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PHASE PARTITIONING OF PROTEINS IN AQUEOUS TWO PHASE POLYMER SYSTEMS IN PRESENCE OF SALTS

BY

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled, "Phase Partitioning of Proteins in Aqueous Two Phase Polymer Systems in Presence of Salts" submitted by Aparna Gupta in partial fulfillment of the requirements for the degree of Master of Science.

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ABSTRACT

A new model is proposed to describe phase partitioning of proteins in aqueous two phase polymer mixtures. The proposed model combines the Flory-Huggins excess Gibbs free energy model for polymer systems with the thermodynamic model proposed by Chen and Evans (1982, 1986) for electrolyte systems. In the model, the excess Gibbs free energy due to size differences is accounted for by the Flory Huggins model. The long range ion-ion interactions are represented by the Pitzer Debye Hückel model (Pitzer, 1973) while the Nonrandom Two Liquid Theory (Renon, 1968) is used to represent short range interactions between ions.

The proposed model is applied successfully to study phase partitioning of proteins in aqueous two phase polymer systems in the presence of salts. In particular, we have studied phase partitioning of lysozyme, α-chymotrypsin and bovine serum albumin in PEG 3350 / dextran T-70 / water and PEG 8000 / dextran T-500/water systems. The experimental data of King et al. (1988) are used to estimate model parameters. Salt-water interactions needed for the model are directly used from Chen and Evans (1982, 1986). Polymer-protein interaction parameters are regressed. Furthermore, new Flory-Huggins parameters are regressed to describe the phase equilibria of PEG 3350 / dextran T-70 / water and PEG 8000 / dextran T-500 / water systems. The comparison of calculated and experimental results indicates that the proposed model gives a good correlation

of binodal curves and also for salt effects on the phase partitioning of proteins in aqueous two phase polymer systems.

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Dedicated to my parents

Yash Pal Chaddah and Rakesh Chaddah

and Siblings

Varinder Chaddah, Pradeep Chaddah and Alpana Bajaj

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LIST OF SYMBOLS

Symbols	Description
a	activity
d	Density
D	Dielectric Constant
Dx	Dextran
exp	Exponential
f	Fugacity
G	Gibbs free energy
g	Gibbs free energy (for one mole)
I	Ionic Strength
k	Boltzman's Constant
K	Partition Coefficient
M	Molecular Weight
m	Molar volume
n	Number of moles
N	Number of point
PEG	Polyethylene Glycol
O.F.	Objective Function

T	Temperature
w	Weight fraction
x	Mole fraction
Z	Charge on specie

SUBSCRIPTS

a	Anion
c	Cation
ca	Salt
i	Component i
j	Component j
m	Molecular specie
p	Protein

SUPERSCRIPTS

comb	Combinatorial
E	Excess

ex	Excess
FH	Flory Huggins
lc	Local composition
M	Mixing
PDH	Pitzer Debye Huckel

Greek Symbols

α	Nonrandomness factor
γ	Activity coefficient
θ	Temperature dependent constant
μ	Chemical potential
ρ	Density
Σ	Summation
τ	Chen's Interaction Parameter
Φ	Volume fraction
χ	Flory Huggins interaction parameter

CHAPTER 1

INTRODUCTION

Proteins are widely used as pharmaceuticals. After these biochemicals are made, they must be separated and purified. These separations are difficult and frequently cost more than the initial manufacture. This has resulted in an increased interest in the development of efficient methods for the separation, concentration and purification of biological products from fermentation and cell culture media. The competitive advantage in biochemical production depends not only on innovations in molecular biology but also on innovation and optimization of separation processes. The purpose of this study is to propose a thermodynamic model which could be used for biochemical separations.

Thermodynamics plays an important role in the development and production of biochemicals with the main application of thermodynamics in the design of separation operations particularly in the separation of biochemical products from dilute aqueous solutions. The study of phase equilibrium behaviour of biomolecules is very important because it helps in understanding partition mechanisms. It is also an important tool in the design and optimization of downstream recovery processes.

In this area of protein extraction there has been massive and continued investment on a worldwide basis by all the pharmaceutical companies (Smith,

1988). Liquid-liquid extraction has been used extensively in the chemical and petrochemical industries. It is of particular interest in biochemical separations of proteins. The interest in this technology is because it is readily scalable and it can be operated on a continuous basis. A system which is widely used in the extraction of proteins is the aqueous two phase polymer system. Aqueous two phase polymer systems are formed when two polymers are dissolved together above certain concentrations. The advantage of these systems is that both phases are aqueous (of water content 85 to 99% by weight). This allows the partition of biomolecules under nondenaturing conditions, between two immiscible phases with each phase being rich in a specific polymer.

A comprehensive model for partitioning of proteins in the presence of salts is not available. In the past, researchers have concentrated on aqueous systems containing non-electrolytes but phase behaviour of proteins depends on the type and concentration of salts present. Salts are added to aqueous solutions in order to buffer the solution. Salts also stabilise proteins. By varying salt type and concentration, selectivity and yield of protein can be manipulated.

The purpose of this study is to develop a model that could be used for predicting the liquid-liquid phase separation of proteins in aqueous two phase polymer systems in presence of salts. Proteins studied are lysozyme, bovine serum albumin and α-chymotrypsin.

CHAPTER 2

LITERATURE REVIEW

Several models have been proposed to represent solubility of proteins in aqueous two phase polymer systems but all these models provide only partial information about specific aspects of partitioning. However, a comprehensive and model which can be used to correlate protein partition behaviour has not been developed as yet. Aqueous two phase systems are gaining interest because of their broad applications in biochemical separations. Since there was a lack of a useful and predictive model for particle partitioning, many researchers attempted to develop new models (Baskir et al., 1989).

Baskir et al. (1989) note that Bronsted developed an approximate expression to describe the effect of particle size on partitioning in aqueous two phase systems as early as 1931. He proposed that particle partitioning is very sensitive to the molecular weight of the protein. He developed an approximate expression to represent the effect of particle size on the partition coefficient, K, given by

$$K = y/x = \exp(M \lambda / kT) \qquad (2.1)$$

where y and x are the mole fractions in the two phases of a protein with molecular

weight M and λ is a constant depending on both the phase system and the protein. The above equation demonstrates that the particle molecular weight determines the protein partitioning to a large extent.

Flory (1942) and Huggins (1942) formulated some basic principles for athermal polymer mixtures. They proposed a model to calculate the excess Gibbs free energy of a mixture of polymers in a common solvent.

Scott (1948) presented an analysis of the polymer incompatibility problem using Flory-Huggins theory. He also derived analytic expressions for the plait point for the mixture of two polymers and a solvent. He showed that the phase separation results from the interaction energy between segments of two polymers. The interaction of the polymer segment with solvent has little influence on the phase separation.

Brooks et al. (1985) developed a lattice model to represent the partitioning of biomolecules by extending the theory proposed by Flory and Huggins for polymer solvent systems to multicomponent systems containing proteins. He assumed the system contains four components (water, polymer, polymer and the biomolecule). He modelled the biomolecule as a polymer and assumed that all the polymeric molecules (including biomolecule) have the same interaction energy with the solvent molecule (i.e. $\chi_{12}=\chi_{13}=\chi_{14}=\chi$). The partition coefficient for the biomolecule was found by equating the chemical potentials for the biomolecule in the two phases. The partition coefficient of the bimolecule is given by,

$$\ln K_4 = \ln (\phi_{41}/\phi_{42}) = m_4 [(1-\chi)(\phi_{11} - \phi_{12}) + (1/m_2 - \chi_{24})(\phi_{21} - \phi_{22}) + (1/m_3 - \chi_{34})(\phi_{31} - \phi_{32})] \quad (2.2)$$

where ϕ_{i1} and ϕ_{i2} are the volume fractions of component i in the two phases respectively, and m_i is the size of solute relative to the water. Equation (2.2) shows that the partitioning of protein is directly proportional to the molecular size of the protein and to the polymer concentration difference between the two phases ($\phi_{21} - \phi_{22}$). Furthermore, note from Equation (2.2) that the interaction between the protein and the polymers determine the protein partition coefficient (χ_{24} and χ_{34}).

Gustafsson et al. (1986) investigated the mechanism that leads to the phase separation of aqueous polymer systems. Their study demonstrated that an important factor governing phase separation is the direct molecular interaction between two monomer units of the polymer. The force of interaction between these two monomer units should be repulsive for the phase separation to occur. However, their analysis did not consider the size and shape of the entire polymer molecule as an important factor affecting phase separation. They also regressed Flory-Huggins model parameters for the system Dextran (molecular weight 23,000), PEG 6000 (molecular weight 6000) and water. They used the experimental data given by Albertsson (1971) to regress model parameters.

Kang and Sandler (1987) in their study used the UNIQUAC model to explain the thermodynamics of liquid - liquid phase behaviour of the dextran-PEG-water system. They determined UNIQUAC interaction parameters for several dextran-

PEG systems. They also used the model proposed by Flory and Huggins to determine the interaction parameters for the aqueous polymer systems of PEG 6000 (molecular weight 6000) - Dextran 17 (molecular weight 23,000), PEG 6000 - Dextran 24 (molecular weight 40,500), PEG 6000 - Dextran 37 (molecular weight 83,000) and PEG 6000 - D48 (molecular weight 180,000). In order to estimate the interaction parameters they used the LLE data of polymer solutions given by Albertsson (1971). However, their work was limited to the ternary polymer-polymer water system. They did not study the phase partitioning of biomolecules in these aqueous polymer systems.

A systematic study of phase partitioning of proteins in aqueous systems containing polymers and electrolytes has been done only recently.

King et al. (1988) proposed a molecular thermodynamic model based on the osmotic virial equation to represent phase equilibrium for dilute aqueous solutions containing polymers, proteins and salts. They also reported phase diagram data for the two aqueous polymer systems PEG 8000 (molecular weight 8920) - Dextran T-500 (molecular weight 167,000) and PEG 3350 (molecular weight 3690) - Dextran T-70 (molecular weight 37,000) . Experimental data for partitioning of a number of proteins in these aqueous polymer systems in the presence of salts were also given. They determined the osmotic second virial coefficients for aqueous mixtures containing polymers, proteins and salts. In the model, "salt-water" is treated as a single component and the salt molality does not appear explicitly in the equations. The osmotic coefficients reported are therefore

applicable only for a single salt species at specific concentration. It has also been observed that the virial expansion truncated at the second order terms is appropriate only for very dilute polymer solutions.

Haynes et al. (1989) proposed a molecular thermodynamic model for predicting thermodynamic properties of aqueous two phase system containing polymers, proteins and salts. The model combines an extended Debye Hückel approximation and a short range interaction model that includes osmotic virial coefficients for interactions between the various species. In this model the salt composition variables are included specifically. The authors comment that truncation of the osmotic virial expansion after the second term limits the usefulness of the model at higher polymer concentrations.

CHAPTER 3

PHASE EQUILIBRIUM MODELS

In a closed system, at constant temperature and pressure, the conditions for phase equilibrium are (Smith and Van Ness, 1975)

$$\mu_{i1} = \mu_{i2} \tag{3.1}$$

In other words, the chemical potential of substance i must have the same value in each phase of the system. An alternative equation for phase equilibrium may be obtained by noting that

$$\mu_i = RT \ln f_i + \theta_i \tag{3.2}$$

where θ_i is a constant that depends on temperature only. Since all phases are at the same temperature, Equations (3.1) and (3.2) give

$$f_{i1} = f_{i2} \tag{3.3}$$

The above criterion for phase equilibrium requires that for multiple phases at the same temperature and pressure to be in equilibrium, the fugacity of each

component must be same in all the phases.

For non-ideal liquids it is convenient to define a standard state fugacity and activity coefficients so that

$$f_{iL} = \gamma_i x_i f_i^0 \qquad (3.4)$$

where :

γ_i = activity coefficient of component i

f_i^0 = fugacity of i in its standard state.

The activity coefficient is a measure of the nonideality of a component in the liquid phase. In ideal solution, all the activity coefficients are equal to unity. The non-ideality of a solution is represented by the deviation of γ_i from unity. The positive deviation from ideality is defined as $\gamma_i > 1$, and the negative deviations from ideality as $\gamma_i < 1$.

The Gibbs free energy of mixing minus the ideal Gibbs free energy is called the excess free energy and is given by

$$\Delta G^E / RT = \Delta G^M / RT - \Delta G^{M,ideal} / RT \qquad (3.5)$$

Since

$$\Delta G^M / RT = \Sigma_i^N x_i \ln a_i \qquad (3.6)$$

Where a_i is the activity of component i and is defined as the product $\gamma_i x_i$, and

$$\Delta G^{M,ideal} / RT = \Sigma_i^N x_i \ln x_i \qquad (3.7)$$

which gives:

$$\Delta G^E / RT = \Sigma_i^N x_i \ln \gamma_i \qquad (3.8)$$

The activity coefficient can be calculated from the equation

$$\ln \gamma_I = \left(\frac{\partial(ng^E/RT)}{\partial n_I}\right)_{T, P, n_{J \neq I}} \qquad (3.9)$$

3.1 Excess Gibbs Free Energy Models

Walas (1985) has presented a compact summary of many models which are commonly used to represent the excess Gibbs free energy of mixtures. Some examples of these models are Van Laar, Margules, Wilson, NRTL, UNIQUAC and UNIFAC models.

The equations proposed by Margules in (1895) are the earliest cited by Walas (1985). The expression for the excess Gibbs free energy of a binary system is

$$G^E / RT = x_1 x_2 [A_{12} x_1 + A_{21} x_2] \qquad (3.10)$$

Thus we get the following expressions for the activity coefficients:

$$\ln\gamma_1 = [A_{12} + 2 (A_{21} - A_{12} x_1)] x_2^2 \qquad (3.11)$$

$$\ln\gamma_2 = [A_{21} + 2 (A_{12} - A_{21} x_2)] x_1^2 \qquad (3.12)$$

In these equations A_{12} and A_{21} are the parameters to be regressed from the experimental data.

In the van Laar equation the excess Gibbs energy is related to mole fractions in the reciprocal form, given by

$$G_{ex} / RT = ABx_1x_2 / (Ax_1 + Bx_2) \qquad (3.13)$$

This gives the following equations for the activity coefficients

$$\ln\gamma_1 = A [Bx_2 / (Ax_1 + Bx_2)]^2 \qquad (3.14)$$

$$\ln\gamma_2 = B [Ax_1 / (Ax_1 + Bx_2)]^2 \qquad (3.15)$$

In the Wilson model it is postulated that interactions between molecules depend primarily on "local concentrations" which he expressed as volume fractions. The local concentrations are defined in terms of probabilities using a Boltzman distribution. Wilson's equation gives the following expression for the excess Gibbs free energy of a binary system

$$G^E / RT = - x_1 \ln (x_1 + \Lambda_{12} x_2) - x_2 \ln (x_2 + \Lambda_{21} x_1) \qquad (3.16)$$

Thus, the following expressions for the activity coefficients are obtained

$$\ln \gamma_1 = - \ln (x_1 + \Lambda_{12} x_2) + \beta x_2 \qquad (3.17)$$

$$\ln \gamma_2 = - \ln (x_2 + \Lambda_{21} x_2) - \beta x_1 \qquad (3.18)$$

where

$$\beta = \Lambda_{12} / (x_1 + \Lambda_{12} x_2) - \Lambda_{21} / (x_2 + \Lambda_{21} x_1) \qquad (3.19)$$

The NRTL (Non-Random Two liquid) theory was derived by Renon and Prausnitz (1968). The following expression was obtained for the excess Gibbs free energy of mixing,

$$\frac{\Delta G^E}{RT} = \sum_{i=1}^{N} x_i \frac{\sum_{j=1}^{N} \tau_{ji} G_{ji} x_j}{\sum_{k=1}^{N} G_{ki} x_k} \qquad (3.20)$$

where

$$\tau_{ij} = (g_{ij} - g_{jj}) / RT \; ; \; g_{ij} = g_{ji}, \qquad (3.21)$$

g_{ij} is the energy of interaction between i-j pair of molecules

$$G_{ij} = \exp(- \alpha_{ij} \tau_{ij}) \; ; \; G_{ii} = 1.0 \qquad (3.22)$$

and

$$\alpha_{ij} = \alpha_{ji} \qquad (3.23)$$

Coefficient α_{ij} is called the nonrandomness constant of the i-j pair of molecules. The following expression for the activity coefficient is obtained from the NRTL model

$$\ln \gamma_i = \frac{\sum_{j=1}^{N} \tau_{ji} G_{ji} x_j}{\sum_{k=1}^{N} G_{ki} x_k} + \sum_{j=1}^{N} \frac{x_j G_{ij}}{\sum_{k=1}^{N} x_k G_{kj}} \left(\tau_{ij} - \frac{\sum_{l=1}^{N} x_l \tau_{lj} G_{lj}}{\sum_{k=1}^{N} x_k G_{kj}} \right) \qquad (3.24)$$

The NRTL equation has three parameters (τ_{12} ,τ_{21} and α_{12}) for a binary mixture. The NRTL equation unlike Wilson's equation, can represent liquid-liquid phase equilibria. It can also be extended to multicomponent mixtures with only binary interaction parameters. However, the NRTL equation does not include specific terms for mixtures which have molecules of different sizes (for example, a mixture containing polymer molecules).

The UNIQUAC (Universal Quasi - Chemical) equation of Abrams and Prausnitz (1975) is also based on the two liquid model of Scott and uses the local composition concept. In this model the excess Gibbs free energy is assumed to be made up of two contributions; a combinatorial part due to the differences in the sizes and shapes of molecules and a residual part due to the energy of interaction between unlike molecules. This model is capable of predicting liquid-liquid equilibria. It can also be applied to multicomponent mixtures using only the binary

interaction parameters.

$$G^E = G^E_{combinatorial} + G^E_{residual} \tag{3.25}$$

where

$$\frac{G^E_{combinatorial}}{RT} = \sum_I x_I \ln \frac{\phi_I}{x_I} + \frac{z}{2} \sum_I q_I x_I \ln \frac{\theta_I}{\phi_I} \tag{3.26}$$

and

$$\frac{G^E_{residual}}{RT} = -\sum_I q_I x_I \ln[\sum_J \theta_J \tau_{JI}] \tag{3.27}$$

also

$$\theta_I = \frac{q_I x_I}{\sum_J q_J x_J} \tag{3.28}$$

$$\phi_I = \frac{r_I x_I}{\sum_J r_J x_J} \tag{3.29}$$

$$\tau_{JI} = \exp[\frac{-u_{JI}}{RT}] \tag{3.30}$$

In the above equations q_i and r_i are the surface and volume parameters, u_{ji} are interaction parameters and z is the coordination number.

The UNIFAC (Uniquac Functional Group Activity Coefficient) model is a group contribution model based on the UNIQUAC model. Group interaction parameters and size parameters have been correlated for a large number of group combinations, including some biomolecules, (Gupta and Heidemann, 1990), providing a broad predictive capability for the kinds of mixture to which UNIQUAC can be applied.

In this work some of the mixtures considered consist of both polymeric and nonpolymeric substances. Hence a few of the theories available to describe polymer solutions will be discussed briefly. Flory(1942) and Huggins (1942) formulated some basic principles for athermal polymer mixtures (athermal mixtures are those whose components can be mixed with zero enthalpy of mixing). The details of this model are presented in Chapter 4.

An extension of Wilson's equation has been proposed by Heil and Prausnitz (1966) for describing thermodynamic properties of strongly nonideal polymer solutions. It makes use of the local volume fraction concept of Wilson and contains two adjustable parameters per binary pair. It can readily be extended to multicomponent systems. However, it has only been used to describe the vapour-liquid phase behaviour of systems containing polymers.

Various models have been proposed for mixtures containing electrolytes. These are reviewed by Zemaitis et al. (1986) and Renon (1986). These models generally combine the electrostatic theory of Debye and Hückel with modifications of well known methods for nonelectrolyte systems.

Pitzer and Mayorga (1973) proposed a successful model based on the combination of an extended Debye Hückel form (which accounts for long range forces) with the virial expansion (which accounts for short range forces). Later Cruz and Renon (1978), Chen et al.(1982) and Sander et al.(1984) replaced the virial expansion with various local composition models.

In the model proposed by Cruz and Renon(1978), the excess Gibbs free energy is assumed to be the sum of the Debye Hückel term, the Born contribution arising from the composition dependence of the dielectric constant of the mixture and a local composition term obtained using the NRTL equation.

In the model proposed by Chen et al.(1982, 1986) the excess Gibbs free energy is assumed to be a sum of the Debye Hückel term and the NRTL term. However, in Chen's model two assumptions are made; "like ion repulsion" and "local electroneutrality". The "like ion repulsion" assumption states that the local composition of cations around cations and of anions around anions is zero. This implies that repulsive forces between ions of like charge are extremely high. The local electroneutrality assumption states that the distribution of cations and anions around a central molecular species is such that the net local ionic charge is zero.

Chen's model can be used to calculate activity coefficients of components in aqueous electrolyte systems as well as mixed solvent electrolyte systems over the entire range of electrolyte concentrations. The model calculates the activity coefficients of ionic species and molecular species in aqueous electrolyte systems as well as in mixed solvent electrolyte systems. When the electrolyte

concentrations become zero the model reduces to the Renon NRTL model.

Some of the applications of Chen's electrolyte model are shown in Figure 3.1 and Figure 3.2. These Figures have been recalculated as a part of this thesis research in order to confirm results reported by Chen and his coworkers. Figure 3.1 presents the mean ionic activity coefficients of various electrolytes in water. The parameters used for this system are given in Table 3.1 and are taken from Chen et al. (1982). The Figure shows that Chen's model for electrolytes can be used to calculate mean ionic activity coefficients of 1-1, 3-1, 1-2, 2-2, and 3-2 electrolyte systems. As shown by Chen et al. (1982), the model provides an accurate correlation of data.

In Figure 3.2, calculated liquid-liquid phase behaviour is compared with the experimental liquid-liquid data for acetonitrile - water - potassium acetate system at 303 K. Parameters used for this system are reported in Table 3.2 and are taken from Mock et al. (1986). The experimental data used in this figure are of Renard and Heichelheim (1967). The calculated results are seen to be in good agreement with the experimental data. The Figure demonstrates that Chen's model gives good predictions for the salt effects on liquid-liquid phase behaviour.

Details of this model are presented in the next chapter.

Table 3.1

Binary Interaction Parameters for the Salt-Water Systems used in Figure 3.1 with $\alpha = 0.2$; Chen et al. (1982)

Aqueous Solutions of Salts at 25°C	$\tau_{salt,water}$	$\tau_{water,salt}$
NaCl	-4.5916	9.0234
$PrCl_3$	-5.779	9.420
Na_2SO_4	-4.539	8.389
UO_2SO_4	-6.764	11.201
$Al_2(SO_4)_3$	-7.116	10.646

Table 3.2

Binary Interaction Parameters for the Acetonitrile (m1) - Water (m2) - Potassium Acetate (ca) System, Mock et al. (1986)

$\tau_{m1,m2}^{(1)}$	$\tau_{m2,m1}$	$\tau_{ca,m1}^{(2)}$	$\tau_{m1,ca}$	$\tau_{ca,m2}^{(3)}$	$\tau_{ca,m2}$
1.2871	1.3840	-10.628	32.081	-4.868	9.769

with

(1) $\alpha = 0.3$; (2) $\alpha = 0.05$; (3) $\alpha = 0.2$

Table 3.3

Experimental Tie Line Data for the Acetonitrile - Water - Potassium Acetate System at 25°C [Converted to mole fraction from Tables in Renard and Heichelheim (1967)]

Salt Rich Phase			Acetonitrile Rich Phase		
KAc	Water	Acetonitrile	Water	KAc	Acetonitrile
0.2860	0.664	0.0500	0.9725	0.0270	5.0e-04
0.2370	0.712	0.0510	0.9480	0.0510	1.0e-03
0.1650	0.780	0.0550	0.9166	0.0830	4.0e-04
0.0995	0.838	0.0625	0.8470	0.1525	7.5e-04
0.0633	0.861	0.0757	0.7824	0.2165	1.1e-03
0.0250	0.850	0.1250	0.6194	0.3792	1.4e-03

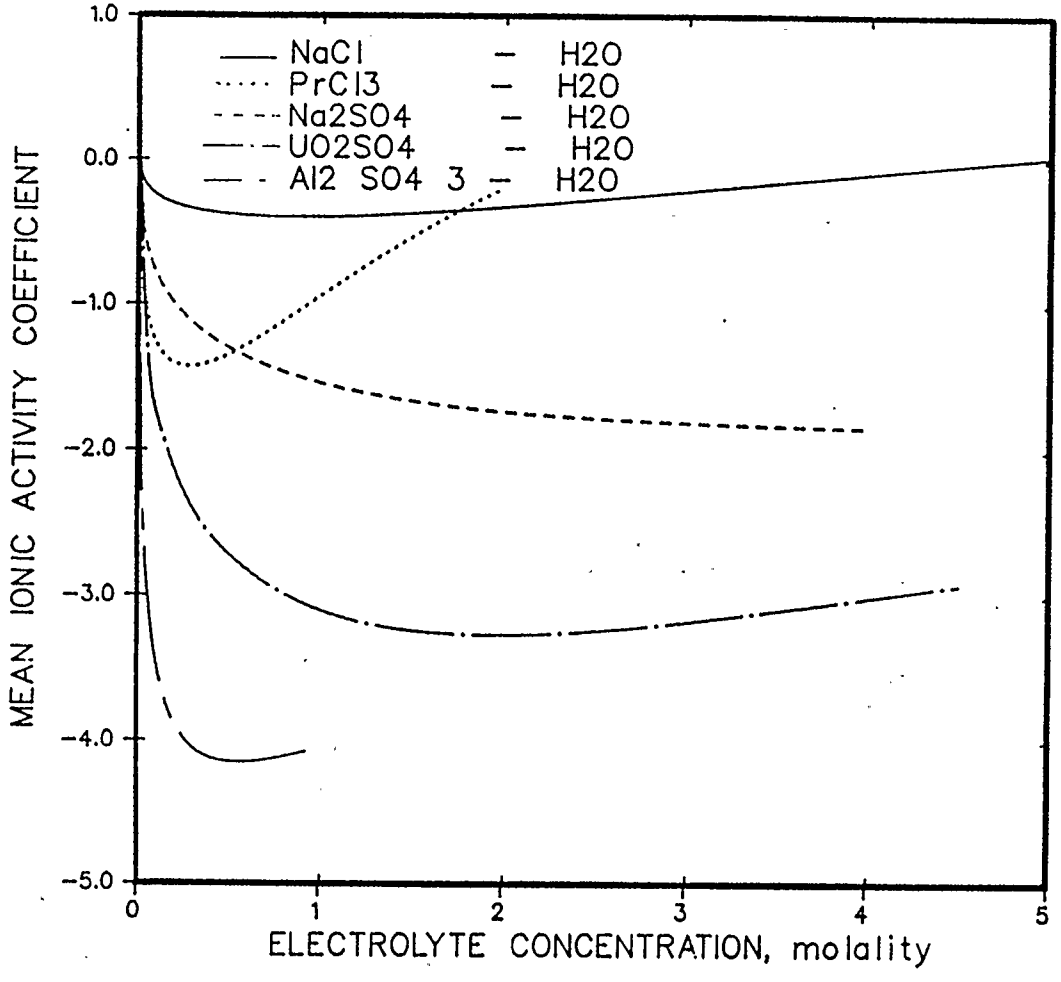

Figure 3.1 : Mean Ionic Activity Coefficients of Various Electrolytes at 298.15

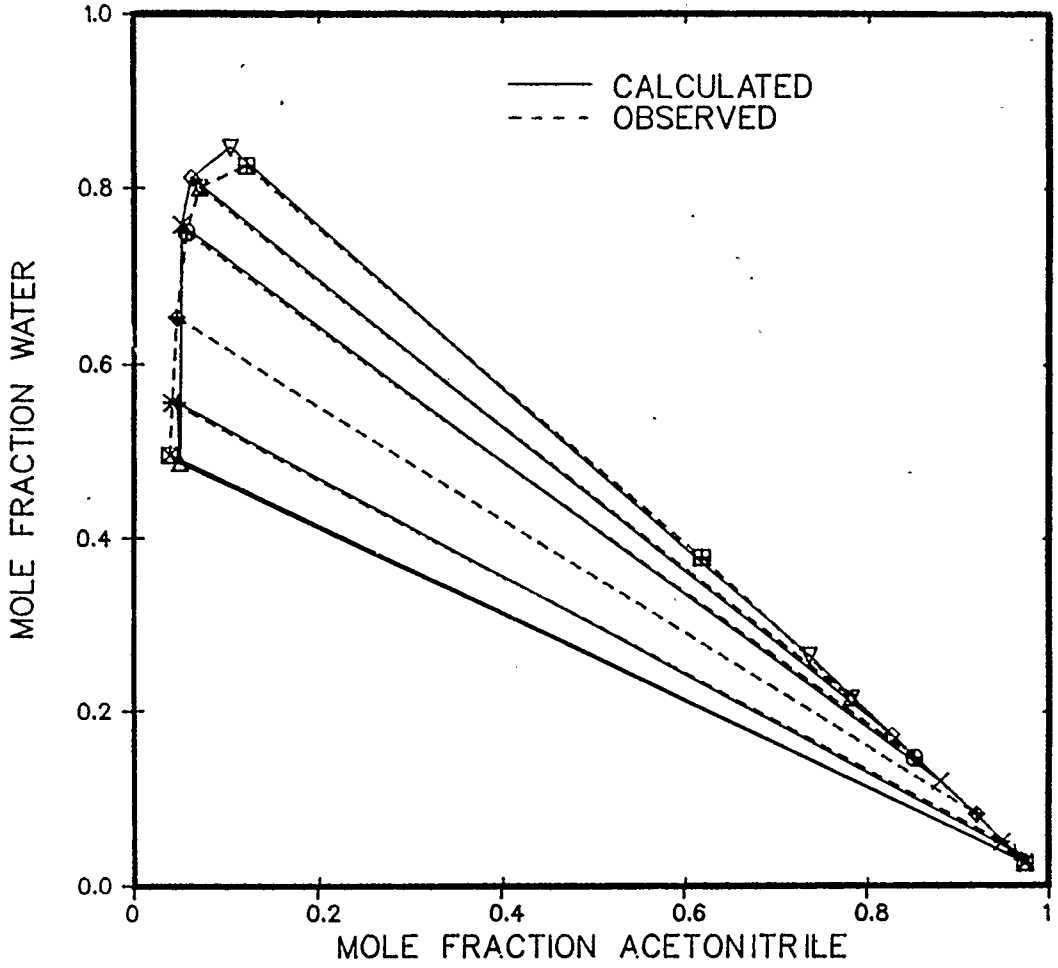

Figure 3.2 : Liquid – Liquid Equilibrium Calculations for Acetonitrile – KAc –Water System at 303 K

CHAPTER 4

MODEL DEVELOPMENT

As pointed out by Forciniti et al. (1991), when aqueous solutions of incompatible polymers such as polyethylene glycol (PEG) and dextran (Dx) are mixed above critical concentrations, a liquid-liquid phase separation occurs. Proteins added to the resulting two phase mixture generally tend to partition unequally between the phases. Thus proteins can be extracted from dilute aqueous solutions using liquid-liquid separation. This technique is gentle enough for the fragile protein molecules and is thus preferred for the isolation of proteins.

In this thesis an attempt has been made to assemble a comprehensive theory that can correlate the phase partitioning of proteins in aqueous two phase polymer systems in the presence of salts. The proposed model combines the excess Gibbs free energy model of Flory (1942) with the excess Gibbs free energy model proposed by Chen (Chen and Evans, 1982).

This combination of models permits inclusion explicitly of the salt concentration as a variable. In this respect it may posess advantages over the osmotic virial expansion used by King et al. (1988) in which the osmotic coefficient values must be re-correlated at each salt compositions.

The Electrolyte Non-Random, Two Liquid model proposed by Chen et al.(1982, 1986) is a versatile model for the calculation of activity coefficients. Using

only binary parameters this model can represent aqueous electrolyte systems as well as mixed solvent electrolyte systems over the entire range of electrolyte concentrations. A distinct advantage in using Chen's model is that the salt-water interaction parameters have been reported by Chen et al. (1982, 1986). These parameters needn't be regressed. This electrolyte model reduces to the NRTL model when electrolyte concentrations become zero. The electrolyte model proposed by Chen et al. (1982, 1986) has also been applied successfully to describe phase behaviour of amino acids (Chen et al.,1989) and antibiotics (Zhu et al, 1990). Chen et al. (1989) successfully represented the liquid - solid equilibrium behaviour of amino acids and small peptides as a function of temperature, ionic strength, solvent compositions and pH. They also reported the binary interaction parameters τ_{ij} for various water-amino acid, salt-amino acids and amino acid-amino acid pairs. Based on a similar theoretical framework, Zhu et al.(1990) represented the liquid - solid and liquid - liquid equilibrium behaviour of β-lactam antibiotics as functions of temperature, ionic strength, solvent composition and pH.

Salts are added to aqueous solutions of proteins to help buffer the solution and stabilise the proteins. Salts have a marked effect on phase partitioning of proteins. In aqueous solution proteins have a net positive or negative charge, depending on the pH of the solution. It has been observed (Albertsson, 1971; King et al., 1988) that some of the salts when added to the aqueous two phase solution of PEG and Dextran create an electric potential difference between the phases.

This electric potential causes unequal partitioning of the charged proteins. For example, phosphate and sulphate salts when added to aqueous two phase systems create 3 - 7mV potential difference between the phases. The reason for this is that the sulphate and phosphate ions have more affinity for the lower dextran rich phase than for the upper PEG rich phase (Albertsson,1971). The electric potential difference created by a salt between the phases is given by (Abbott and Hatton, 1988)

$$\Delta \psi = RT \ln(K^-/K^+) / (z^+ + z^-) \qquad (4.1)$$

where $\Delta \psi$ is the electric potential difference between the two phases, z^+ and z^- are the net charges on the cation and anion of the salt and K^-/K^+ is the ratio of anion and cation hypothetical partition coefficient between the two phases in the absence of potential difference. However, it has been observed that when KCl is added to an aqueous two phase polymer mixture of Dextran and PEG, the potassium and chloride ions have a similar affinity for the two phases. Thus KCl does not generate a measurable potential difference between the two phases.

In this work, we focus our attention on the partitioning behaviour of proteins only at the isoelectric point. Thus possible effect of potential difference on the phase partitioning of proteins is neglected. Also, both Dextran and PEG are modelled as monodisperse polymers(i.e., polymers with a uniform molar mass).

4.1 THE PROPOSED MODEL

The Flory-Huggins model was developed to account for size differences between the species. In the Chen et al. (1982, 1986) model the long range ion -ion interactions are accounted for by the Pitzer Debye Hückel model (Pitzer,1973) and the Non Random Two Liquid theory (Renon and Prausnitz, 1968) is used to represent the short range interactions between ions and molecules. The present proposal is to combine these models as follows:

$$G^{ex} / RT = G^{ex,FH} / RT + G^{ex,PDH} / RT + G^{ex,lc} / RT \qquad (4.2)$$

The above equation gives the following expression for the activity coefficient of a component i, γ_i

$$\ln \gamma_i = \ln \gamma_i^{FH} + \ln \gamma_i^{PDH} + \ln \gamma_i^{lc} \qquad (4.3)$$

4.1.1 Flory Huggins Contribution

Flory(1942) and Huggins (1942) derived an expression for the entropy of mixing ΔS_M of a long flexible chain molecule in solution from statistical geometrical considerations. According to the model, the polymer molecule consists of a number of building blocks called "segments" which are connected to one another in a

regular sequence. Proceeding from a quasi-crystalline model and assuming that each solvent molecule or segment of a polymer chain occupies only one lattice site, they showed that (Papavicza and Prausnitz, 1976)

$$\Delta S_M^{comb} = - R \Sigma n_i \ln \Phi_i \qquad (4.4)$$

where n_i is the number of moles of component i and Φ_i is the volume fraction given by

$$\Phi_i = n_i m_i / \Sigma n_j m_j \qquad (4.5)$$

In the above equation, m_i is the number of segments in a polymer molecule and is calculated as the ratio of the molar volumes of the polymer and solvent (subscript s)

$$m_i = v_i / v_s \qquad (4.6)$$

Equations (4.5) and (4.6) lead to alternate expressions for calculating the volume fraction of a component. These are

$$\Phi_i = x_i v_i / \Sigma x_j v_j \qquad (4.7)$$

and

$$\Phi_i = w_i/\rho_i \ / \Sigma w_j/\rho_j \qquad (4.8)$$

where w_i is the weight fraction of component i in the solution and ρ_i is its corresponding mass density. Since the mixture is assumed to be athermal, the Gibbs free energy of mixing is given by

$$\Delta G^M /RT = \Sigma n_i \ln \Phi_i \qquad (4.9)$$

For one mole of mixture, Equation (4.9) can be written as

$$\Delta g^M /RT = \Sigma x_i \ln \Phi_i \qquad (4.10)$$

The Gibbs free energy of mixing minus the Gibbs free energy of mixing of an ideal solution is called the excess free energy and is given by

$$\Delta g^E / RT = \Sigma x_i \ln (\Phi_i / x_i) \qquad (4.11)$$

An expression for activity coefficient can be calculated using

$$\ln \gamma_I = \left(\frac{\partial (n g^E/RT)}{\partial n_I}\right)_{T,P,n_j} \qquad (4.12)$$

In order to apply the Flory-Huggins theory to real polymer solutions, (i.e. solutions which are not athermal) it is necessary to consider the intermolecular forces occurring between the mixture's components. These enthalpic contributions

are generally introduced by van Laar type interaction terms in the Flory-Huggins model. Hence, the Flory-Huggins model for the excess gibbs free energy of mixing is given by (Kang and Sandler, 1987)

$$\frac{\Delta g^E}{RT} = \left(\sum_i \frac{\Phi_i}{m_i} \ln \Phi_i + \sum_i \sum_{j>i} \chi_{ij} \Phi_i \Phi_j \right) \sum_i x_i m_i - \sum_i x_i \ln x_i \qquad (4.13)$$

where χ_{ij} is the interaction parameters between i and j components.

The activity of each component is obtained by differentiation of the excess Gibbs energy of mixing with respect to the number of moles of component i, n_i (Equation 4.12). For real polymer mixtures, the activity coefficient of a component is given by (Kang and Sandler,1987),

$$\ln \gamma_i = \ln(\Phi_i / w_i) + 1 - m_i \sum_j \frac{\Phi_j}{m_j} + m_i \sum_j \chi_{ij} \Phi_j - m_i \sum_j \sum_{k>j} \chi_{jk} \Phi_j \Phi_k \qquad (4.14)$$

In our case, since the mixture consists of polymers, water, salts and proteins, w_i in the above equations is the actual weight fraction of components in solution.(ie w_i of all the components sum up to 1.0). Also note that for the ions and proteins, the Flory-Huggins contribution is disregarded as the Flory-Huggins expressions only take care of interactions between polymer and solvent molecules.

4.1.2 Long Range Interaction Contribution

The Debye Hückel equation proposed by Pitzer (1973, 1980) is used by Chen and coworkers (1982, 1986) to account for long range ion-ion interaction. They have also used this expression in the thermodynamic modelling to describe solid - liquid equilibrium behaviour of amino acids (Chen et al., 1989) and to represent solid-liquid and liquid-liquid equilibrium behaviour of antibiotics (Zhu et al., 1988; Zhu et al. 1990). The Pitzer Debye Hückel formula is normalised so that $\gamma_i = 1$ at mole fractions of unity for solvents and at mole fractions of zero for electrolytes.

The Pitzer Debye Hückel expression for the activity coefficient is,

$$\ln \gamma^{*PDH} = - (1000/ M_s)\ A\phi\ [(2\ Z_i / \rho) \ln (1 + \rho I_x^{1/2}) + (Z_i^2 I_x - 2 I_x^{3/2})/ (1 + \rho I_x^{1/2})] \qquad (4.15)$$

In this expression, z_i represents the absolute charge on the ionic species and I_x is the ionic strength on a mole fraction basis, and is given as

$$I_x = 1/2 \sum x_i\ Z_i^2 \qquad (4.16)$$

and M_s is the molecular weight of the solvent (which is water in our case).

ρ is the closest approach parameter of the Pitzer Debye Hückel equation, and

$A\phi$ is the Debye Hückel Parameter, given by

$$A\phi = 1/3(2 \pi N_o d)^{1/2} + (e^2 / DkT)^{3/2} \qquad (4.17)$$

N_o is the Avogadro's Number, d is the density of solvent, D is the Dielectric constant of water, T is the temperature in kelvins, k is the Boltzman's constant and x_i is the mole fraction of component i.

Chen et al. calculated the Debye Hückel parameter for aqueous electrolyte systems by the following expression

$$\begin{aligned} A\phi = & -61.44534 \exp ((T - 273.15) / 273.15) \\ & + 2.864468 (\exp((T-273.15) / 273.15))^2 \\ & + 183.5379 \ln (T / 273.15) - 0.6820223 (T - 273.15) \\ & + 0.0007875695 (T^2 - (273.15)^2) + 58.95788(273.15/T) \end{aligned} \qquad (4.18)$$

4.1.3 Short Range Interaction Contribution

The short range interaction contributions between ion and ion, molecule and molecule, and ion and molecule are accounted for by using the local composition concept. The modified form of the Non-Random Two Liquid theory was presented by Chen et al (1986).

The excess Gibbs free energy due to the short range interactions are represented by

$$\frac{\Delta g^{e,lc}}{RT} = \sum_m x_m \frac{\sum_j \tau_{jm} G_{jm} x_j}{\sum_k G_{km} x_k} + \sum_c x_c \sum_a \left(\frac{x_a}{\sum_{ka} x_{ka}}\right) \frac{\sum_j X_j G_{jc,ac} \tau_{jc,ac}}{\sum_k X_k G_{kc,ac}}$$

$$+ \sum_a x_a \sum_c \left(\frac{x_c}{\sum_{kc} x_{kc}}\right) \frac{\sum_j X_j G_{ja,ca} \tau_{ja,ca}}{\sum_k X_k G_{ka,ca}} \qquad (4.19)$$

The activity coefficients of the molecule, cation and anion are obtained by differentiation using Equations (4.12) and (4.19). These are given by Chen and Evans (1986).

The activity coefficient equation for molecular components is given by:

$$\ln\gamma_m^{lc} = \frac{\sum_j \tau_{jm} G_{jm} X_j}{\sum_k G_{km} X_k} + \sum_{km} \frac{X_{km} G_{m,km}}{\sum_k X_k G_{k,km}} \left(\tau_{m,km} - \frac{\sum_k X_k G_{k,km} \tau_{k,km}}{\sum_k X_k G_{k,km}}\right)$$

$$+ \sum_c \sum_a \frac{X_a}{\sum_{ka} X_{ka}} \frac{X_c G_{mc,ac}}{\sum_k X_k G_{kc,ac}} \left(\tau_{mc,ac} - \frac{\sum_k X_k G_{kc,ac} \tau_{kc,ac}}{\sum_k X_k G_{kc,ac}}\right)$$

$$+ \sum_a \sum_c \frac{X_c}{\sum_{kc} X_{kc}} \frac{X_a G_{ma,ca}}{\sum_k X_k G_{ka,ca}} \left(\tau_{ma,ca} - \frac{\sum_k X_k G_{ka,ca} \tau_{ka,ca}}{\sum_k X_k G_{ka,ca}}\right) \qquad (4.20)$$

The activity coefficient equation for cations is given as:

$$\frac{1}{Z_c}\ln\gamma_c^{lc} = \sum_m \frac{X_m G_{cm}}{\sum_k X_k G_{km}} \left(\tau_{cm} - \frac{\sum_k X_k G_{km} \tau_{km}}{\sum_k X_k G_{km}}\right)$$

$$+ \sum_{ka} \frac{X_{ka}}{\sum_{kaa} X_{kaa}} \frac{\sum_k X_k G_{kc,kac} \tau_{kc,kac}}{\sum_k X_k G_{kc,kac}}$$

$$+ \sum_a \sum_{kc} \frac{X_{kc}}{\sum_{kcc} X_{kcc}} \frac{X_a G_{ca,kca}}{\sum_k X_k G_{ka,kca}} \left(\tau_{ca,kca} - \frac{\sum_k X_k G_{ka,kca} \tau_{ka,kca}}{\sum_k X_k G_{ka,kca}}\right) \quad (4.21)$$

The activity coefficient equation for anions is given by:

$$\frac{1}{Z_a}\ln\gamma_a^{lc} = \sum_m \frac{X_m G_{am}}{\sum_k X_k G_{km}} \left(\tau_{am} - \frac{\sum_k X_k G_{km} \tau_{km}}{\sum_k X_k G_{km}}\right)$$

$$+ \sum_{kc} \frac{X_{kc}}{\sum_{kcc} X_{kcc}} \frac{\sum_k X_k G_{ka,kca} \tau_{ka,kca}}{\sum_k X_k G_{ka,kca}}$$

$$+ \sum_c \sum_{ka} \frac{X_{ka}}{\sum_{kaa} X_{kaa}} \frac{X_c G_{ac,kac}}{\sum_k X_k G_{kc,kac}} \left(\tau_{ac,kac} - \frac{\sum_k X_k G_{kc,kac} \tau_{kc,kac}}{\sum_k X_k G_{kc,kac}}\right) \quad (4.22)$$

Where:

X_j = Effective Liquid Phase mole fraction

= $x_j C_j$

C_j = z_j (for ions)

C_j = 1.0 (for molecules)

G_{ji} = $\exp(-\alpha_{ji} \tau_{ji})$

α_{ji} = Nonrandomness factor

τ_{ji} = $(g_{ji} - g_{ii}) / RT$

g_{ji} = Energy of interaction between j-i species

g_{ii} = Energy of interaction between i-i species

X_{ca} = Effective mole fraction of salt

X_a, X_{ka}, X_{kaa} = Effective mole fraction of anions

X_c, X_{kc}, X_{kcc} = Effective mole fraction of cations

Also:

$G_{ji,ki} = \exp(-\alpha_{ji,ki} \tau_{ji,ki})$

$\tau_{ji,ki} = (g_{ji} - g_{ki}) / RT$

$\alpha_{ji,ki}$ = Nonrandomness factor

Also, the following relationships are assumed in the model

$X_{am} = X_{cm}$ (follows from the assumption of electroneutrality) (4.23)

$G_{am} = G_{cm}$ (4.24)

$\alpha_{am} = \alpha_{cm} = \alpha_{ca,m}$ (4.25)

$\alpha_{mc,ac} = \alpha_{ma,ca} = \alpha_{m,ca}$ (4.26)

It may be inferred from the above equations

$$\tau_{am} = \tau_{cm} = \tau_{ca,m} \quad (4.27)$$

$$\tau_{mc,ac} = \tau_{ma,ca} = \tau_{m,ca} \quad (4.28)$$

The variable $\tau_{ma,ca}$ and $\tau_{mc,ac}$ can be computed from τ_{im}

$$\tau_{ma,ca} = (g_{ma} - g_{ca}) / RT$$

$$= (g_{ma} - g_{mm}) / RT + (g_{mm} - g_{ca}) / RT$$

$$= \tau_{am} - \tau_{ca,m} + \tau_{m,ca} \quad (4.29)$$

Similarly

$$\tau_{mc,ac} = \tau_{cm} - \tau_{ca,m} + \tau_{m,ca} \quad (4.30)$$

CHAPTER 5

EXPERIMENTAL DATA USED

Some of the variables which affect phase partitioning of proteins are ionic composition, pH, size of protein molecule and also the molecular weight of the two polymers and their concentrations (Albertsson,1971). In addition, the electric potential difference between the phases caused by the addition of certain buffer ions also affects the phase partitioning. The effects of these variables have been studied by various workers (Albertsson,1971 ; Brooks,1984; Johansson et al. 1974).

King et al. (1988) conducted a systematic study and presented data for the phase partitioning of three proteins in dextran-PEG-water systems in the presence of certain buffer ions. The proteins they studied are bovine serum albumin, α-chymotrypsin and lysozyme. We have used their experimental data to regress our model parameters.

Table 5.1 reports the molecular weights of polymers and proteins which were used in the King et al. (1988) study. The experimental phase compositions for PEG 8000/dextran T-500/H_2O and PEG 3350/dextran T-70/H_2O are given in Tables 5.2 and 5.3. The measured partition coefficients of proteins (albumin, α-chymotrypsin and lysozyme) are given by King et al. (1988) as a function of tie line length. These experimental data points have been reported in Tables 5.4 - 5.15

It has been conventional to refer to "tie line length" as the independent variable in two-phase polymer-polymer aqueous systems. Let w_1 and w_2 be the weight percents of the two polymers in one equilibrium phase and w'_1 and w'_2 be the polymer weight percents in the second phase. The "tie-line length" for this equilibrium is

$$L = [(w_1 - w'_1)^2 + (w_2 - w'_2)^2]^{1/2} \qquad (5.1)$$

In the polymer-polymer-water ternary system, as in other ternary systems, a given tie line can be reached in an overall mixture made from the two equilibrium phases mixed in any ratio.

When the weight percent of two polymers together is greater than about 20%, away from the plait point in these polymer-polymer-water systems, the two phases are virtually dextran and water (with no polyethylene glycol) and polyethylene glycol and water (with virtually no dextran). The essentially complete exclusion of one polymer by the other at these conditions can be seen in Tables 5.2 and 5.3.

The experimental setup and techniques used by King et al.(1988) are discussed below:

Experimental

Materials

Polymers:	PEG 8000, PEG 3350 (PEGs were purchased from Union Carbide Corporation)
	dextran T-70, dextran T-500 (Dextrans were purchased from Pharmacia Inc.)
Proteins:	bovine serum albumin
	α-chymotrypsin
	lysozyme
Salts:	potassium chloride
	potassium phosphate
	potassium sulphate

Phase Diagram Measurements:

Two phase systems were formed by dissolving PEG and dextran in water. The phases were then allowed to separate and equilibrate in a constant temperature bath. To measure PEG and dextran compositions in ternary polymer(2)-polymer(3)-water and polymer(2)-polymer(3)-buffer systems Size Exclusion High Performance Liquid Chromatography was used. SEC is a variation of chromatography which separates macromolecules by size.

Protein partition coefficient measurements:

Protein partition coefficients were determined by ultraviolet spectrophotometry. These partition coefficients were measured in aqueous and in salt solutions containing either KCl, KH_2PO_4 or K_2SO_4 in concentrations of 50 or 100mM. The protein partition coefficient was assumed to be independent of its own concentration by King et al., 1988. The amounts of protein added by King et al. for determining protein partition coefficients were approximately 0.5, 1.5 and 2.5 mg/mL for lysozyme, chymotrypsin and albumin respectively. They chose these protein concentrations to assure a measurable UV absorbance and economical use of protein. They also assumed that the polymer concentrations were unchanged by the dilute concentrations of added solutes.

It should also be noted that the pH's measured for these aqueous salt polymer solutions were approximately 5.5 for 50-mM KH_2PO_4 and 7.5 for both 50-mM KCl and 50- and 100-mM K_2SO_4.

Polymer-Polymer-Salt-Water System:

For polymer-polymer-salt-water systems, King et al.(1988) have presented some data points indicating the effect of salt on the location of the binodal curves in their polymer-polymer-water systems. However, they mention that " *At the concentrations studied here, the effect is negligible; however at higher salt concentrations (>0.1 M), phosphates and sulphates have been shown to reduce polymer concentrations at the critical point*".

Table 5.1

Molecular Weights of Polymers and Proteins

COMPONENT	Mn*	Mw*
PEG 3350	3,690	3,860
PEG 8000	8,920	11,800
Dextran T-70	37,000	74,540
Dextran T-500	167,000	509,000
Lysozyme		18,350
α-chymotrypsin		28,500
Albumin		90,000

Mn* : Number average molecular weight

Mw* : Weight average molecular weight

Table 5.2

Phase - Diagram Data for PEG 8000 / Dextran T-500 / Water at 25°C

(King et al.,1988)

Total Wt%			Top Wt%			Bottom Wt%		
Dextran	PEG	H_2O	Dextran	PEG	H_2O	Dextran	PEG	H_2O
4.90	3.44	91.66	1.63	4.98	93.39	6.85	2.33	90.82
4.94	4.10	90.96	0.68	5.91	93.41	10.31	1.60	88.09
4.99	4.30	90.71	0.34	6.68	92.98	11.75	1.70	86.55
5.93	4.49	89.58	0.18	7.23	92.59	13.67	1.42	85.91
5.00	5.70	89.30	0.10	7.90	92.00	15.00	1.10	83.90
7.02	5.07	87.91	0.07	8.24	91.69	16.30	1.00	83.70
7.39	5.86	86.75	0.03	9.29	90.68	17.57	0.61	82.88
8.32	7.16	84.52	0.01	11.14	88.85	23.05	0.58	76.37

Table 5.3

Phase - Diagram Data for PEG 3350 / Dextran T-70 / Water at 25°C

(King et al.,1988)

Total Wt%			Top Wt%			Bottom Wt%		
Dextran	PEG	H_2O	Dextran	PEG	H_2O	Dextran	PEG	H_2O
6.47	7.22	86.31	4.70	7.87	87.43	11.25	5.14	83.61
6.47	7.22	86.31	3.63	8.69	87.68	13.73	3.39	82.88
6.57	7.64	85.79	1.47	10.23	88.30	15.56	2.85	81.59
5.58	9.15	85.27	0.59	11.42	87.99	19.50	2.01	78.49
8.19	8.70	83.11	0.79	12.40	86.81	21.32	2.25	76.43
8.21	9.76	82.03	0.48	13.73	85.79	24.19	2.14	73.67
9.78	11.39	78.83	0.19	16.22	83.59	29.44	1.37	69.19

Table 5.4

Experimental Protein Partition Coefficient vs. Tie Line Length for Lysozyme PEG 3350 / Dextran T-70 / KCl Water System (King et al.,1988)

Tie Line Length	Partition Coefficient
20.20	1.70
22.92	2.20
26.66	2.22
32.90	2.70

Curve fit : ln K_p = 0.0309 * (Tie Line Length)

* The overall KCl concentration is 50mM.

Table 5.5

Experimental Protein Partition Coefficient vs. Tie Line Length for Lysozyme PEG 3350 / Dextran T-70 / KH_2PO_4 Water System (King et al.,1988)

Tie Line Length	Partition Coefficient
15.00	0.950
20.83	0.930
22.92	0.925
26.67	0.900
32.67	0.850

Curve fit : $\ln K_p = -0.00295 *$ (Tie Line Length)

* **The overall KH_2PO_4 concentration is 50mM.**

Table 5.6

Experimental Protein Partition Coefficient vs. Tie Line Length for Albumin PEG 3350 / Dextran T-70 / KCl Water System (King et al.,1988)

Tie Line Length	Partition Coefficient
21.0	0.08
23.0	0.06
26.5	0.015

Curve fit : ln K_p = -0.1370 * (Tie Line Length)

* **The overall KCl concentration is 50mM.**

Table 5.7

Experimental Protein Partition Coefficient vs Tie Line length for Albumin in PEG 3350 / Dextran T-70 / KH_2PO_4 Water System (King et al.,1988)

Tie Line Length	Partition Coefficient
15.0	0.26
21.0	0.1
23.0	0.06

Curve fit : ln K_p = -0.1115 * (Tie Line Length)

* **The overall KH_2PO_4 concentration is 50mM.**

Table 5.8

Experimental Protein Partition Coefficient vs Tie Line length for Albumin in PEG 3350 / Dextran T-70 / K_2SO_4 Water System (King et al.,1988)

Tie Line Length	Partition Coefficient
15.75	0.33
21.0	0.20
22.875	0.09
22.875	0.09
26.0	0.09
33.0	0.02

Curve fit : $\ln K_p = -0.0997 * (\text{Tie Line Length})$

* **The overall K_2SO_4 concentration is 50mM.**

Table 5.9

Experimental Protein Partition Coefficient vs. Tie Line Length for Albumin PEG 8000 / Dextran T-500 / KCl Water System (King et al.,1988)

Tie Line Length	Partition Coefficient
12.50	0.290
17.50	0.125
18.00	0.124
19.50	0.075

Curve fit : ln K_p = -0.1200 * (Tie Line Length)

* **The overall KCl concentration is 50mM.**

Table 5.10

Experimental Protein Partition Coefficient vs. Tie Line Length for Albumin PEG 8000 / Dextran T-500 / KH_2PO_4 Water System (King et al.,1988)

Tie Line Length	Partition Coefficient
12.5	0.390
18.0	0.175
17.8	0.125
20.0	0.088

Curve fit : ln K_p = -0.1077 * (Tie Line Length)

* **The overall KH_2PO_4 concentration is 50mM.**

Table 5.11

Experimental Protein Partition Coefficient vs. Tie Line Length for Albumin PEG 8000 / Dextran T-500 / K_2SO_4 Water System (King et al.,1988)

Tie Line Length	Partition Coefficient
12.5	0.48
18.0	0.27
18.0	0.35
18.5	0.20
18.5	0.32

Curve fit : $\ln K_p = -0.0730 * (\text{Tie Line Length})$

* **The overall K_2SO_4 concentration is 50mM.**

Table 5.12

Experimental Protein Partition Coefficient vs. Tie Line Length for α-Chymotrypsin PEG 3350 / Dextran T-70 / KCl Water System (King et al.,1988)

Tie Line Length	Partition Coefficient
20.5	1.375
22.86	1.75
27.14	1.625

Curve fit : ln K_p = -0.0193 * (Tie Line Length)

* **The overall KCl concentration is 50mM.**

Table 5.13

Experimental Protein Partition Coefficient vs. Tie Line Length for α-Chymotrypsin PEG 3350 / Dextran T-70 / KH_2PO_4 Water System (King et al.,1988)

Tie Line Length	Partition Coefficient
6.9	0.88
15.1	0.80
20.1	0.68
23.0	0.63
27.0	0.52
32.5	0.40

Curve fit : $\ln K_p = -0.0245 *$ (Tie Line Length)

* **The overall KH_2PO_4 concentration is 50mM.**

Table 5.14

Experimental Protein Partition Coefficient vs. Tie Line Length for α - Chymotrypsin PEG 8000 / Dextran T-500 / KCl Water System (King et al.,1988)

Tie Line Length	Partition Coefficient
12.5	1.25
17.5	1.25
18.5	1.50
20.0	1.75

Curve fit : ln K_p = (Tie Line Length) (- 0.00583 + 0.1497 * (Tie Line Length))

* **The overall KCl concentration is 50mM.**

Table 5.15

Experimental Protein Partition Coefficient vs. Tie Line Length for α - Chymotrypsin PEG 8000 / Dextran T-500 / KH_2PO_4 Water System (King et al.,1988)

Tie Line Length	Partition Coefficient
12.5	0.7550
16.5	0.6875
18.5	0.6250
20.0	0.5625
25.0	0.4900

Curve fit : $\ln K_p = -0.0239 * (\text{Tie Line Length})$

* **The overall KH_2PO_4 concentration is 50mM.**

CHAPTER 6

DETERMINATION OF MODEL PARAMETERS

Interaction parameters needed in the Flory-Huggins and Chen models were regressed using the experimental data of King et al. (1988).

For each dextran, PEG, water ternary system there are potentially three adjustable Flory-Huggins parameters χ_{ij} and nine parameters for the local composition model (τ_{ij} and α_{ij}). In addition, the Flory-Huggins model requires values of polymer molar volumes. The data available are not sufficient to fit such a large number of parameters and steps were taken to fix as many as possible from a priori information.

For each of the polymer-water and polymer-polymer pairs, the NRTL parameters (τ_{ij} and α_{ij}) were all set to be zero. The non-idealities in the free energies for these pairs were reduced to the Flory-Huggins terms alone since this model is more appropriate for dealing with large molecule/small molecule interactions.

Kang and Sandler (1987) correlated data of Albertsson (1971) for PEG-dextran-water systems. They proceeded by fixing specific volumes of dextran and PEG at 0.626 and 0.832 cm^3/g, respectively. The molar volumes are found as the product of molar mass and density.

There were then three χ_{ij} parameters available to fit the ternary data of PEG 8000 - dextran T-500 - water and PEG 3350 - dextran T-70 - water systems which have been presented in Tables 5.2 and 5.3.

The Flory-Huggins parameters χ_{ij} were estimated by minimising the objective function (Kang and Sandler, 1987),

$$O.F. = \sum_{k=1}^{Npoints} \sum_{j=1}^{2} \sum_{i=1}^{3} \delta_k \left(\frac{w_{ijk} - w'_{ijk}}{w_{ijk} + w'_{ijk}} \right)^2 \qquad (6.1)$$

where δ_k is the weight of each data point, w'_{ijk} is the measured weight fraction of the ith component in the jth phase and for the kth data point and w_{ijk} is the calculated weight fraction. The three points closest to the plait point in each of the Tables 5.2 and 5.3 were given weight $\delta_k = 3$. The next two were given $\delta_k = 2$ and the rest were weighted $\delta_k = 1$. The objective function was minimised using Powell's algorithm. The algorithm was adapted from Himmelblau (1972). In the minimisation procedure, each of the overall mixtures of Tables 5.2 and 5.3 was flashed with a given set of parameters. The procedure was assumed to be converged when values of χ_{ij} changed by less than 0.00001 in successive iterations, as was suggested by Himmelblau (1972).

The converged values for the χ_{ij} for the PEG 3350 / dextran T-70 / water system and the PEG 8000 / dextran T-500 / water system are reported in Table 6.2.

For the mixture of water, PEG, dextran, salt and protein only three Flory Huggins parameters are considered ($\chi_{water\text{-}PEG}$, $\chi_{water\text{-}dextran}$ and $\chi_{dextran\text{-}PEG}$) and others were set to be zero since the Chen model was expected to correlate other effects.

The τ's for the water-salt pair are taken from Chen and Evans (1982, 1986) and are reported in Table 6.1. The τ's for the salt-protein pair, KCl-polymer pair and water-protein pair were set to be zero as it was observed that the partition coefficient of protein is insensitive to these values of τ_{ij}. The partition coefficient of protein was most sensitive to the protein-polymer interactions. This is in accordance with the behaviour observed by King et al (1988), who have also utilized protein-polymer interactions in their model.

The data of King et al. (1988) that were presented in Tables 5.4 - 5.15 showing the protein partition coefficient versus tie-line length provide the basis for regressing protein-polymer interaction parameters in the Chen model. The data in these tables were read from figures in the King et al. (1988) manuscript by digitizing enlarged copies of the figures.

The data were then smoothed by curve fitting . The function used for curve fitting was $\ln K = a * L + b * L^2$; where L is the tie line length and a and b are constants. This form was chosen as the simplest expression that has the essential character of the data. Note that K = 1 at a plait point (when tie line length, L, is zero). Further, K is positive at any tie line length. In fact, for only one of the data sets (α-chymotrypsin in PEG 8000 / dextran T-500 / KCl water) was it advantageous to use a non-zero value of parameter b.

The values obtained for a and b are given at the bottom of Tables 5.4-5.15 where the partition coefficient data was presented.

There are potentially three NRTL parameters required in the Chen model for a given polymer-protein pair with a given salt. These three parameters were reduced to only one by assuming; $\tau_{ij} = \tau_{ji}$ and $\alpha_{ij} = -1$. The symmetry in τ_{ij} in the NRTL model was also assumed by Zhu et al. (1990) in dealing with biomolecules. The proposal that $\alpha_{ij} = -1$ is a suitable universal value for the NRTL model was made by Marina and Tassios (1973).

Numerical experiments with typical small positive values of $\alpha_{ij} = 0.2$, 0.3 and 0.4 failed to describe the protein partition coefficient behaviour. With a small positive α_{ij}, it was observed that even large values of τ_{ij} gave equal partitioning of the proteins.

An additional step was to assume that τ_{ij} was nonzero for a given protein with only one of the two polymers. This reduced the number of NRTL parameters to be estimated for a given system (with a specific salt) to only one.

The three proteins studied had different preferences for different phases depending on the nature of protein. Albumin preferentially goes into the dextran rich bottom phase while lysozyme and α-chymotrypsin prefer the PEG-rich top phase in the presence of KCl but they prefer the bottom dextran rich phase in the presence of other salts studied. While regressing interaction parameters it was observed that when $\tau_{Dextran\text{-}protein}$ is taken to be positive (and $\tau_{PEG\text{-}protein}$ equal to zero) the protein would preferentially go into the PEG rich phase and, likewise, if

$\tau_{PEG\text{-}protein}$ was positive and $\tau_{Dextran\text{-}protein}$ was zero, the protein would partition, preferentially into the dextran rich phase. On this basis, it was decided to set one of the polymer-protein τ_{ij} equal to zero and to regress the other.

The following objective function was used in regressing the one non-zero protein-polymer τ parameter.

$$O.F. = \sum_{k=1}^{Npoints} (\ln K_p - \ln K'_p)^2 \qquad (6.2)$$

In the above equation, K_p is the calculated equilibrium ratio. The calculation was done by flashing mixtures with the overall compositions given in Tables 5.2 and 5.3 with 50mM salt and the experimental amount of protein added. The flash calculation produced also a "tie-line length". This length was inserted into the smoothing functions for the experimental data (i.e.; $\ln K = a * L + b * L^2$) to produce a value for the "experimental" partition coefficient (K'_p in equation 6.2)

The objective function was minimised using Powell's algorithm. The same technique was used as in minimising the objective function of 6.1. Converged values of these parameters for the three different proteins are reported in Tables 6.3 - 6.7.

Large values of the protein-polymer binary interaction parameters (τ_{ij}) were required with $\alpha_{ij} = -1$ to describe the phase behaviour of proteins. This is justified as proteins are high molecular weight compounds and the aqueous solutions have

low concentration of proteins (typically 1-2 g/L). Thus large values of τ_{ij} are required.

Table 6.1

Binary Interaction parameters for Salt Water Pairs

with α=0.2; Chen et al. (1982); Chen and Evans (1986)

Aqueous Solutions of Salts at 25°C	$\tau_{w,ca}$	$\tau_{ca,w}$
KCl	8.1354	-4.1341
KH_2SO_4	9.2470	-4.9640
K_2SO_4	8.9320	-4.1160

Table 6.2

Flory-Huggins Parameters for Aqueous Polymer Systems of Various Dextrans and PEGS at 25° C

Dextran	PEG	$\chi_{Dx\text{-}PEG}$	$\chi_{Dx\text{-}water}$	$\chi_{PEG\text{-}water}$
Dx T-70	PEG 3350	0.0603	0.47	0.50
Dx T-500	PEG 8000	0.0309	0.52	0.53

Table 6.3

Binary Interaction Parameters For the Partitioning of Lysozyme and α-Chymotrypsin in Different Dextran-PEG-KCl Water Systems.

Protein	$\tau_{Dx\ T\text{-}70,protein}$	$\tau_{Dx\ T\text{-}500,protein}$	$\alpha_{Dx\ T\text{-}70,protein}$	$\alpha_{Dx\ T\text{-}500,protein}$
Lysozyme	6.223	-	-	-1.0
α - chymotrypsin	5.772	7.069	-1.0	-1.0

For the above parameters

$\tau_{12} = \tau_{21}$ and $\alpha_{12} = \alpha_{21}$

Table 6.4

Binary Interaction Parameters for the Partitioning of Lysozyme, α-Chymotrypsin in Different Dextran-PEG-KH_2PO_4 Water Systems

Protein	$\tau_{PEG\ 3350,protein}$	$\tau_{PEG\ 8000,protein}$	$\alpha_{PEG\ 3350,protein}$	$\alpha_{PEG\ 8000,protein}$
Lysozyme	2.945	-	-	-1.0
α - chymotrypsin	4.830	5.222	-1.0	-1.0

For the above parameters

$\tau_{12} = \tau_{21}$ and $\alpha_{12} = \alpha_{21}$

Table 6.5

Binary Interaction Parameters For the Partitioning of Bovine Serum Albumin in Different Dextran-PEG-KCl Water Systems.

Protein	$\tau_{PEG\ 3350,protein}$	$\tau_{PEG\ 8000,protein}$	$\alpha_{PEG\ 3350,protein}$	$\alpha_{PEG\ 8000,protein}$
Bovine Serum Albumin	5.853	6.679	-1.0	-1.0

For the above parameters

$\tau_{12} = \tau_{21}$ and $\alpha_{12} = \alpha_{21}$

Table 6.6

Binary Interaction Parameters for the Partitioning of Bovine Serum Albumin in Different Dextran-PEG-KH_2PO_4 Water Systems

Protein	$\tau_{PEG\ 3350,protein}$	$\tau_{PEG\ 8000,protein}$	$\alpha_{PEG\ 3350,protein}$	$\alpha_{PEG\ 8000,protein}$
Albumin	5.749	6.250	-1.0	-1.0

For the above parameters

$\tau_{12} = \tau_{21}$ and $\alpha_{12} = \alpha_{21}$

Table 6.7

Binary Interaction Parameters for the Partitioning of Bovine Serum Albumin in Different Dextran-PEG-K_2SO_4 Water Systems

Protein	$\tau_{PEG\ 3350,protein}$	$\tau_{PEG\ 8000,protein}$	$\alpha_{PEG\ 3350,protein}$	$\alpha_{PEG\ 8000,protein}$
Albumin	5.550	5.929	-1.0	-1.0

For the above parameters

$\tau_{12} = \tau_{21}$ and $\alpha_{12} = \alpha_{21}$

CHAPTER 7

RESULTS AND DISCUSSION

7.1 : Polymer-Polymer-Salt-Water System

Figure 7.1 shows the phase diagram (binodal) for the PEG 3350 - dextran T-70 and water system. The predicted binodal curve is calculated from the proposed model. The experimental data for this system are given in Table 5.3 and are taken from King et al. (1988). The regressed interaction parameters for this system are given in Table 6.2. The Figure shows that for this ternary system the model gives a good correlation for the binodal curve. However, the slope of the calculated tie line is somewhat different from the experimental one.

Also shown in Figure 7.1 are points on the binodal curve calculated by King et al. (1988) from their osmotic virial equation. The points were located by digitizing a curve as shown in Figure 2 of their paper. Calculated tie-lines were not shown.

Figure 7.2 similar to Figure 7.1 but for the system PEG 8000 - dextran T-500 -water. The experimental data for the binodal curve and tie lines have been presented in Table 5.2. Also shown are calculated points extracted from Figure 7 of the King et al. (1988) manuscript.

It is apparent from Figures 7.1 and 7.2 that the Flory-Huggins model is at least effective as the model used by King et al. (1988) in correlating these data.

It should be noted that the plait point in the Flory-Huggins model is always at nearly equal mole percentages of the two polymers, as was shown by Scott (1948), and is insensitive to the water-polymer χ_{ij} values. This fact accounts for the deviation of the calculated tie lines from the experimental ones. However, the King et al. (1988) model apparently does not overcome this difficulty.

Comparing Figure 7.1 and Figure 7.2 we see that, as the molecular weight of the polymer is increased, the two-phase liquid region becomes larger. It is noted that $\chi_{PEG\text{-}Dextran}$ for the PEG 8000 - dextran T-500 system is smaller than $\chi_{PEG\text{-}Dextran}$ for the PEG 3350 - Dextran T-70 and water system. This is in accordance with Scott's (1948) analysis that as the polymer molecular weight increases phase incompatibility becomes a rule rather than an exception.

Figure 7.3 shows the effect of salt (KCl) on the calculated binodal curve for the PEG 8000 - dextran T-500 - water system. The binary interaction parameters for this system are given in Table 6.2. It should be noted that binary interaction parameters for the water - KCl pair are taken from Chen and Evans (1982, 1986) and are reported in Table 6.1. This Figure has been included to illustrate that polymer concentrations in the two phases are unchanged by the addition of 50mM of salt.

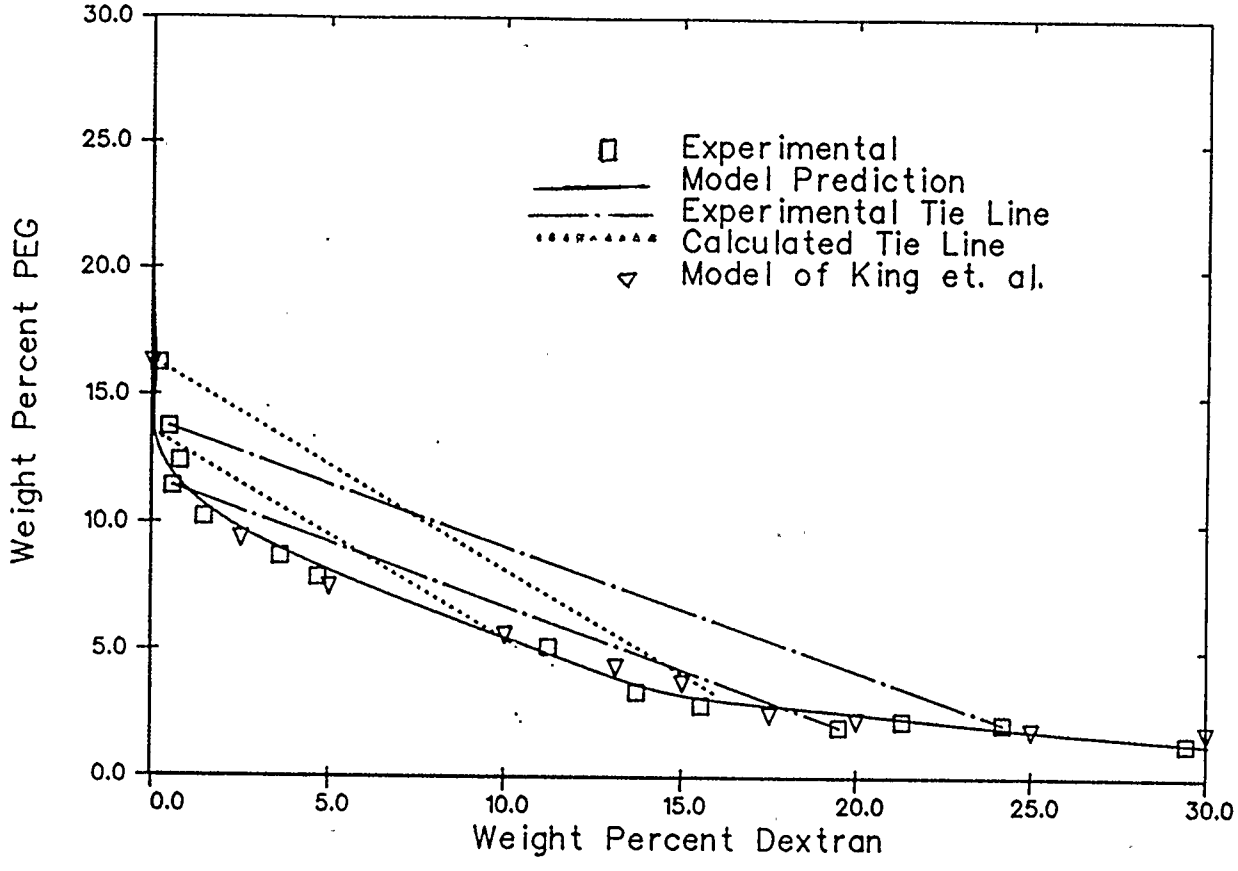

Figure 7.1: Experimental and Predicted Binodal curves for PEG 3350, Dextran T-70 and Water system

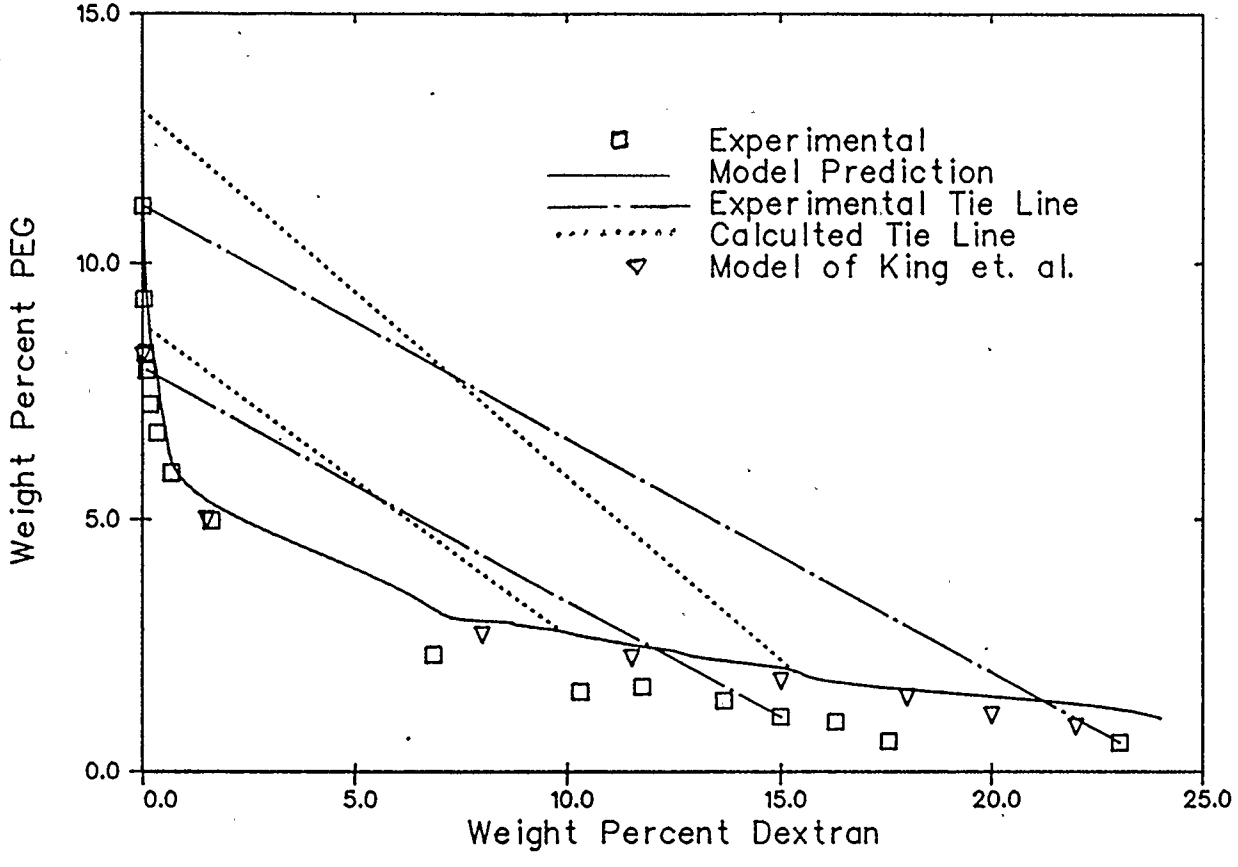

Figure 7.2 : Experimental and Predicted Binodal curves for PEG 8000, Dextran T-500 and Water System

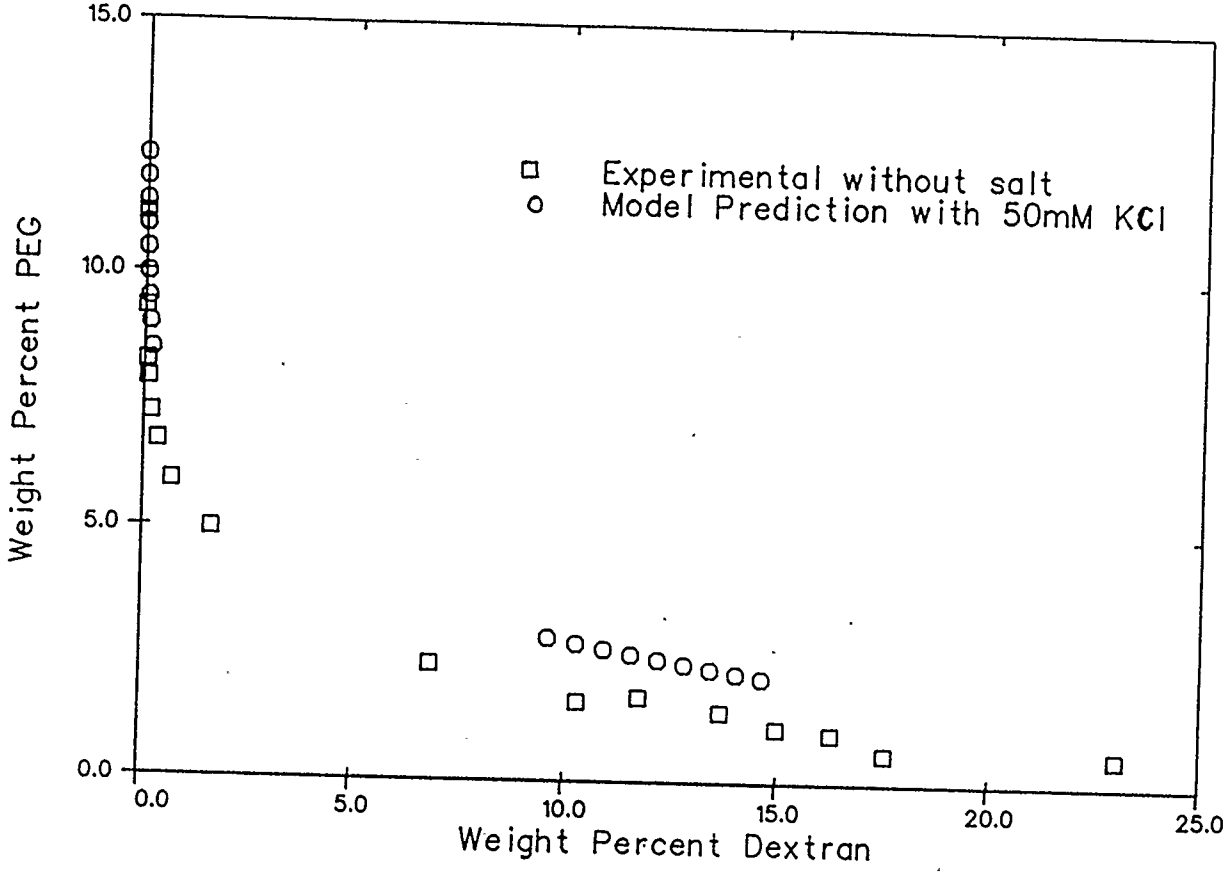

Figure 7.3 : Experimental and Predicted Binodal Curves for PEG 8000, Dextran T-500 Salt and Water system

7.2 : Protein Partitioning

Results for protein partitioning in the different aqueous two-phase salt systems are shown in Figures 7.4-7.15.

The protein partition coefficient is plotted against tie line length. Tie line length is the measure of composition difference between the two phases and is defined as:

$$\text{Tie Line Length} = [\ (D''- D')^2\ + (P''- P')^2\ ]^{1/2} \qquad (7.1)$$

where D and P are in weight percent.

The partition coefficient of protein is defined as:

$$K_p = w_{p,top} / w_{p,bottom} \qquad (7.2)$$

where $w_{p,top}$ is the weight fraction of protein in the PEG-rich top phase and $w_{p,bottom}$ is the weight fraction of protein in the dextran-rich bottom phase. King et al. (1988) have defined their K_p in terms of molality in the top and bottom phases. The partition coefficient on a weight percent basis can be calculated from the partition coefficient on a molality basis by multiplying by the ratio of water weight percents in the two phases; i.e.;

$$K_p = w_{H2O,top} / w_{H2O,bottom}\ (m_{p,top} / m_{p,bottom}) \qquad (7.3)$$

The model proposed in this study predicts the partition coefficient of water to be very near unity over the range of the experimental data. Thus the calculated K_p values on molality and weight percent bases are virtually identical.

The Figures contain data points and the curves showing the model predictions of King et al.(1988) that were extracted from the Figures contained in their manuscript.

In preparing Figures 7.4-7.15, flash calculations were performed using the proposed model with parameters shown in Tables 6.3-6.7. The calculated partition coefficient was plotted against the tie-line length resulting from these flash calculations.

The scattered nature of the King et al.(1988) data is clear in these Figures. It is also clear that some problems exist in the model results plotted in the King et al. (1988) Figures. For instance, it is not always clear that their partition coefficient reaches 1.0 at zero tie-line length. On some curves (see Figures 7.6, 7.8 and 7.10), the partition coefficient goes to zero at a finite tie-line length, which is unphysical. Also, the curve in Figure 7.13 shows an unexpected minimum. Probably these irregularities are only due to flawed plotting or perhaps to difficulty in converging calculations for very dilute solutions near a plait point. In any case, definitive comparison with the results of King et al. (1988) is impossible.

It is possible to state, however, that the present model provides a reasonable correlation of all the data sets, whether the partition coefficient is an increasing function of tie-line length (Figures 7.4, 7.12 and 7.13) or a decreasing

function of tie-line length (all other Figures).

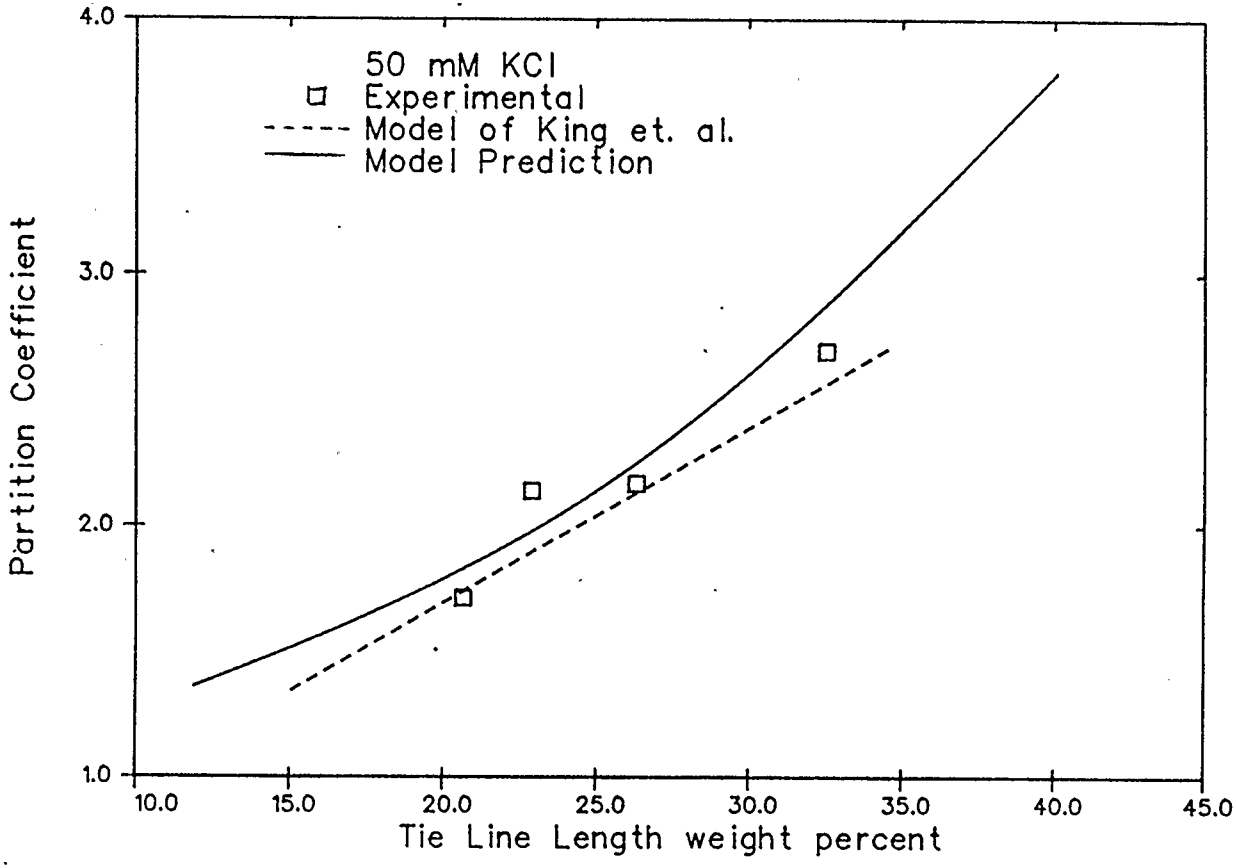

Figure 7.4 : Experimental and Predicted Partition Coefficients vs.Tie Line length for Lysozyme in PEG 3350, Dextran T-70, Salt-Water system

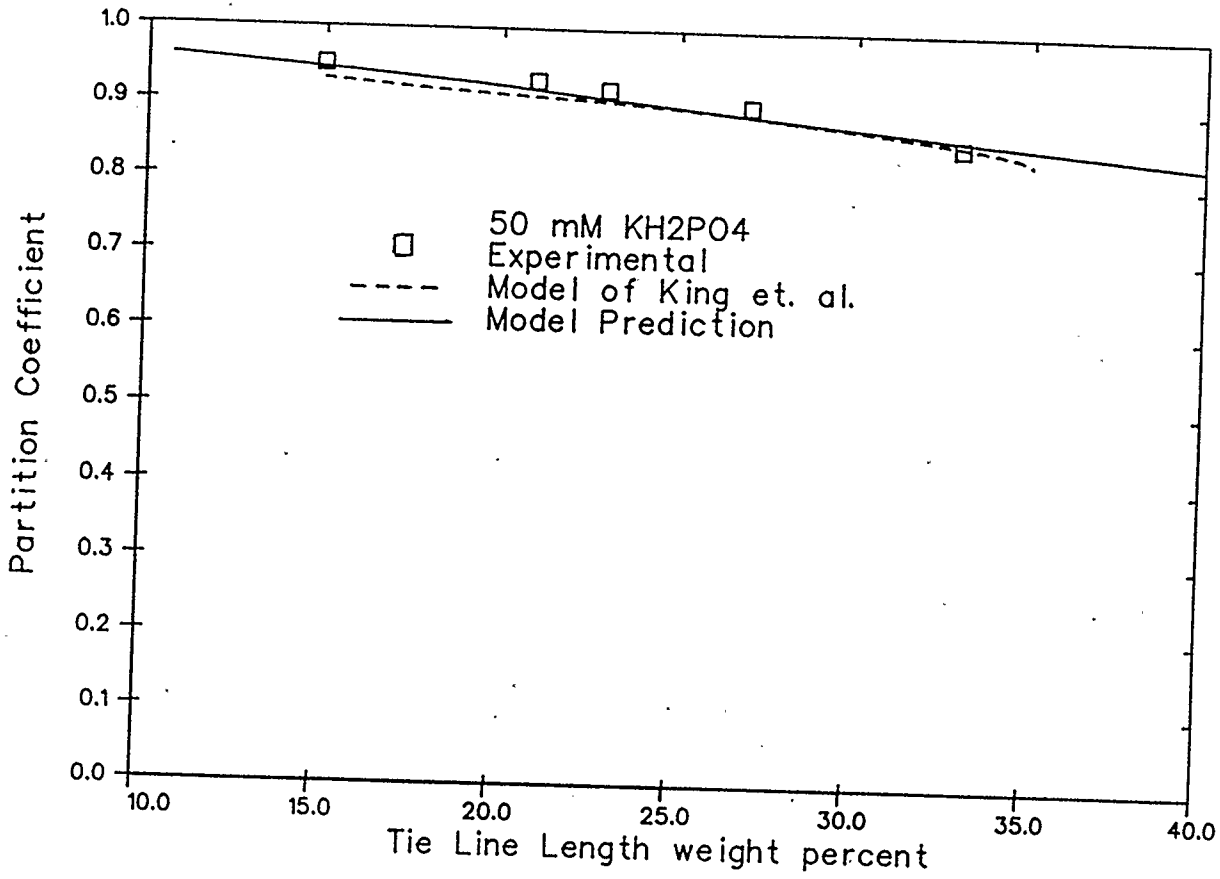

Figure 7.5 : Experimental and Predicted Partition Coefficients vs.Tie Line Length for Lysozyme in PEG 3350, Dextran T-70, Salt-Water system

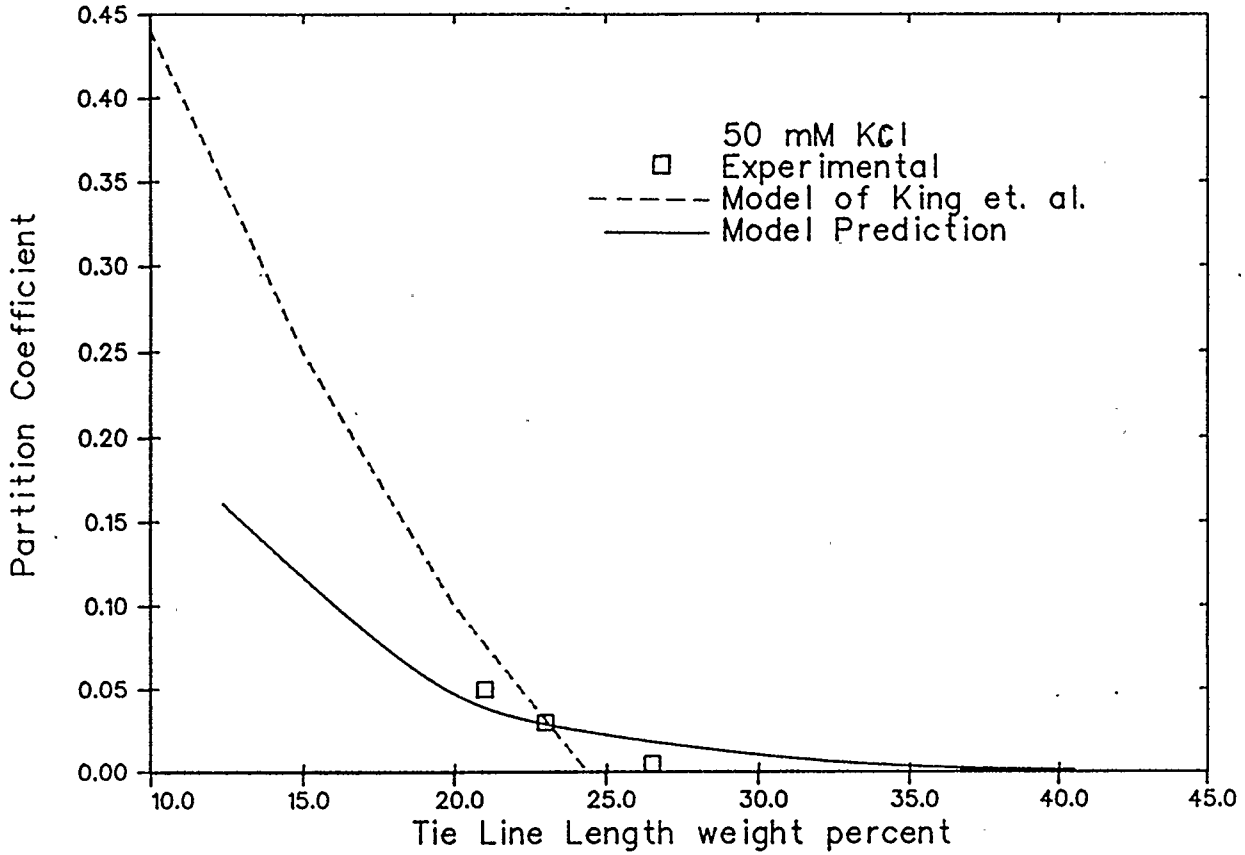

Figure 7.6 : Experimental and Predicted Partition Coefficients vs. Tie Line Length for Albumin in PEG 3350, Dextran T-70, Salt-Water system

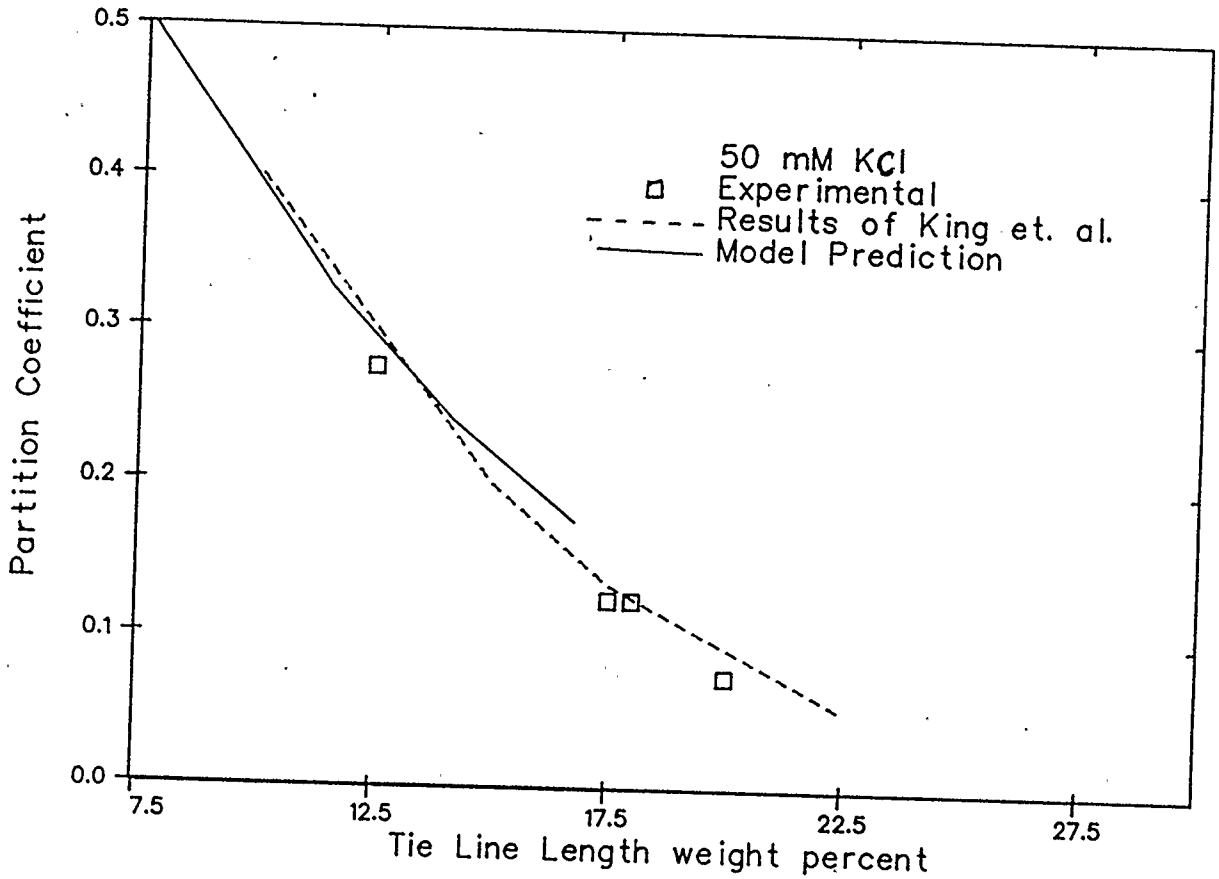

Figure 7.7 : Experimental and predicted partition coefficient vs. tie line length for Albumin in PEG 8000, Dextran T–500, Salt–Water system

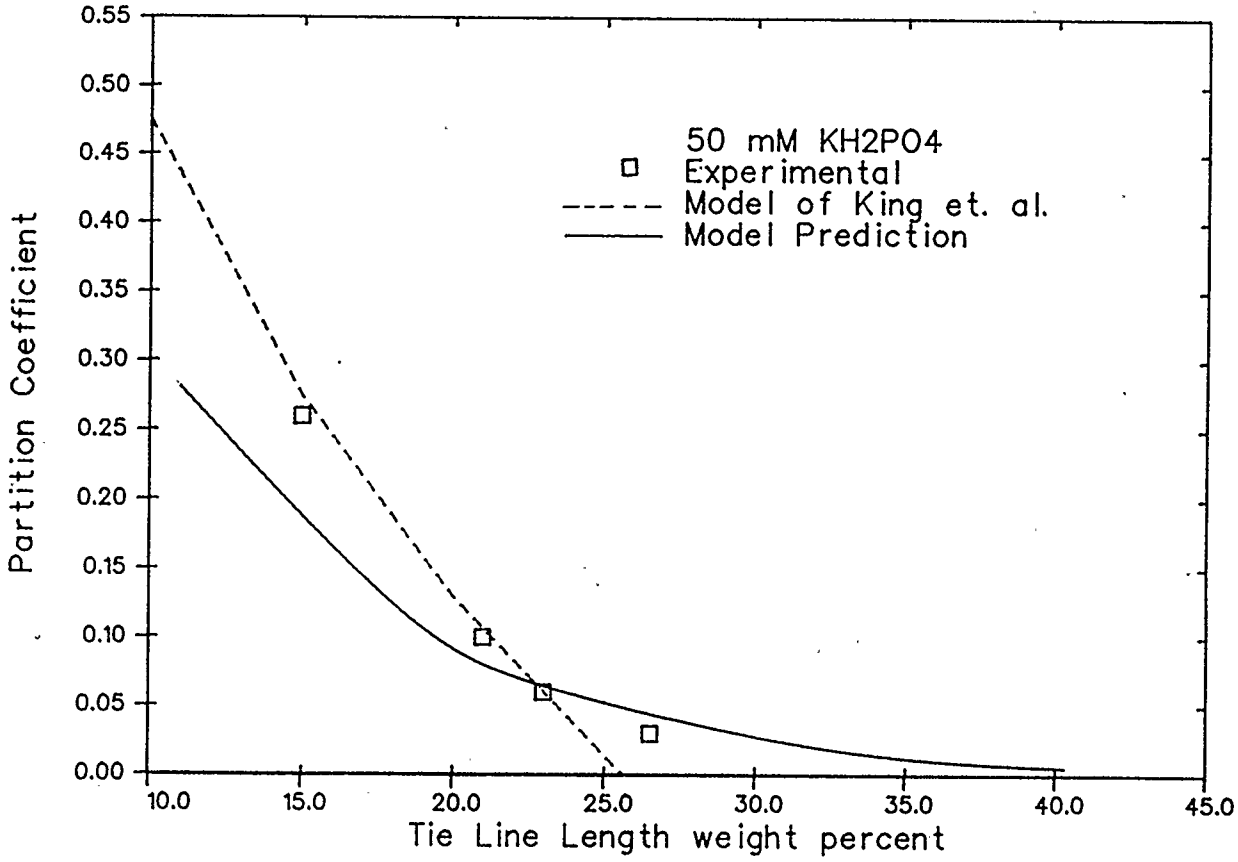

Figure 7.8 : Experimental and Predicted Partition Coefficients vs. Tie Line Length for Albumin in PEG 3350, Dextran T-70, Salt-Water system

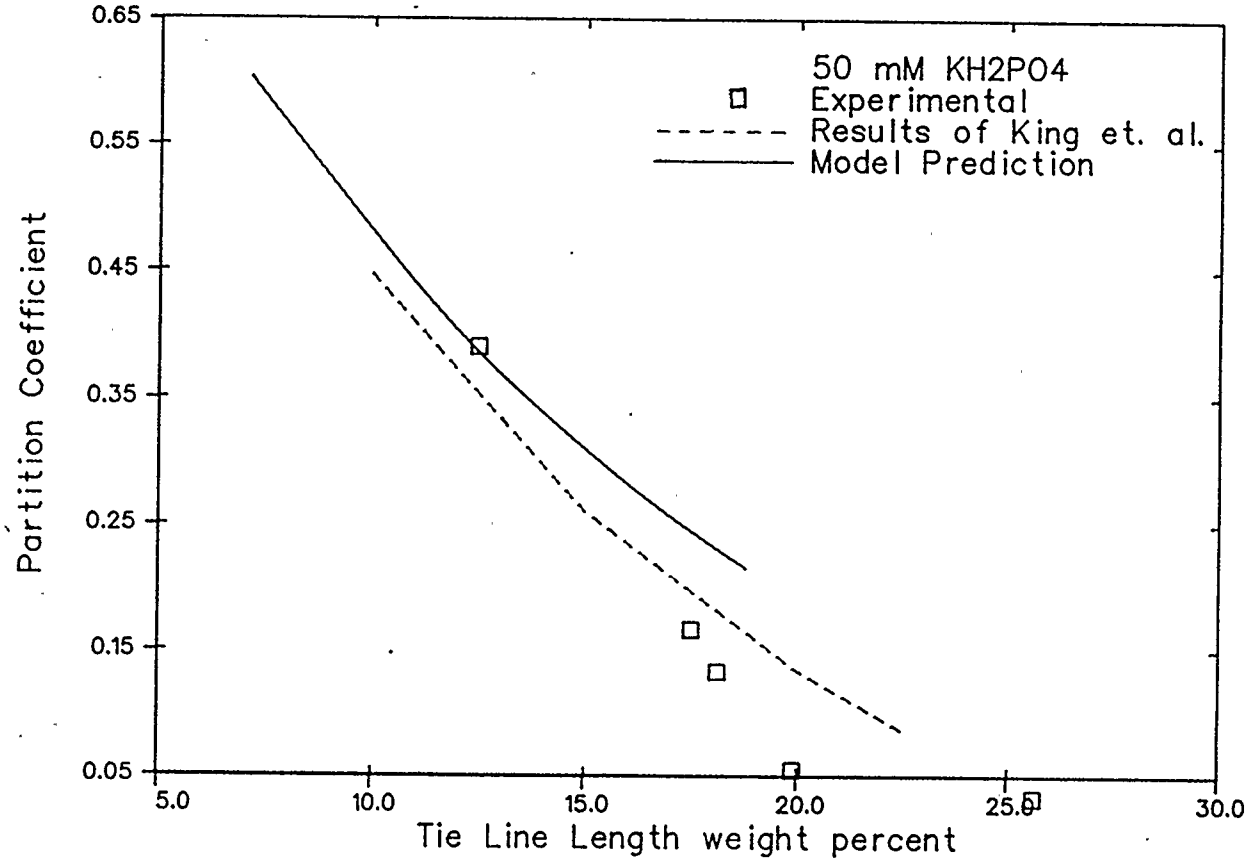

Figure 7.9 : Experimental and predicted partition coefficient vs. tie line length for Albumin in PEG 8000, Dextran T-500, Salt-Water system

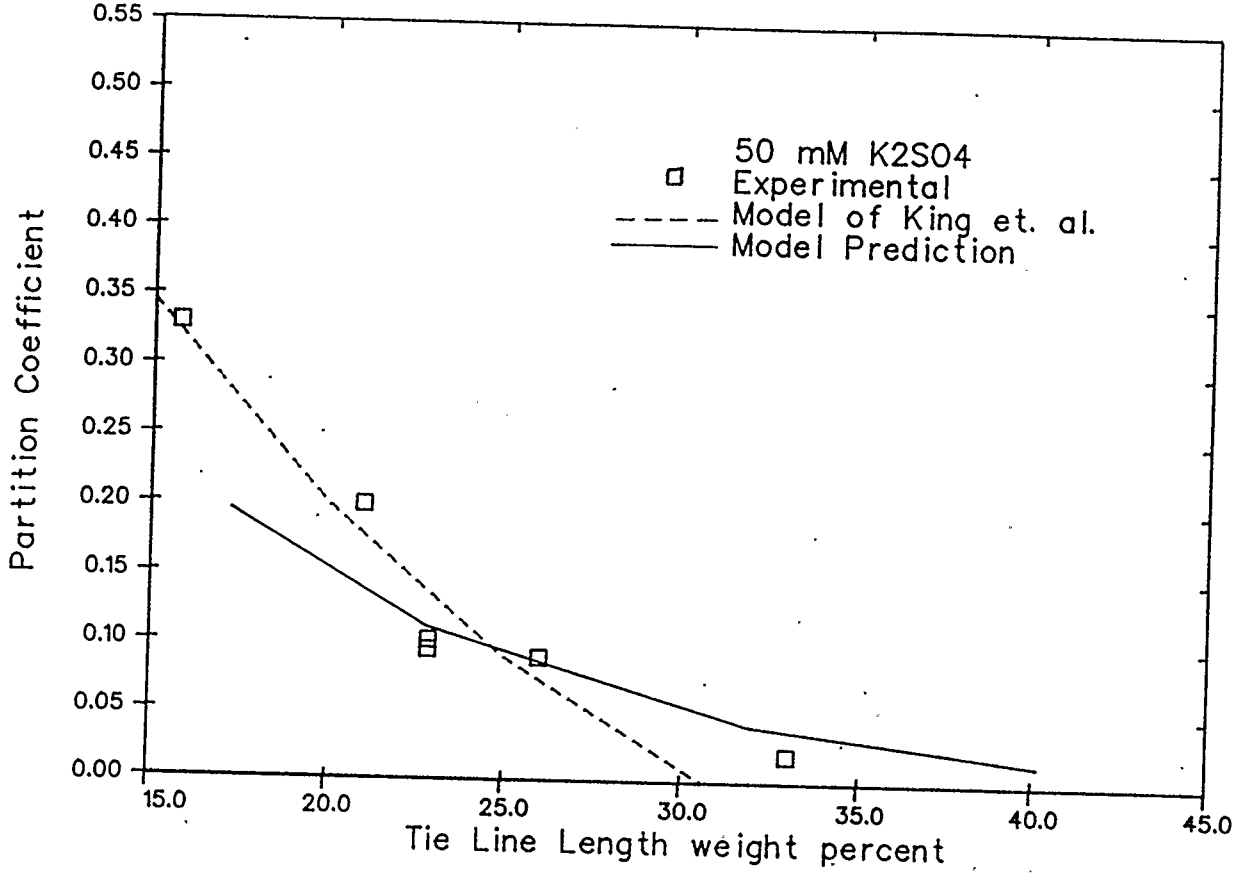

Figure 7.10 : Experimental and Predicted Partition Coefficients vs. Tie Line Length for Albumin in PEG 3350, Dextran T-70, Salt-Water system

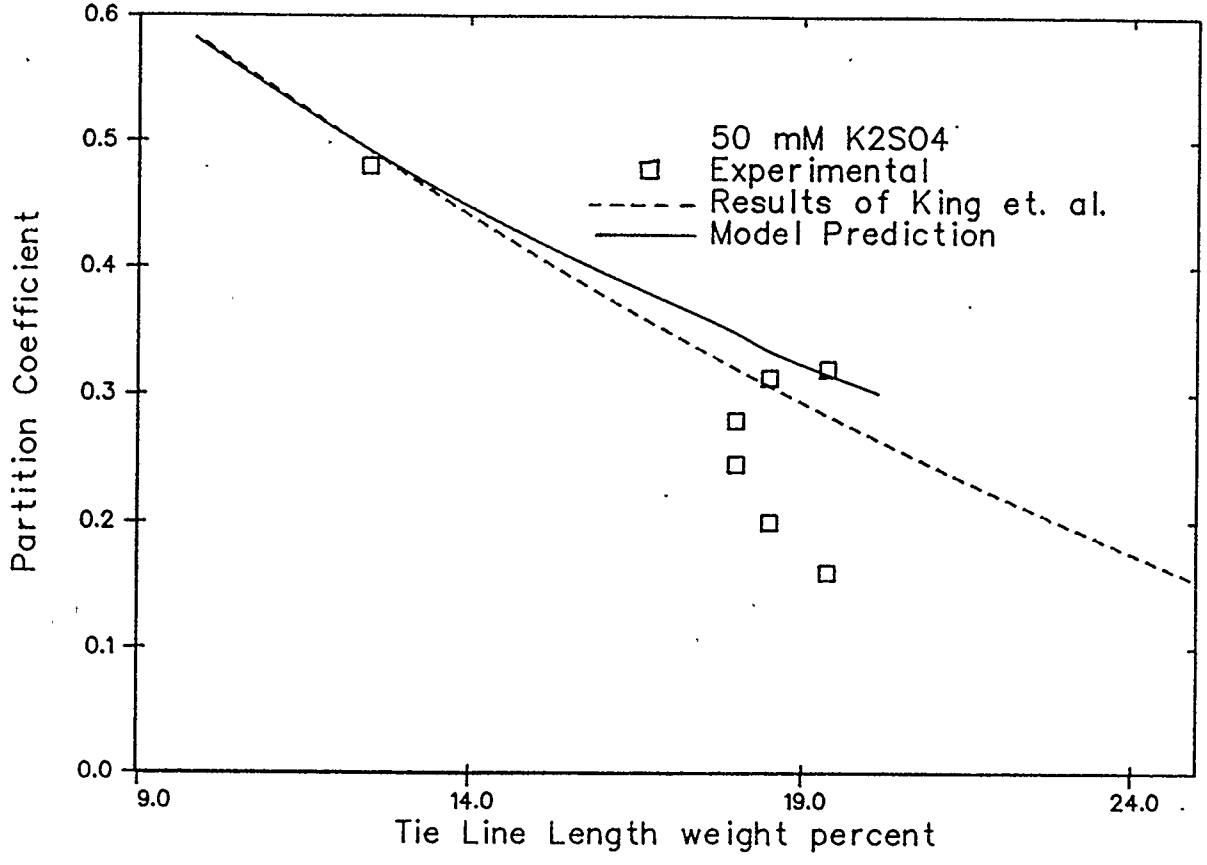

Figure 7.11: Experimental and predicted partition coefficient vs. tie line length for Albumin in Dextran T-500, Salt-Water system

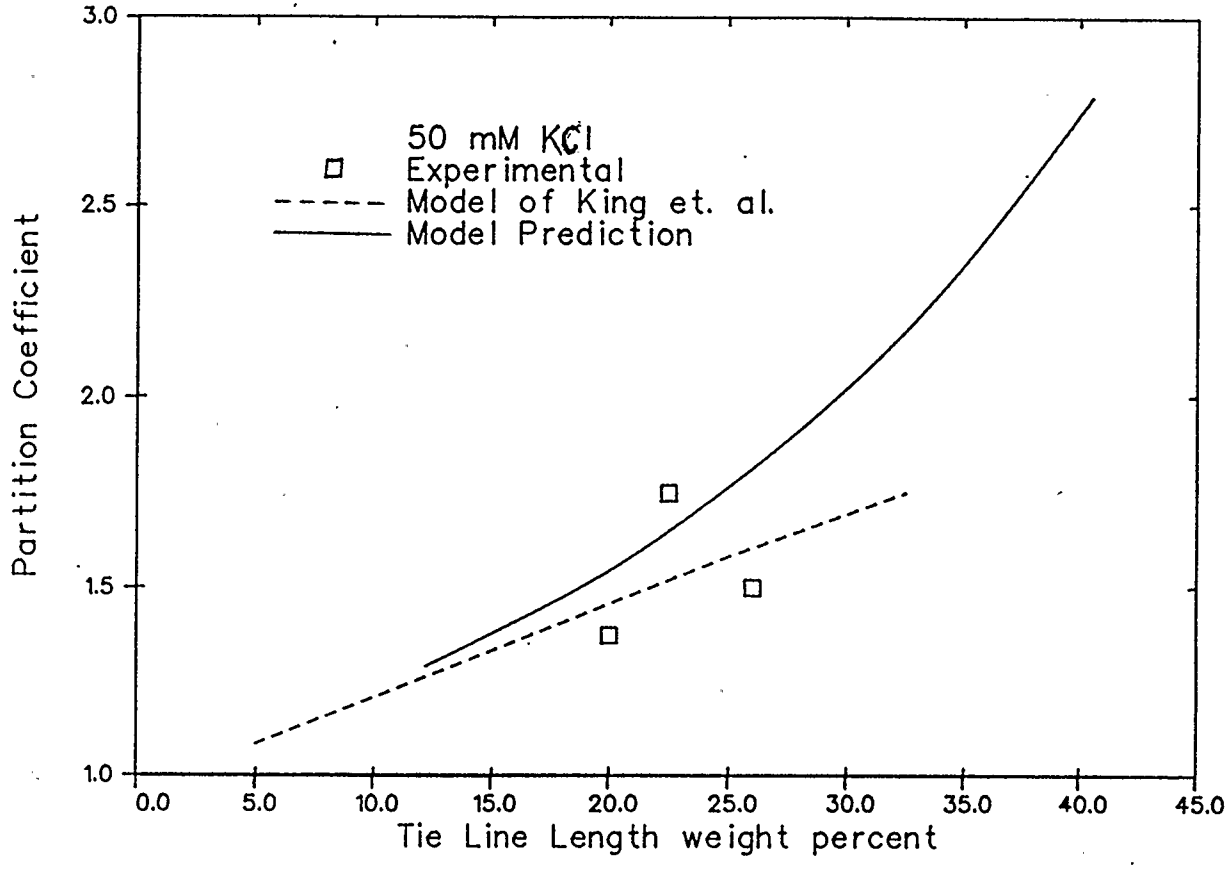

Figure 7.12 : Experimental and Predicted Partition Coefficients vs. Tie Line Length for Chymotrypsin in PEG 3350, Dextran T-70, Salt-Water system

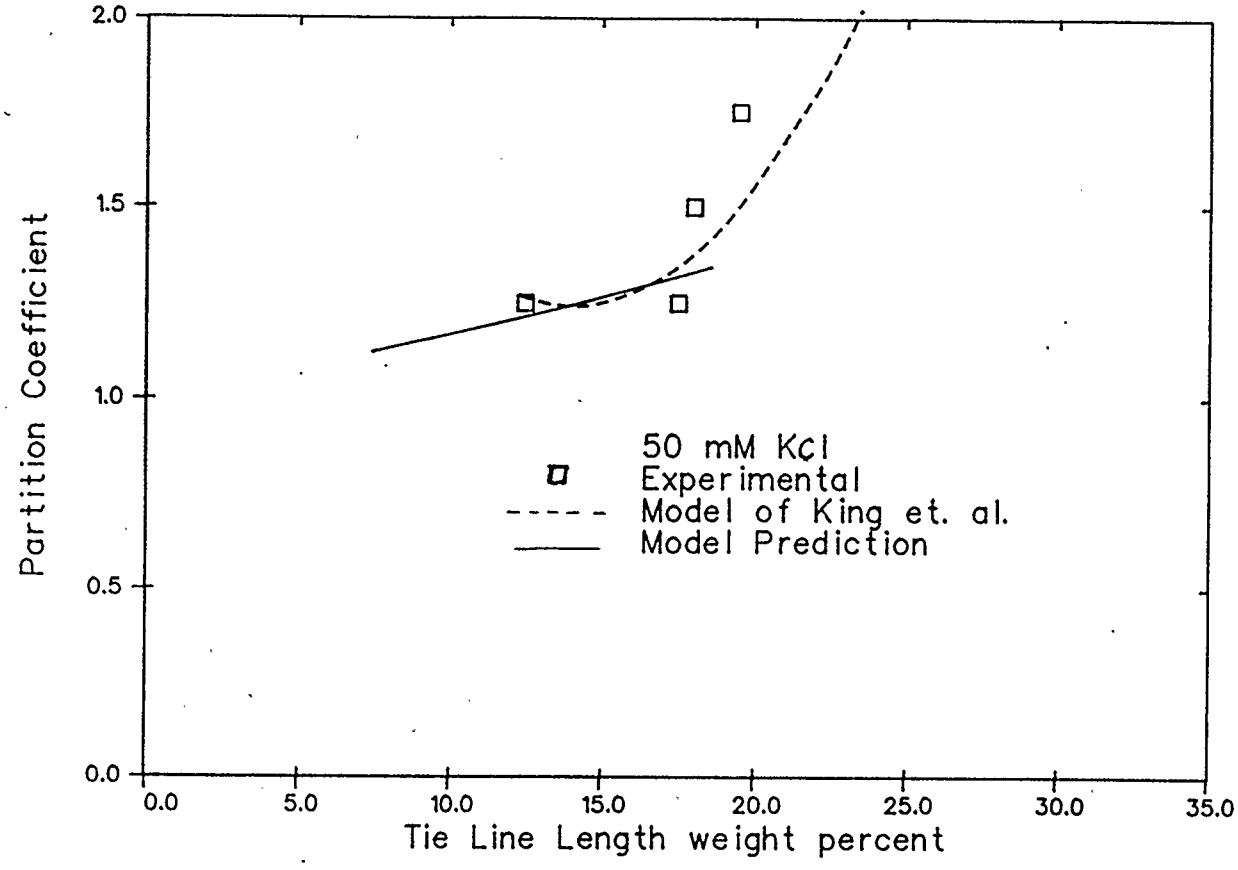

Figure 7.13 :Experimental and Predicted Partition Coefficients vs. Tie Line Length for Chymotrypsin in PEG 8000, Dextran T-500, Salt-Water system

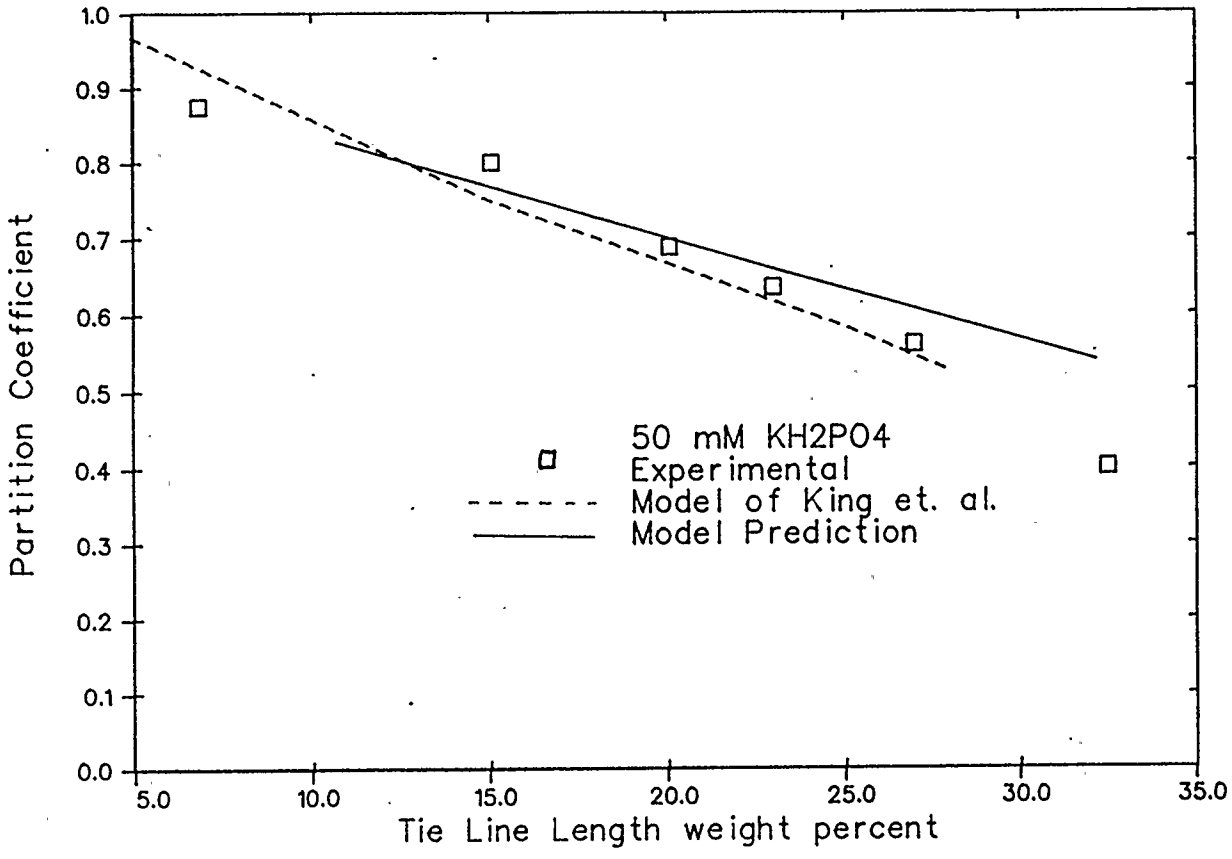

Figure 7.14 : Experimental and Predicted Partition Coefficients vs. Tie Line Length for Chymotrypsin in PEG 3350, Dextran T-70 Salt-Water system

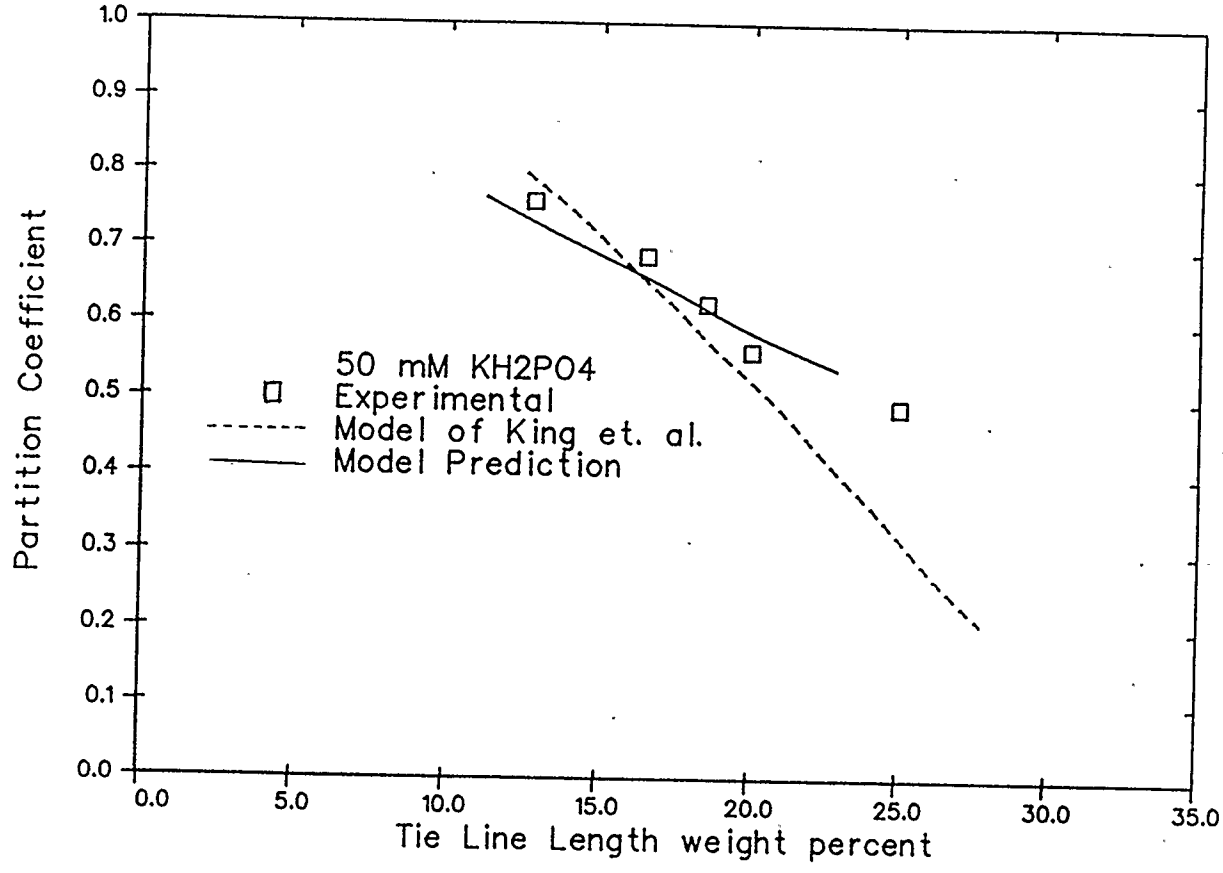

Figure 7.15 :Experimental and Predicted Partition Coefficients vs. Tie Line Length for Chymotrypsin in PEG 8000, DextranT-500, Salt-Water system

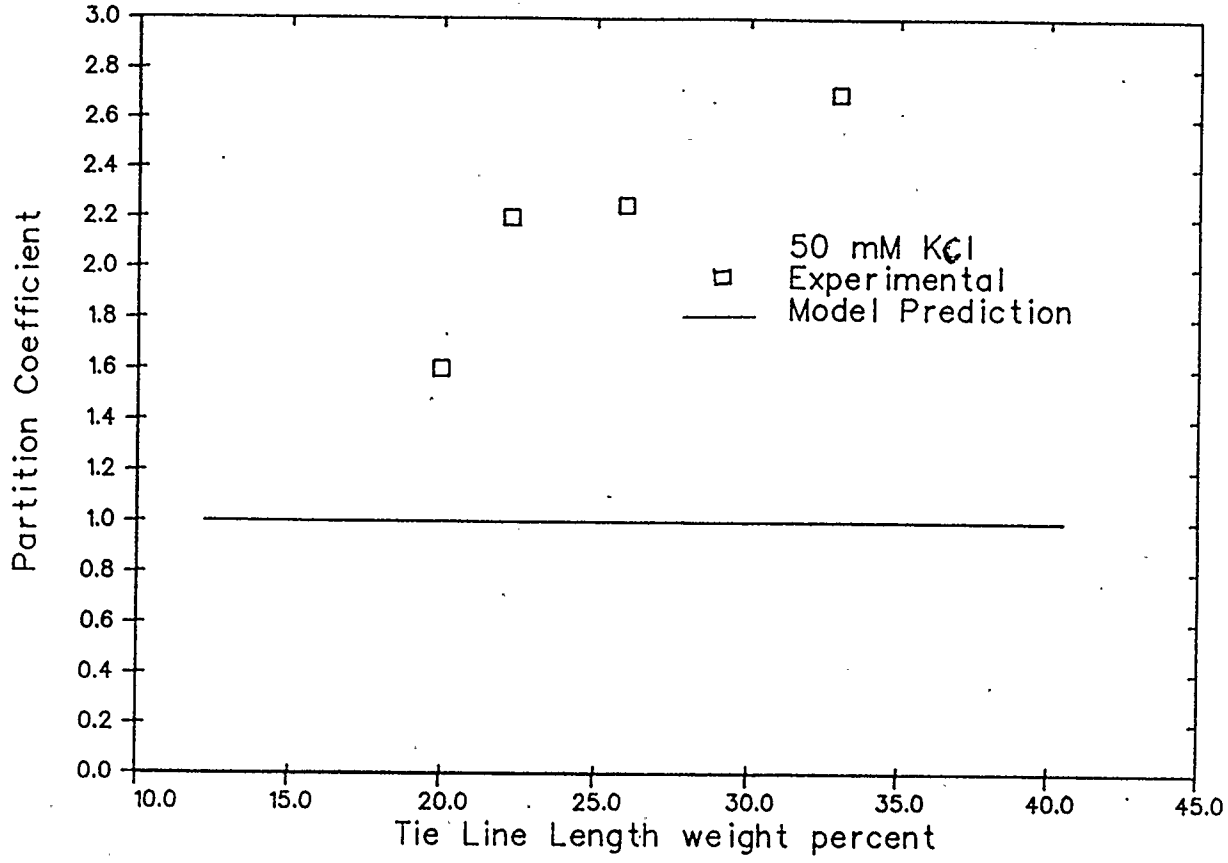

Figure 7.16: Experimental and predicted partition coefficient vs. tie line length for Lysozyme in PEG 3350, Dextran T-70, Salt-Water system. The Interaction Parameter tow for Dextran Protein is 25.0 alpha IS 0.2

CHAPTER 8

CONCLUSIONS

The main thrust of this work has been to develop a comprehensive model that could be used in protein separation processes.

The proposed thermodynamic framework combines the excess Gibbs free energy model proposed by Chen and Evans(1982, 1986) for electrolytes in mixed solvents with the Flory-Huggins Gibbs free energy model for polymer-solvent systems. The resulting model can be applied to mixtures of water and other solvents, polymers, proteins and salts.

The Flory-Huggins part of the model provides an adequate correlation of data for aqueous two phase polymer systems. The effects of salts on the phase separations are also adequately described by the contributions from Chen's electrolyte model.

The proposed model has been used to correlate data on the phase partitioning of proteins in aqueous two phase polymer systems with chloride, sulphate and phosphate salts in the mixture. The data available for correlation is somewhat scattered and exists at only limited salt concentrations but is adequately represented by the correlations.

The advantage of the proposed model is that the salt-water interaction parameters are available from Chen and coworkers(1982, 1986). The specific volumes needed in the Flory-Huggins model can be obtained a priori from the work

of Kang and Sandler(1987) and there are relatively few parameters required to complete the model.

The parameters for protein polymer interactions depend on the specific salt in the mixture. There were simply insufficient data to overcome this limitation in the model. It would be highly desirable if additional tie-line data or data of other kinds for these systems could be provided.

In principle, the Chen model with additional Flory-Huggins terms (as proposed in this thesis) could be used to correlate data for mixtures with other kinds of large bio-molecules or with solvents other than water. This would be a topic for further research.

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