N^{\text{top-phase}} = (1 - f_i) \cdot N \quad (1.12)$$

where,

$N^{\text{top-phase}}$ = total moles in top phase

$N^{\text{bottom-phase}}$ = total moles in bottom phase

The total moles in respective phases are defined as $N^{\text{top-phase}}$ and $N^{\text{bottom-phase}}$. The mole of component i in each phase is calculated using Equation (1.5) and (1.6) to calculate the volume fraction of component i . The NLP is formulated as:

$$\min(\Delta G^{\text{two-phase}} - \Delta G^{\text{one-phase}}) + \sum_i w_i \left(\frac{\Delta \mu_i^{\text{top-phase}} - \Delta \mu_i^{\text{bottom-phase}}}{\Delta \mu_i^{\text{top-phase}}} \right)^2 \quad (1.13)$$

$$\left(\frac{\Delta \mu_i^{\text{top-phase}} - \Delta \mu_i^{\text{bottom-phase}}}{\Delta \mu_i^{\text{top-phase}}} \right)^2 \leq \text{tol}_1 \quad \forall i = 1, \dots, n \quad (1.14)$$

$$\Delta G^{\text{two-phase}} < \Delta G^{\text{one-phase}} \quad (1.15)$$

$$\phi_2^{\text{top-phase}} - \phi_2^{\text{bottom-phase}} > \text{tol}_2 \quad (1.16)$$

$$\phi_2^{\text{bottom-phase}} - \phi_2^{\text{top-phase}} > \text{tol}_2 \quad (1.17)$$

$$0 \leq \phi_i^k \leq 0.3 \quad \forall i = 1, \dots, n \quad k = \text{top-phase, bottom phase} \quad (1.18)$$

where :

μ_i^k - change in the chemical potential on mixing for component i in phase k

w_i - static weighing factor for the chemical potential difference between the phases for component i .

In equation (1.13), the chemical potential difference term added as a penalty term to the original objective function, due to the Gibbs energy of mixing difference surface alone is too flat, hindering this approach from finding the global minimum. There also exist more than two inflection points where the tangents between the smaller inflections give false local minima [3].

1.5 Problem Statement

Many possible combinations of polymer and salt can be used to extract protein at different magnitudes and efficiencies. The selection is exhaustive. The prediction on the behaviour of ATPES and protein partitioning in ATPES is complicated and not straightforward. Furthermore, there are plenty of combinations of polymer-salt systems that can be attempted in order to get the best partitioning coefficient. In this project, the protein extraction is simulated using a thermodynamic modeling approach developed by Ahmad [3] in MATLAB based on Flory-Huggins theory. It is too expensive to predict the partitioning behavior by using a mathematical approach. This approach will be applied to predict LLE behavior in a few chosen polymer-salt systems to its applicability.

1.6 Objective

- To familiarize with the application of thermodynamic modeling and simulation framework by Ahmad [3] to predict the LLE behavior of polymer-salt ATPES.
- To perform literature research on suitable polymer-salt ATPES model and application of study.
- To calculate LLE data using the modeling approach by Ahmad [3] for PEG-Magnesium Sulphate system.
- To determine whether PEG-Magnesium Sulphate system can offer a suitable medium for protein extraction.

1.7 Scope of study

This project focuses on develop an ATPES which will be used for protein extraction via modeling and review approach proposed by Ahmad [3] in Matlab. In order to develop best ATPES, initially a lot of literature reviews need to be done. It is very essential to understand the concept of ATPES. Then the factors affecting ATPES need to be studied. Finally apply all the information on the polymer salt system and complete the mathematical modeling.

CHAPTER 2: LITERATURE REVIEW

In this chapter, journals and articles which contain experimental and modeling results in polymer-salt ATPES partitioning will be discussed to analyze and identify most suitable polymer salt system and to get some experimental data to compare with the result of this project.

2.1 Review on experimental approach

Malathy Jayapal and her team studied liquid-liquid equilibrium for an aqueous two-phase system containing PEG 2000 + potassium citrate + water at three different temperatures of (25,35 and 45) °C [15]. They fitted the binodal curve to an empirical equation relating the concentration of PEG 2000 and potassium citrate, and estimated the coefficients for the respective temperatures. They correlated the tie line compositions using Othmer-Tobias and Bancroft equations and reported the parameters. In the result they found that, the effect of temperature on the binodal curve which shows that, as temperature increases the solubility also increases and hence the two-phase region expands. They also studied the effect of polymer weight in two phase partitioning where they use two different polymers PEG 2000 and PEG 6000. The effect of molecular weight shows that, as the polymer molecular weight increases the binodal curve shifts down, indicating a clear increase in two-phase region.

Mastro B.D. and Azhar M. did experiment to study aqueous two-phase partitioning between PEG8000 + Magnesium Sulphate and PEG8000 + Sodium Sulphate at 35 °C [43]. Experimental results were then correlated with a mass-fraction based NRTL activity coefficient model. New interaction parameters were estimated with the Simplex method. The results show that sodium sulphates provides a larger two-phase region, showing a wider range of application than magnesium sulfate. Energy interaction parameters for the original NRTL model were estimated, and the mean deviations

2.2 Review on modeling approach

Ahmad [3] did a heuristic design approach to investigate ATPES as protein extraction technique. She developed Flory-Huggins model [1,2] as a thermodynamic modeling in Matlab to calculate liquid-liquid equilibrium behavior in aqueous polymer mixture. This work is based on minimization of Gibbs energy of mixing. She studied and demonstrated the applicability of her of her approach by simulating thermodynamic behavior of water + PEG6000+ D_xT500 and water + PEG6000 + Na₃PO₄ system which contains phosphofructokinase and ovalbumin. At the end of the project she compared the results with the calculations of Johansson [87] and the experimental data of Albertsson [9]. She found that, as the concentration of PEG or salt increase, the length of the tie line increase as well. Furthermore, she also discovered that the addition of protein which is phosphofructokinase and ovalbumin, did not change the LLE behaviour significantly. In the end, she found that the behaviors correspond to the observations made by Johansson et al. [35] and Albertsson et al. [44].

Ali Haghtalab and Babak Mokhtarani used UNIQUAC-NRF model to study the phase behavior of aqueous two-phase polymer salt system [45]. The system investigated were PEG + ammonium sulphate + water and PEG + monohydrogen phosphate + water for five different molecular weight of PEG (1000,1540,2000,4000,6000). Debye-Huckel equation based on Fowler-Guggenheim equation used to calculate electrostatic interaction of ions. The results of overall relative deviation of UNIQUAC-NRF, UNIQUAC and UNIFAC are compared. It is found that, UNIQUAC-NRF have better accuracy than other models. Although the UNIQUAC-NRF model is superior to both UNIQUAC and the extended UNIFAC models, it may noted that the extended UNIFAC equation can be used both for correlation and prediction, but the UNIQUAC-NRF and UNIQUAC equations are a correlative model.

CHAPTER 3: METHODOLOGY

3.1 Modeling Using MATLAB

MATLAB is used as a modeling simulation language to calculate the prediction of the LLE behavior in an ATPES system. The figure below shows the step taken to accomplish this project.

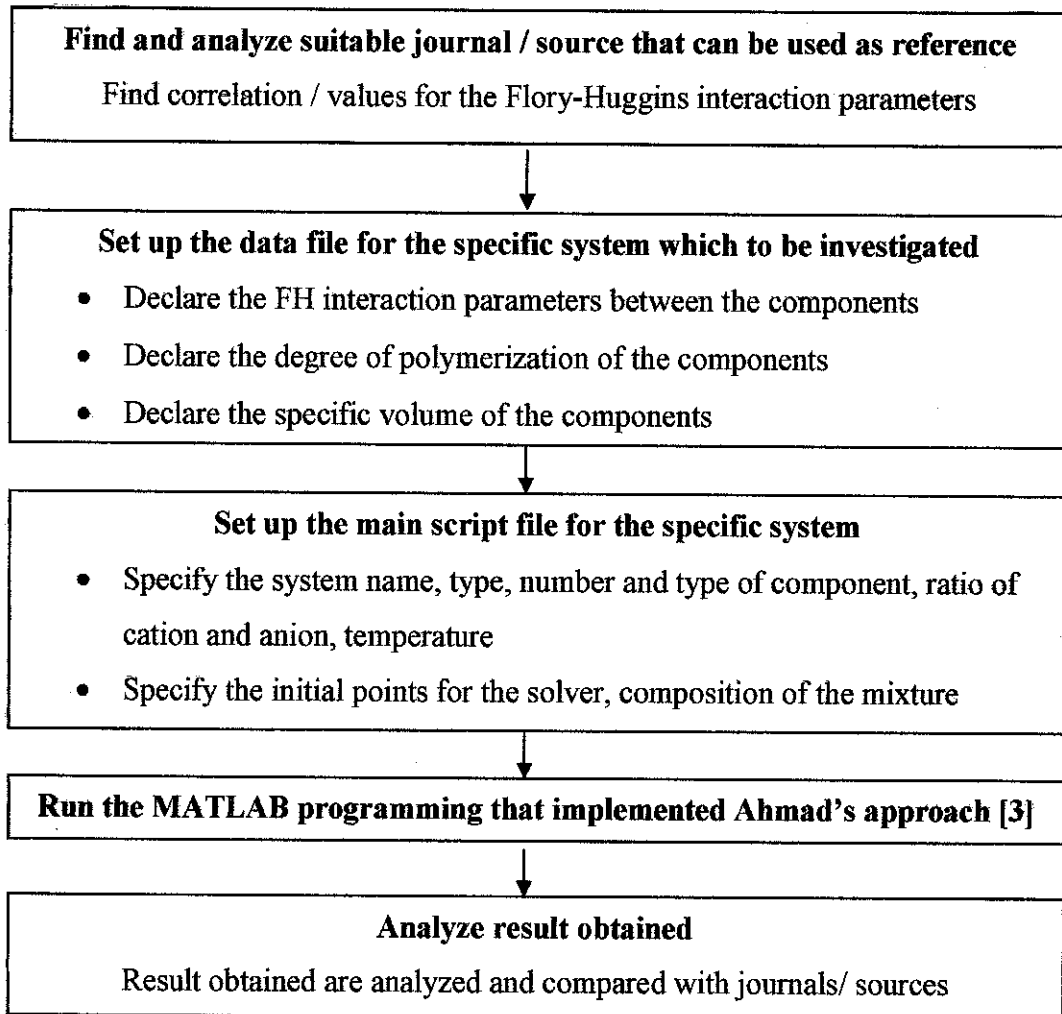


Figure 3.1: Steps in extending the approach by Ahmad [3] on predicting the LLE behavior based on an aqueous polymer system.

The figure 3.1 shows the steps taken to accomplish this project. The steps are extended approach of Ahmad [3] on predicting LLE behavior on polymer-salt ATPES. The first step is to find and analyze suitable journals or sources that can be used as reference. The correlation or value for the Flory-Huggins (FH) interaction parameters is determined. Then, the data file for the system is set up. The FH interaction parameters between the components, the degree of polymerization of the components and the specific volume of the components is declared. As for next step, the main script file for the system is prepared. In this main script the system name, type, number of component, type of component and temperature is specified. The initial points for the solver and composition of the mixture are also specified.

3.1 Flow Chart

The figure below shows the methodology of this project. [4]

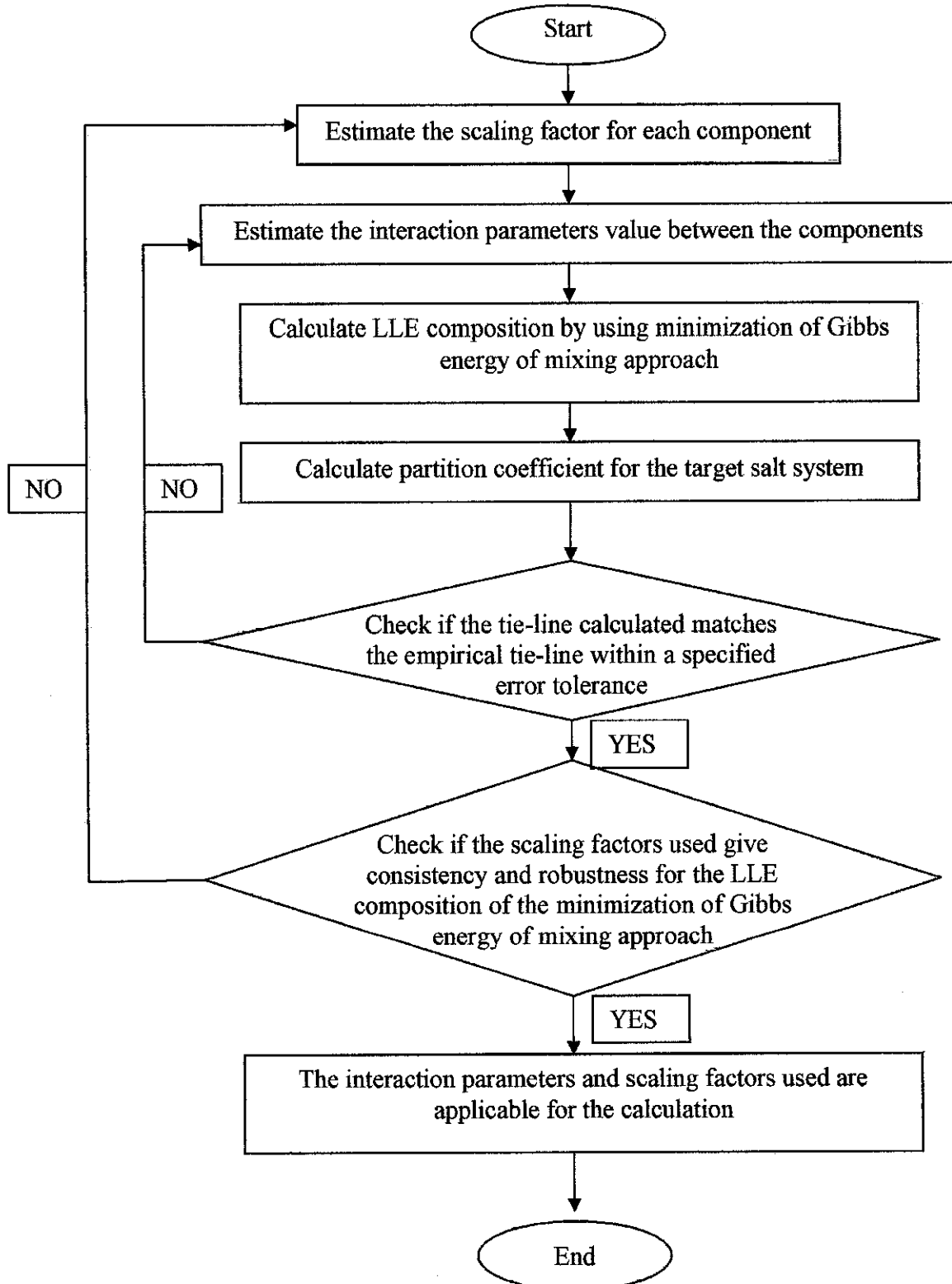


Figure 3.2: Flow chart of the modeling approach

Figure 3.2 shows that, manual partial enumeration strategy in determining the model parameters used in the ATPES modeling. It explains the calculation steps that are used in extending the approach by Ahmad [3]. First, the scaling factors values are estimated for each component in the particular ATPES system in study. This is used as a starting point to determine the correct scaling factor and interaction parameters for each component for an ATPES system. Next, the interaction parameters values between the components are estimated. By using the value that been estimated, the LLE compositions and the partition coefficient for the target polymer system are calculated. The tie-line produced by modeling approach is compared with the empirical tie-line to ensure it's within the specified error tolerance.

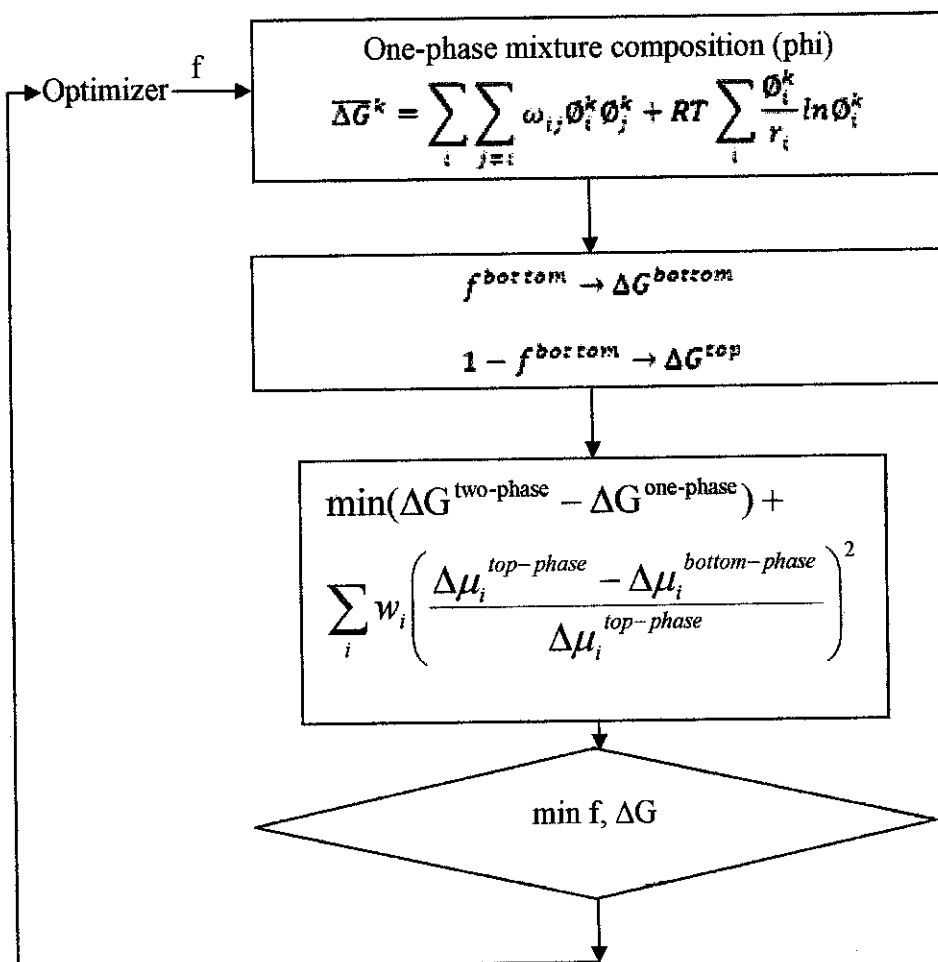


Figure 3.3: Algorithm of the MATLAB solver [3].

Figure 3.3 shows how the MATLAB coding is written in order to solve for the minimum ΔG . The initial value of 'f' is given to the optimizer to minimize the Gibbs energy difference. 'f' is the ratio of mole fraction of each component in bottom phase to top phase. If the 'f' value positive means the mole fraction of bottom phase is higher than the mole fraction of top phase. The optimizer will optimize the 'f' value iteratively. The SOLVOPT optimizer will optimize the value of f and ΔG by using modified Shor's r-algorithmn.

CHAPTER 4: RESULT AND DISCUSSION

In this chapter, the results of the modeling approach to determine the effect of using Magnesium Sulphate salt on the LLE behavior of polymer-salt ATPSs is presented and discussed. In this project Magnesium Sulphate is allowed to mix with PEG in water. The mixture is then reached equilibrium upon reaching min Gibbs energy.

Subscripts: 1 = water, 2 = PEG, 3 = Mg^{2+} , 4 = SO_4^{2-}

Table 4.1: Scaling Factors and interaction parameters for water-PEG-MgSO₄ system

System	System Composition (Volume Fraction)	Interaction parameters (J/mol)	Degree of polymerization and temperature	Scaling factors
PEG-MgSO ₄	water 86%	$w_{12} = 100$	$M_1 = 1$	$1e-1$
	PEG 10%	$w_{13} = -10000$	$M_2 = 100$	10
	Mg^{2+} 2%	$w_{14} = -30541$	$M_3 = 1$	$1e-2$
	SO_4^{2-} 2%	$w_{23} = -1265$	$M_4 = 1$	$1e-4$
		$w_{24} = -3084$	$T = 293.15K$	
	$w_{34} = -125587$			

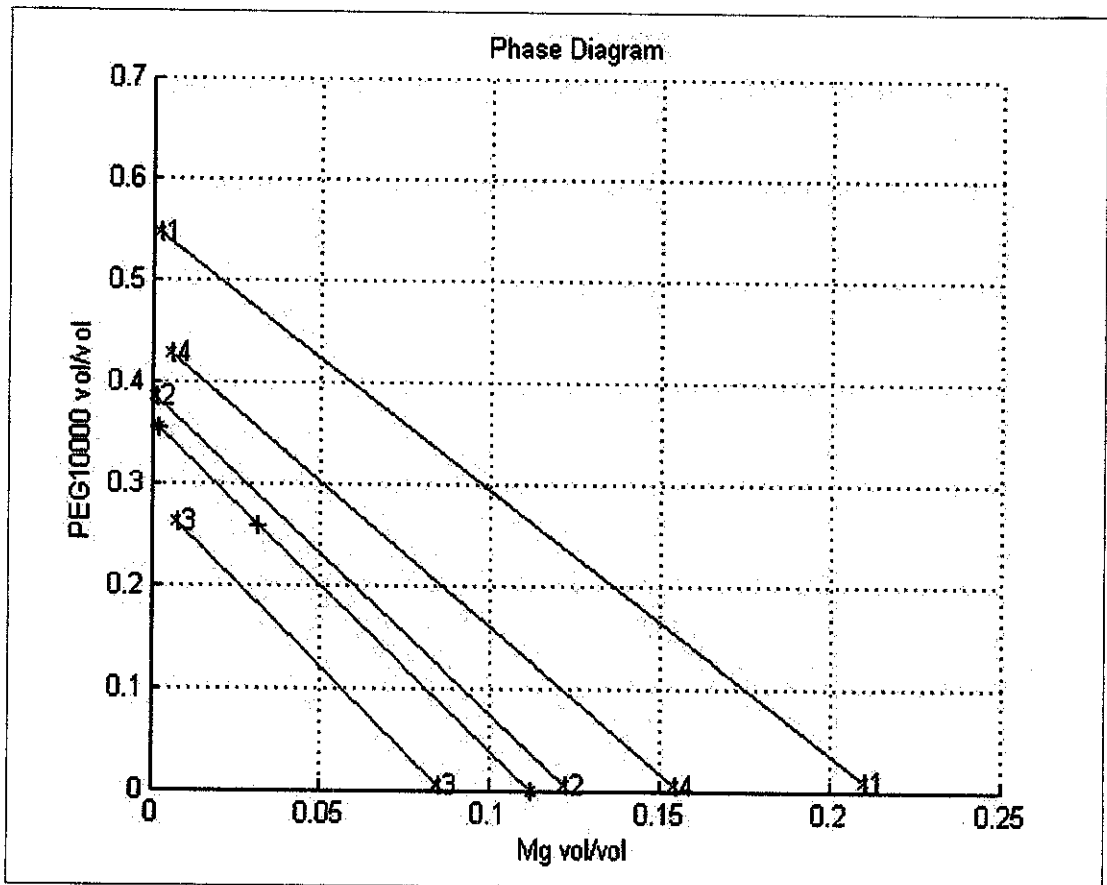


Figure 4.1: Phase diagram for water-PEG- MgSO_4 from literature review with calculated tie line

Water- PEG- MgSO_4 system is investigated. Table 4.1 shows the interaction parameters for water-PEG- MgSO_4 system. The table shows the composition of mixture is 86% of water and 10% of PEG while the salt is 4% is total. The thermodynamic calculations are done at 293.15K. The Figure 4.1 shows the phase diagram for water-PEG- MgSO_4 system. The blue lines represent the data obtained from literature review [46]. The data obtained from H.Rasa's journal to be compared with the result obtained from simulation. First of all, the data which is in weight fraction is converted to mole fraction. The calculations are shown in appendix B. Then the volume fraction of PEG and salt is plotted as PEG vs Mg graph. The red line is the calculated tie line from MATLAB simulation for water-PEG- MgSO_4 system. The interaction parameters are adjusted so that the slope of the red line is parallel with the lines obtained from literature data. The interaction parameters are given in Table 4.1. In the red line, the

mixture composition is set as [0.6769 0.2599 0.0316 0.0316], which means the coordinate of the point is [0.2599,0.0316]. The mixture composition is the middle point in red line. Then it forms two points at the top and bottom which represent composition of the system at the top and bottom. Connecting all three points we get tie lines.

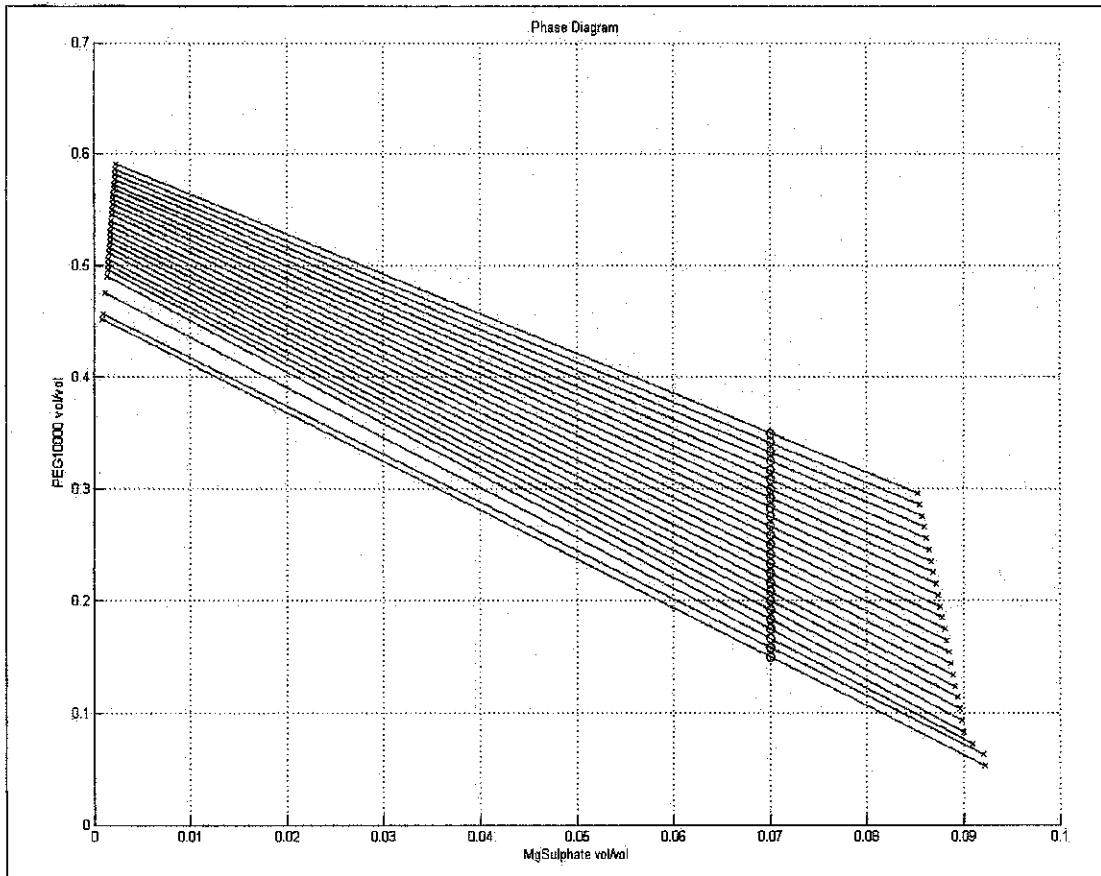


Figure 4.2: Phase diagram for water-PEG- MgSO₄ from adjusted scaling factors

Figure 4.2 shows the phase diagram for water-Peg10000-MgSO₄ system from adjusted scaling factors. The scaling factors are adjusted so that the calculated tie lines are parallel. The scaling factors are tabulated in Table 4.1. The top phase is rich with PEG and the bottom phase is rich with salt. This is due the density and solubility difference between the PEG1000 and salt components. The salt component disassociates fully in water to form Mg²⁺ and SO₄²⁻ ions whereby the PEG is not soluble in water. The bottom phase which is the salt has relatively higher density than PEG makes it to sink at bottom of the solution. Theoretically, as the concentration of the PEG and salt increase in

water, the length of tie lines which connect the top, bottom and mixture phases increase. But as shown in the Figure 4.2, the length of the tie line increase with increase in salt concentration and decrease in PEG concentration. But still the result shows that the system is converged to an optimum value of 'f' and the PEG10000-MgSO₄ system in water is robust due to very minimal difference in gradient of the tie lines.

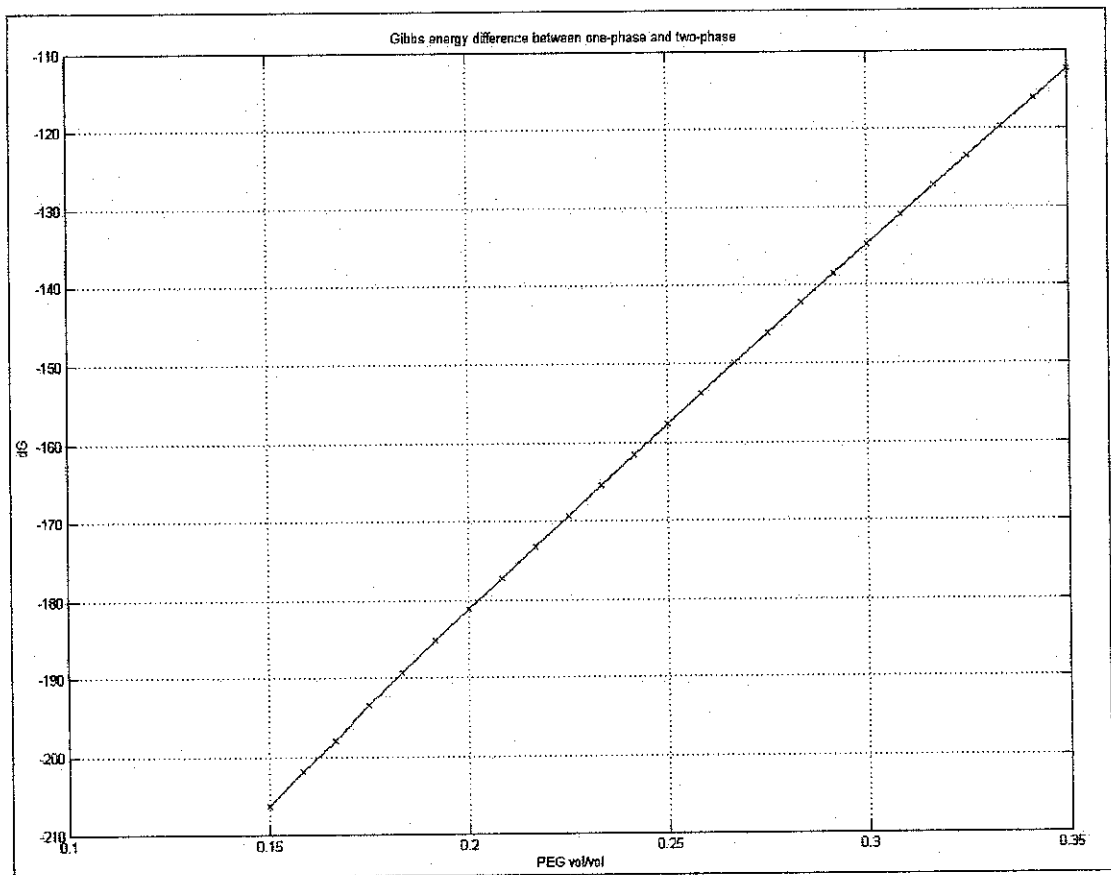


Figure 4.3: Gibbs energy of mixing difference for water-PEG10000-MgSO₄

Figure 4.3 shows the difference in Gibbs energy for water-PEG10000-MgSO₄ system. The Gibbs energy increases smoothly with increasing PEG concentration. The consistency and reliability of the approach in calculating LLE behavior is demonstrated by the plot of Gibbs energy of mixing difference in Figure 4.3.

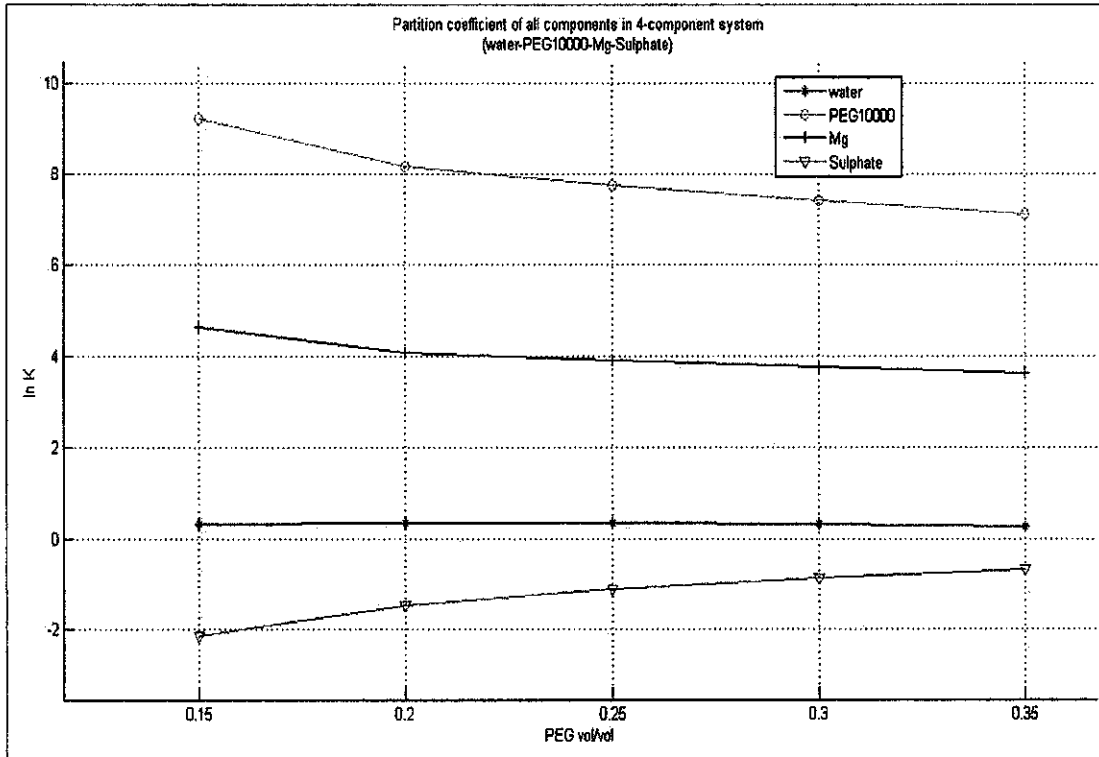


Figure 4.4: Partition coefficient for water-PEG10000-MgSO₄ system

Figure 4.4 shows the partition coefficient of each component in water-PEG10000-MgSO₄ system. The top phase is formed by PEG10000 has the partition coefficient, k value more than 1 ($K > 1$). Hence the $\ln K$ value for PEG is more than 0 ($\ln K > 0$). As explained above, the reason for PEG1000 to be at the top phase is due to lesser density and its characteristic which is insoluble in water. The variation in partition coefficient value as the PEG volume fraction increases is very low displays the consistency and reliability of the approach. The high difference in $\ln K$ value for PEG10000 and salt shows that the system can be a suitable medium for protein extraction to take place.

CHAPTER 5: CONCLUSION & RECOMMENDATION

Aqueous two-phase extraction systems (ATPES) are particularly suited for separation and purification of proteins from crude material like cell extracts, down stream broth, culture filtrate, because insoluble cell debris often partitions in one of the two phases. These systems are classified in two categories. The first category is consisted of an aqueous solution of two polymers such as polyethylene glycol and dextran, and the second is an aqueous solution of a polymer with a salt. However, polymer-salt aqueous two-phase systems have some advantages such as low price, low viscosity and a short time for phase splitting. The liquid-liquid behavior of the polymer-salt system is captured by using modeling and simulation. From the literature review, many journals been reviewed and Rasa et. al.[46] is chosen to compare the result obtained from simulation. From the literature review it is found that Flory-Huggins theory is suitable for this thermodynamic calculation. PEG10000- MgSO_4 system was studied in water. Upon phase separation it observed that the PEG10000 is at top phase and MgSO_4 is at bottom phase due to density and solubility in water. Furthermore, it is found that as the concentration of PEG and salt increase in top and bottom phases as the length of the tie line increase. If the tie line is longer means the phase separation between PEG10000 and Magnesium Sulphate is more in water. This result shows that the Flory-Huggins theory is able to represent the behavior of polymer-salt aqueous two phase system. The partition coefficient, K value shows that the PEG has K value more than 1. The significant difference in partition coefficient value between PEG10000 and MgSO_4 in water suggest that this system is suitable to become protein extraction medium. Lastly, the generalized thermodynamic framework by Ahmad [3] is applicable to predict the phase separation in PEG10000- MgSO_4 in water. As for recommendation, the application of this project needs to be expanded to predict protein behaviour in this ATPES. A detailed study need to be done to apply this method in large scale industry.

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APPENDICES

Appendix B: Manual Calculation of volume fraction from weight fraction

ϕ = volume fraction

ρ = density

w = weight fraction

$$\rho = 1/\Sigma(w*v)$$

At mixture composition and 3rd row:

$$w = [0.6147 \ 0.2837 \ 0.0508 \ 0.0508]$$

$$v = [1 \ 0.832 \ 0.5653 \ 0.5653]$$

$$\rho = 1/0.9082 = 1.1011$$

$$\phi_i = \rho v_i w_i$$

$$= 1.1011 \times 1 \times 0.6147 = 0.6748$$

$$\text{Therefore } \phi = [0.6748 \ 0.2599 \ 0.0316 \ 0.0316]$$

The answer from this calculation is compared with the answer obtained in MATLAB.

```
>> phi_lit.mix(3,:)
```

$$\text{ans} = [0.6769 \ 0.2599 \ 0.0316 \ 0.0316]$$

It is found that the answers are the same and hence the codings are correct.

Appendix C

Date file for water-PEG-MgSulphate system

```
% Data file for WaterPEG10000MgSulphate
% reference: Johansson et al. (1998)

% FH interaction parameter between water-water
omega.water_water = 0;

% FH interaction parameter between water-PEG
omega.water_PEG = 100;

% FH interaction parameter between water-Mg2+
omega.water_Mg = -10000;

% FH interaction parameter between water-SO42-
omega.water_Sulphate = -30541;

% FH interaction parameter between PEG-PEG
omega.PEG_PEG = 0;

% FH interaction parameter between PEG-Mg2+
omega.PEG_Mg = -1265;

% FH interaction parameter between PEG-SO42-
omega.PEG_Sulphate = -3084;

% FH interaction parameter between Mg2+-Mg2+
omega.Mg_Mg = 0;

% FH interaction parameter between Mg2+-SO42-
omega.Mg_Sulphate = -125587;

% FH interaction parameter between C6H5O73--C6H5O73-
omega.Sulphate_Sulphate = 0;

% specifying anion-cation ratio
ratio = 1;

% degree of polymerisation
r.water = 1;
r.PEG = 100;
r.Mg = 1;
r.Sulphate = 1;

% specific volume [cm3/g]
% Kang and Sandler 1987
v.water = 1;
v.PEG = 0.832;
v.Mg = 1/1.769;
v.Sulphate = 1/1.769;
```

```

% specifying the component
comp.c1 = 'water';
comp.c2 = 'PEG10000';
comp.c3 = 'Mg';
comp.c4 = 'Sulphate';

% source:
% /results_LLEgrid_3comp_type2_tocompare_mylog.mat

w_lit.mix = [1-(2*0.1411)-0.2562  0.2562  0.1411  0.1411
             1-(2*0.0508)-0.2837  0.2837  0.0508  0.0508
             1-(2*0.0378)-0.2408  0.2408  0.0378  0.0378
             1-(2*0.0781)-0.2549  0.2549  0.0781  0.0781];

w_lit.top = [1-(2*0.0028)-0.5902  0.5902  0.0028  0.0028
             1-(2*0.0010)-0.4303  0.4303  0.0010  0.0010
             1-(2*0.0121)-0.2972  0.2972  0.0121  0.0121
             1-(2*0.0091)-0.4712  0.4712  0.0091  0.0091];

w_lit.bot = [1-(2*0.2803)-0.0079  0.0079  0.2803  0.2803
             1-(2*0.1812)-0.0045  0.0045  0.1812  0.1812
             1-(2*0.1321)-0.0051  0.0051  0.1321  0.1321
             1-(2*0.2204)-0.0047  0.0047  0.2204  0.2204];

[m,n] = size(w_lit.mix);
para.v = [v.water v.PEG v.Mg v.Sulphate];

% loop to convert each row of w into phi
for i = 1:m

    % mixture composition
    phi_lit.mix(i,:) = Calc_w2phi(w_lit.mix(i,:),para);

end

[m,n] = size(w_lit.top);

for i = 1:m

    % top composition
    phi_lit.top(i,:) = Calc_w2phi(w_lit.top(i,:),para);

    % bottom composition
    phi_lit.bot(i,:) = Calc_w2phi(w_lit.bot(i,:),para);

end

% assigning the system type
type = 2;

% plotting the results
h = plot_LLE(1,phi_lit,'bx-',type,comp,1,1)

```

```
% saving the data in a mat file
save waterPEG10000MgSulphate;
```

Appendix D

Main script file for water-PEG-MgSulphate system

```
% script file to run file Calc_split_solvopt
% in order to calculate split compositions for a 4-component
% system containing water, polymer1, anion and cation
% using Gibbs minimization routine
% using SolvOpt - optimizer based on Shor's Algorithm

% clearing memory and closing all figures
clear all;
close all;

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

% setting the save data file
SaveFile = sprintf('results_run_mindG_salt_4comp');

% declaring global variables
global R;
global TolF;
global TolX;
global Toldmu;
global ToldmuO;
global MaxIter;

% specifying the values
R = 8.314;
TolF = 1e-8;
TolX = 1e-10;
Toldmu = [ 1 1 1 1 ];
MaxIter = 1000000;

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

% specifying the ATPS
sysname = 'waterPEG10000MgSulphate';
type = 2;

% specifying number of component
no_comp = 4;

% specifying the component
comp.c1 = 'water';
comp.c2 = 'PEG10000';
comp.c3 = 'Mg';
comp.c4 = 'Sulphate';
```

```

% specifying the system temperature
T = 273.15 + 20;

% loading the ATP-protein system parameters
parameter = GetParameters(type,T,sysname);
% specifying initial total amount of the mixture (one-phase system)
NO = 1;

% specifying the composition for the mixture (one-phase system)
vol.PEG = 0.1;
vol.Mg = 0.03;
vol.Sulphate = 0.01;
vol.water = 1 - vol.PEG - vol.Mg - vol.Sulphate;
phi = [ vol.water vol.PEG vol.Mg vol.Sulphate ];
phi = [0.6769    0.2599    0.0316    0.0316];

% specifying initial points
s0 = [ 0.6 0.3 0.1 0.1 ];

% solving the factors/fractions of mole
[f_split,phi_split,mole_split,Ntot_split,dG_split,dU_split,K_split,options] ...
    = Calc_split_solvopt(phi,NO,parameter,s0,type);

% finding the chemical potential difference
[U,dU] = Calc_dU(phi_split.top,phi_split.bot,parameter);

% analyzing results
if (options(9) > 0)
    conv_flag = 1;
else
    conv_flag = 0;
end

% displaying partition coefficient
if ( conv_flag )
    disp(sprintf('\nMinimization routine converged to solutions:'));
    disp(sprintf('\nFraction of mole = [ %.4f %.4f %.4f %.4f
]',f_split));
    disp(sprintf('\nGibbs energy difference = %.4f\n',dG_split));
else
    disp(sprintf('\nMinimization routine did NOT converge to
solutions\n'));
end

% plotting the results
% finding the size of the input matrix
[row,col] = size(parameter.phi_lit.top);

% assigning the figure number
figure(1);
hold on;

```

```

% plotting the literature tie line
for i = 1:row
    h = plot([parameter.phi_lit.top(i,3) parameter.phi_lit.bot(i,3)],
...[parameter.phi_lit.top(i,2) parameter.phi_lit.bot(i,2)], 'bx-');
end

% printing text on literature tie line
for i = 1:row
    text([parameter.phi_lit.top(i,3) parameter.phi_lit.bot(i,3)], ...
        [parameter.phi_lit.top(i,2)
parameter.phi_lit.bot(i,2)], sprintf('t%d', i));
end

% labeling the figure
title('Phase Diagram');
unit_label = 'vol/vol';
ylabel(sprintf('%s %s', comp.c2, unit_label));
xlabel(sprintf('%s %s', comp.c3, unit_label));
grid on;

% plotting the mixture composition
plot(phi(3), phi(2), 'r+');

% plotting the tie line from the minimization approach
h = plot([phi_split.top(3) phi_split.bot(3)], ...
        [phi_split.top(2) phi_split.bot(2)], 'r*-');

% saving the results if converged
if ( conv_flag )
    save(SaveFile);
end

```