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SOLUBILITY MODELS FOR ANTIBIOTICS AND AMINO ACIDS

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,

"SOLUBILITY MODELS FOR ANTIBIOTICS AND AMINO ACIDS"

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ABSTRACT

Liquid solution behaviors of amino acids and antibiotics are studied. The Modified UNIFAC Group-Contribution Model, presented by Larsen et al.(1987), is applied to correlate activity coefficient data for several amino acids dissolved in neutral water. Some new groups are defined. For the new groups, Bondi's size parameters are calculated from the molecular structure. Interaction parameters are regressed from the activity coefficient data. A comparison of predicted and experimental results is presented. For some amino acids, purely predictive activity coefficient ratios (γ/γ^{∞}) are also compared with the experimental values. The amino acids studied are alanine, amino-butyric acid, glycine, hydroxy-proline, proline, serine, threonine and valine. The new groups introduced are *glycine* and *proline*.

Chemical equilibrium theory and Modified UNIFAC, are used to study the effect of pH and temperature on the solubility of amino acids. The values used for amino acid dissociation constants are the accepted experimental values. Additional parameters are correlated from experimental solubility data in neutral water. Some predictions, showing the variation of solubility of amino acids with temperature and the pH of the solution, are presented.

The solubility and activity coefficients of six antibiotics anisomycin, carbomycin A, chloramphenicol, chloramphenicol palmitate, griseofulvin, and hygromycin A are studied. A procedure, based on the Hildebrand-Scatchard equation, to calculate activity of antibiotics using entropy of fusion and melting temperature data, is followed. A constant entropy of fusion of 56.484 *J/mol K* (13.5 *cal/mol K*) is used

for all antibiotics in the study, as suggested by Yalkowsky (1979). Five new antibiotic groups were defined. The size parameters for these new groups were determined from molecular structure. UNIFAC interaction parameters were regressed from solubilities in various solvents.

Antibiotic activity coefficient profiles and solubilities in mixed solvents are also presented. Calculations predicting precipitation of an antibiotic from solution by adding another solvent, are demonstrated. In a typical case the expected recovery of the antibiotic by such precipitation could be 90%.

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Ram Gupta

This work is dedicated to

my parents

Kunj B. Gupta and Keshar D. Gupta

and siblings

Heera, Mahesh, Usha and Vipin

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Chapter 1.

Introduction

Extraction, purification, and crystallization unit operations are some of the most important processes in the manufacturing of the biochemicals. The fine chemical and pharmaceutical industry is mainly concerned with the recovery of antibiotics, amino acids, vitamins, steroids, enzymes, hormones, alkaloids, and many other chemicals from extracts of plant or animal origin. Extraction and purification steps are important because of their high cost. In a typical case the cost involved in bio-separation may be as high as 90% of the total cost of manufacturing. Unfortunately, this area of research has not been given adequate attention and bio-separation units are designed empirically rather than on the basis of rational information.

In the future, the biotechnical industry will have to optimize its bio-separation processes to remain competitive. The company with inefficient bio-separation processes may lose its market, because other companies with economic separation techniques will have lower costs of manufacturing.

In general, bio-separation process are governed by thermodynamics and kinetics. The most important of these two is thermodynamics, which has received the least attention.

Biochemical thermodynamics also plays a key role in drug design and in biological processes. Its importance in the pharmaceutical science is explained by Davis et al. (1974). Concepts of thermodynamics can be applied to biological systems because these processes are essentially physical and chemical changes involving the exchange of energy. These concepts provide the basis for determining important

parameters for rational drug design.

Drug activity, which is one of the most important elements in drug design depends upon two factors (a). structure or 'fit' of the drug molecule to the recepter side, and (b). physical properties (i.e., thermodynamic activity of the drug molecule and its environment) which affect the distribution properties and can have profound effect on the biological activity. One common way to increase drug activity is to increase concentration by increasing dosages. But after a limit, when the thermodynamic equilibrium has been achieved, the activity of the drug cannot be increased by increasing the dosage. Mitscher (1978) explained this fact by the observation of the tetracycline absorption in the human. "With tetracycline given 0.25 g every six hours, the serum level stablizes at about 2.5 μg/ml in 24 hours. Doubling the dose leads to a peak of about 5 μg/ml and further dose increase fails to give any higher blood levels." In many cases, the solubility of the drug can be increased by making some modification in the structure, by increasing thermodynamic activity and consequently biological response. Thermodynamic activity prediction by a group contribution model will be useful in this possibility. A thermodynamic model and its parameters, which represents solution behavior of the drug molecule form the basis of a rational approach to drug design. The knowledge of the activity coefficient or partition coefficient with its variation will allow us to predict solubility, compatibility, distribution, adsorption, membrane permeability etc.

Apart from the drug design, the thermodynamics of biotechnical molecules plays an important role in the manufacturing of biochemicals. This need is well explained by Rao et al.(1983) in his paper entitled "Phase Equilibrium Data Need of Fine Chemical and Pharmaceutical Industry".

Most of the fine chemicals are produced by fermentation (for example enzymes, hormones, penicillins, and steroids) and chemical synthesis (for example analgesic, antipyretic and anti-TB drugs, sulfa drugs, and vitamins). Equilibrium separation process such as absorption, distillation, extraction, crystallization, and drying are required to get a pure product from a fermentation broth or reaction mixture. An optimum design of these separation process should depend upon reliable phase equilibrium data of biochemicals in the process.

Vitamin B_1 (thiamine) has been chemically synthesised for the last 45 years. The main ingredients for the synthesis of vitamin B_1 are amino-pyridine and thiazole. The amino-pyridine is manufactured from pure acetonitrile and some other chemicals. To get pure acetonitrile from an aqueous reaction mixture, an expensive azeotropic distillation is used. Tollefson et al. (1970) suggested that solvent extraction would be a good alternative to replace costly azeotropic distillation. Experimental data to screen some prospective solvents were presented by Rama Rao et al.(1978) and Subba Rao et al. (1979). Amino-pyridine is extracted from the reaction mixture as a batch solvent extraction process, with kerosine as solvent. Rao et al. (1983) reported that the extraction and crystallization units are designed empirically, hence the output is found to fluctuate by as much as 200%. Economy favors the design of a continuous solvent extraction unit, but to design a continuous solvent extraction process may be catastrophic without having reliable phase equilibrium data. By having good phase equilibrium data, not only rational design of the extraction process could be done, but also solvents other than kerosine could be evaluated. Similar problems are associated with production of thiazole (4-methyl 5-hydroxy ethyl thiazole), which is half of the thiamine (vitamin B_1) molecule. To design an efficient continuous extraction equipment, thermodynamic investigation of some ternary systems like

water-thiazole-benzene, water-thiazole-chloroform, and water-thiazole-diethyl ether are important.

Lactic acid, one of the most important industrial biochemicals, is manufactured by fermentation of an inexpensive raw material like molasses. But the lactic acid is expensive because of the high cost involved in its recovery and purification. To make lactic acid one has to use an optimally designed continuous extraction process. A cheap solvent like fusel oil (a distillary product) may prove to be an economical solvent. Some distribution coefficient data for lactic acid between water and fusel oil have been reported by Bansal et al. (1976). These distribution coefficients are affected by the presence of other chemicals , hence a predictive group contribution method to predict them, would be quite helpful in the design of a continuous extraction unit for lactic acid.

Antibiotics are high cost fine chemicals, mostly manufactured by fermentation using inexpensive substrate. In some cases more than 90% of the cost of antibiotics comes from separation processes. Amino acids are used as nutrients for the cells producing antibiotics. To be able to make a rational optimum design of extraction of antibiotics, one needs to know the thermodynamic behavior of the antibiotics, amino acids, and other chemicals present in the fermentation broth or reaction mixture.

In this thesis, an attempt has been made to build a predictive model for the activity coefficients of some antibiotics and amino acids. The antibiotics in the study are anisomycin, carbomycin A, chloramphenicol, chloramphenicol palmitate, griseofulvin, and hygromycin A. These antibiotics are of much commercial importance. Amino acids in the study are alanine, amino-butyric acid, glycine, hydroxy-proline, proline, serine, threonine, and valine. These are 8 out of the 20 most common amino

acids. The effect of pH and temperature on the solubility of *dl*-alanine, glycine, and *dl*-valine is also studied, as in practical situations varying pH and temperatures are encountered. The technique to predict activity coefficients has been demonstrated for these biochemicals and it can also be extended to other biochemicals of importance.

Activity coefficient predictions of amino acids and antibiotics, has had only recent attention. Orella and Kirwan (1987), in their unpublished work have made an attempt to predict relative solubilities of several amino acids (glycine, alanine, phenylalanine, valine, leucine, and iso-leucine) and cephalosporin c (antibiotic) in mixed solvents methanol-water, ethanol-water, 1-propanol-water, and 2-propanol-water. The activity coefficient is considered to be made up of two terms, due to chemical interactions (from UNIFAC), and due to electrostatic interactions (from the extended Kirkwood theory).

Nass (1988) has correlated amino acid activity coefficient and solubility data. She assumed that the activity coefficient is a product of two terms. The first is due to chemical reaction and comes from a chemical reaction equilibrium calculation. The second term is due to physical interaction and is given by Wilson's equation for the activity coefficient. Bondi's volumes (Bondi, 1968) are used to represent the pure component liquid volume ratios. Activity coefficients for alanine, serine, and threonine in water, and solubilities of phenylalanine, tyrosine, and diiodotyrosine in water have been correlated. The correlations are in good agreement with the experimental data. The number of parameters regressed varied from 3 to 10. Several of the parameters could have been taken from the literature instead of being treated as free variables in the regression process.

Chapter 2.

Biochemicals in Study

2.1 Antibiotics

Antibiotics are compounds that inhibit bacterial growth and sometimes cause bacterial death. The useful antibiotics exhibit low toxicity and can be selectively active against particular microorganisms. They are used to destroy micro-organisms in-vivo. Antibiotics have significant and important applications against many human, insect, and plant diseases. There are thousands of antibiotics being produced bio-synthetically, but there are only a few being produced by chemical synthesis. Only some out of several thousands known antibiotics are of commercial importance.

Almost all the antibiotics of commercial importance are manufactured by large scale aerobic fermentation. The companies producing antibiotics have been reluctant to publish process details, however general outlines of the processes are well known. The fermentation media are designed to give maximum production. Carbohydrate sources such as glucose, sucrose, lactose, and/or starch, and nitrogen sources such as urea, ammonium sulfate, soybean meal, cornsteep liquor and/or whey are used. Sometimes chemical compounds are added to the fermentation broth, so that it can react with the naturally produced antibiotic and make a desired product.

After the production cycle, the fermentation broth is taken for harvesting. Microorganisms are removed by filtration or centrifugation. Antibiotic is extracted by solvent extraction, ion-exchange, chromatography, precipitation, crystallization or a combination of these methods. The major cost of manufacturing is involved in the separation processes, because a very low concentration of antibiotic is obtained in the

final fermentation broth.

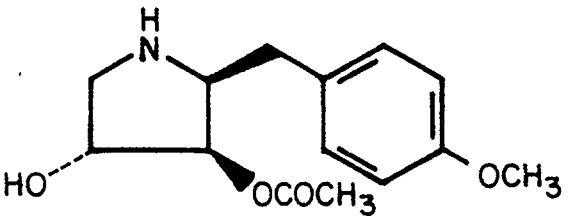
Antibiotics are mostly used in prevention and treatment of disease in humans and animals. They are also used in livestock feed. They have been used in preservation of food and have been sprayed on crops to prevent specific diseases, but these uses have now been abandoned.

Six antibiotics anisomycin, carbomycin A, chloramphenicol, chloramphenicol palmitate, griseofulvin, and hygromycin A are selected for study in this research work. The selection of these antibiotics is based on their commercial importance and availability of the solubility, melting temperature, and chemical structure data. The structures of these antibiotics are given in Figures 2.1a and 2.1b, and chemical formula, melting point, and molecular weight are listed in the Table 2.1 .

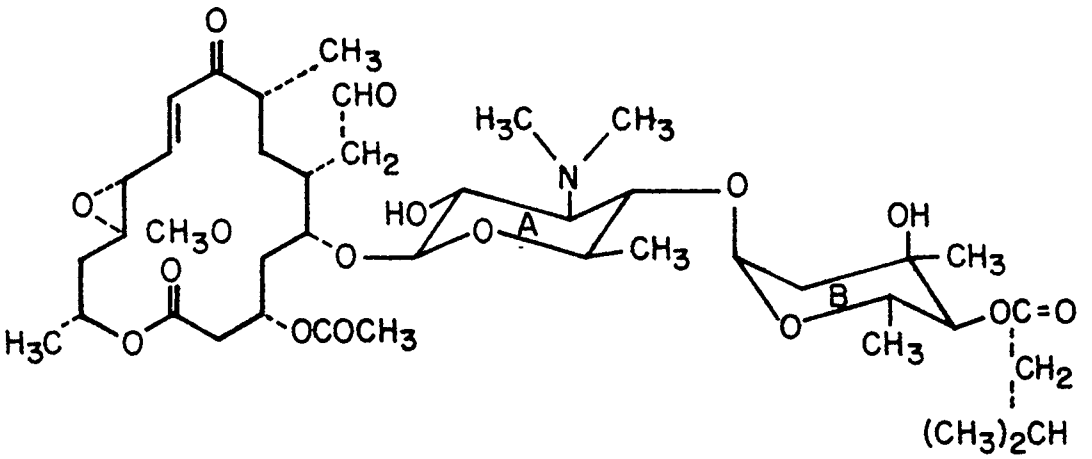
2.2 Amino Acids

Amino acids are the main component of proteins, since every protein can be considered as a polymer of the amino acids. About 20 types of amino acids form most of the proteins. Proteins are found in all living organisms and play an important role. There are 10 essential amino acids, which should be ingested through digestion. The nutritional value of a protein depends upon the presence of essential amino acids in it.

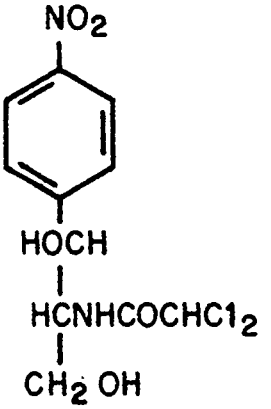
Long ago all *l*-amino acids were manufactured by isolation from protein hydrolysates, but now a number of useful amino acids are being produced economically by fermentation. Some amino acids, including glycine and alanine are produced by chemical synthesis. In fermentive production of amino acids, all raw materials are natural or biologically available substances. Many useful bioproducts are also



ANISOMYCIN

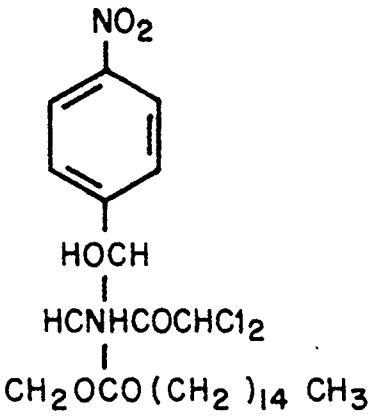


CARBOMYCIN A

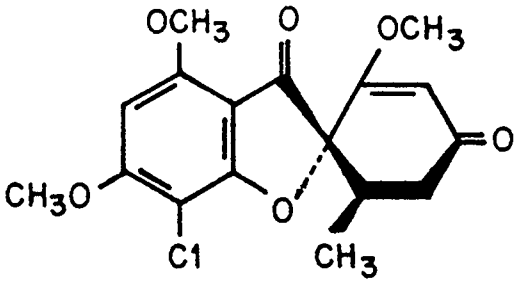


CHLORAMPHENICOL

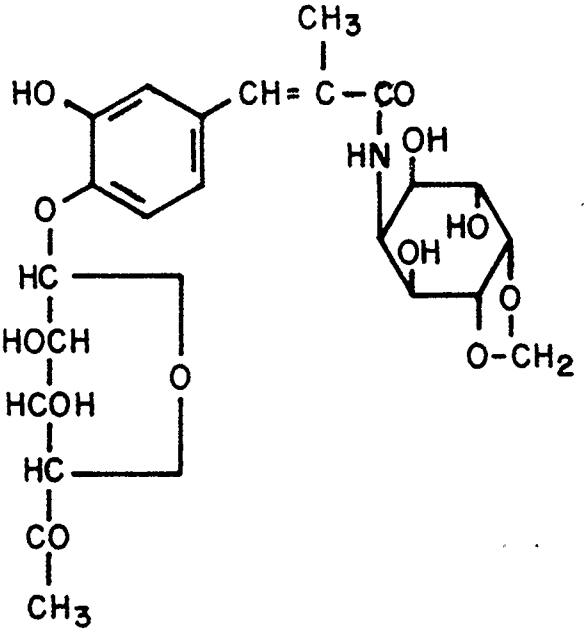
FIGURE 2.1a CHEMICAL STRUCTURE OF ANTIBIOTICS



CHLORAMPHENICOL PALMITATE



GRISEOFULVIN



HYGROMYCIN A

FIGURE 2.1b CHEMICAL STRUCTURE OF ANTIBIOTICS

Table 2.1 Antibiotics in Study

(Chemical Formula, Melting Point, and Molecular Weight)

No.	Antibiotic	Chemical Formula	Melting Temperature (K)	Molecular Weight
1.	Anisomycin	$C_{14}H_{19}NO_4$	413.65	265.30
2.	Carbomycin	$C_{42}H_{67}NO_{16}$	487.15	841.97
3.	Chloramphenicol	$C_{11}H_{12}Cl_2N_2O_5$	424.15	323.14
4.	Chloramphenicol Palmitate	$C_{27}H_{42}Cl_2N_2O_6$	363.15	561.54
5.	Griseofulvin	$C_{17}H_{17}ClO_6$	493.15	352.77
6.	Hygromycin A	$C_{23}H_{29}NO_{12}$	380.15	511.47

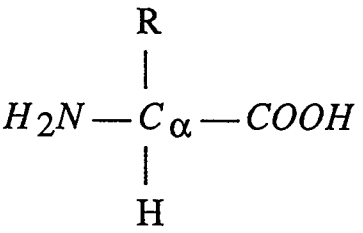


Figure 2.2a Uncharged Amino Acid.

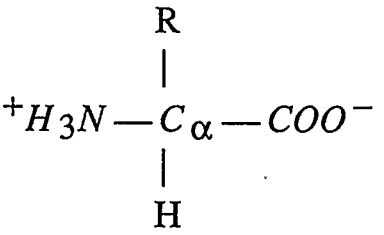


Figure 2.2b Doubly Charged Zwitterion

produced with amino acids. Microorganisms separated from a fermentation broth are high quality proteins, which are used as livestock feed. Various extraction techniques are used to extract and crystallize amino acids from microorganisms.

Amino acids are widely used for livestock feed, as flavor enhancers, in medicine and for nutritional purposes. They have been used in transfusions for many years, to maintain nitrogen metabolism when proteineous food cannot be taken orally. Only very few amino acids are being used in the chemical industry.

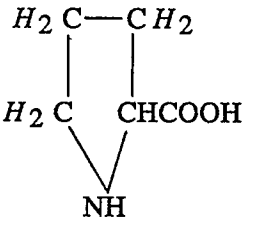
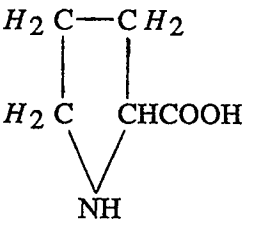
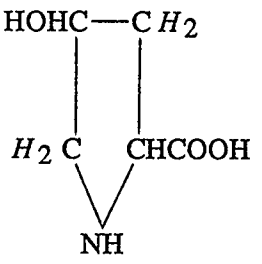
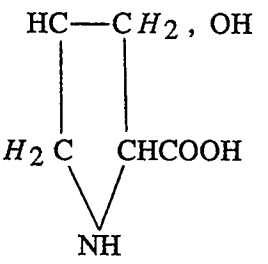
Amino acids have a tetrahedral carbon atom at the center. This α-carbon atom is covalently bonded with a carboxyl group (*COOH*) and with an amino group (NH_2). A third bond is a variable group (*R*) and fourth bond is always a hydrogen atom. Figure 2.1a shows the structure of an amino acid.

All the amino acids except glycine (for which the variable group R is hydrogen) show optical isomerims. This is one of the most interesting fact about amino acids and is due to the asymmetric carbon atom. The two isomers are called dextro ($d-$) and levo ($l-$) isomers, and a mixture of these two would be called the $dl-$ acid.

Amino acids take the form of zwitterion when dissolved in neutral water (Figure 2.2b). The carboxylic group loses a proton and the amino end of the molecule gains a proton. This is the most striking and significant property of amino acids in solution.

Eight amino acids have been considered in the present study ; i.e., alanine, amino-butyric acid, glycine, hydroxy-proline, proline, serine, threonine, and valine. The selection of these amino acids is based on the fact that these are the elementary amino acids, and many other amino acids are made of the groups from these chosen

Table 2.2 Chemical Structure of Amino Acids

Amino Acid	Chemical Structure	Groups
Glycine	$CH_2(NH_2)COOH$	$CH_2(NH_2)COOH$
Alanine	$CH_3CH(NH_2)COOH$	$CH(NH_2)COOH$, CH_3
Amino-Butyric Acid	$CH_3CH_2CH(NH_2)COOH$	$CH(NH_2)COOH$, CH_3, CH_2
Valine	$(CH_3)_2CHCH(NH_2)COOH$	$CH(NH_2)COOH$, $2CH_3$, CH
Serine	$HOCH_2CH(NH_2)COOH$	$CH(NH_2)COOH$, CH_2, OH
Threonine	$CH_3CH(OH)CH(NH_2)COOH$	$CH(NH_2)COOH$, CH_3, CH, OH
Proline		
Hydroxy-Proline		

amino acids. By knowing the properties of these few groups, properties of many amino acids could be predicted from a group contribution method. The chemical structure of these amino acids are presented in the Table 2.2 .

2.2.1 Dissociation of Amino Acids

Amino acids take several ionic forms when dissolved in water. The following reactions occurs:

$$NH_2RCOOH = NH_3{}^+RCOO^- \qquad K_D \qquad (2.1)$$

$$NH_3{}^+RCOOH = H^+ + NH_3{}^+RCOO^- \qquad K_1 \qquad (2.2)$$

$$NH_3{}^+RCOO^- = H^+ + NH_2RCOO^- \qquad K_2 \qquad (2.3)$$

Reaction (2.1) shows formation of the neutral zwitterion with its charge distribution. The zwitterion can accept a proton, as shown in (2.2) to form the positively charged species on the left side of the equation. It can also donate a proton as shown in (2.3) to produce a negatively charged species. The dissociation equilibrium constants K_D, K_1 and K_2 are given by

$$K_D = \frac{[NH_3{}^+RCOO^-]}{[NH_2RCOOH]} \qquad (2.4)$$

$$K_1 = \frac{[H^+]\,[NH_3{}^+RCOO^-]}{[NH_3{}^+RCOOH]} \qquad (2.5)$$

$$K_2 = \frac{[H^+]\,[NH_2RCOO^-]}{[NH_3{}^+RCOO^-]} \qquad (2.6)$$

where the brackets indicate molality units.

Greenstein and Winitz (1961) report that the value for K_D for aliphatic amino acids is of the order of 10^5 to 10^6. This means that the amount of the uncharged amino acid in the aqueous solution is negligible. The amino acids studied for the effect of pH, in this work, have a very high value of K_D, hence it is assumed that the amino acid, when dissolved in water, exists only as the zwitterion and the two net charged species.

On dissolution of the amino acid in neutral water, the solution becomes acidic, since the amino acid behaves like a weak acid. The pH of the solution can be calculated from a mass balance. The reaction model is:

$$A_+ = H^+ + A_\pm \qquad K_1 \qquad (2.7)$$

$$A_\pm = H^+ + A_- \qquad K_2 \qquad (2.8)$$

$$H_2O = H^+ + OH^- \qquad K_w \qquad (2.9)$$

where A represents the amino acid, $A_\pm$ is the neutral zwitterion, and A_+ and A_- are the charged forms.

Suppose that n_o moles of the amino acid are added to 1 kg of water. Let reaction extents ε_1, ε_2 and ε_3 be the moles of H^+ produced in the three reactions (2.7) – (2.9), respectively. Then, supposing ε_1 and ε_2 are very small in comparison with n_o, the equilibrium reactions are.

$$K_1 = \frac{(\varepsilon_1 + \varepsilon_2 + \varepsilon_3)\, n_o}{(-\varepsilon_1)} \qquad (2.10)$$

$$K_2 = \frac{(\varepsilon_1 + \varepsilon_2 + \varepsilon_3)(\varepsilon_2)}{n_o} \qquad (2.11)$$

$$K_w = (\varepsilon_1 + \varepsilon_2 + \varepsilon_3)(\varepsilon_3) \qquad (2.12)$$

These three equations can be solved for ε_1, ε_2 and ε_3, yielding for the hydrogen ion molality.

$$[H^+]^2 = (\varepsilon_1 + \varepsilon_2 + \varepsilon_3)^2 = \frac{K_w K_1 + n_o K_1 K_2}{K_1 + n_o} \tag{2.13}$$

From (2.13), for low n_o

$$[H^+] = (K_w)^{1/2} = 10^{-7} \tag{2.14}$$

that is, pH $= -\log_{10}[H^+] = 7$. On the other hand, as n_o becomes larger

$$[H^+] = (K_1 K_2)^{1/2} \tag{2.15}$$

and

$$pH = \frac{-(\log_{10} K_1 + \log_{10} K_2)}{2} \tag{2.16}$$

or

$$pH = \frac{pK_1 + pK_2}{2} \tag{2.17}$$

The value of pH given by (2.17) is called the "iso-electric" point for the amino acid.

As discussed in chapter 4, typical values for pK_1 and pK_2 are 2.3 and 9.7 respectively. The corresponding pH in all but very dilute solutions of amino acids is pH = 6.0. When the molality n_o is only 5×10^{-3} the pH is reduced to 6.15 .

Chapter 3.

Phase Equilibrium Models

3.1 Activity Coefficients

The activity coefficient is a measure of the non-ideality of a component in the liquid phase. It is one of the most important thermodynamic properties used in the application of thermodynamics to phase equilibria involving liquid phases.

Usually, the activity coefficient is represented by the Greek letter γ. In the most commonly used convention in chemical engineering practice , γ_i is related to the activity and mole fraction as follows:

$$\gamma_i = \frac{a_i}{x_i} \quad , \gamma_i \rightarrow 1 \quad as \quad x_i \rightarrow 1 \tag{3.1}$$

The activity is defined as:

$$a_i = \frac{f_i}{f_i{}^o} \tag{3.2}$$

where f_i is the fugacity of the component i in the solution and $f_i{}^o$ is the fugacity of substance i at its standard state. In the convention of (3.1), the standard state is pure liquid i at the given temperature and pressure. The activity coefficient is related to the partial molar excess Gibbs free energy of component i in the solution:

$$\ln(\gamma_i) = \frac{\overline{G}_i^E}{R\,T} \tag{3.3}$$

For an ideal solution γ_i takes a value of 1 for all x_i. The non-ideality of the 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 deviation from ideality is for $\gamma_i < 1$.

Activity coefficients may be calculated from experimental measurements of vapor-liquid and liquid-liquid equilibrium data. Osmotic pressure, freezing-point depression, boiling-point deviation and vapor pressure lowering measurements are also used to deduce activity coefficients of non-volatile solutes. A solute activity coefficient can be obtained by integrating the Gibbs-Duhem equation:

$$\sum_i x_i \, d\mu_i = 0 \tag{3.4}$$

or

$$\sum_i x_i \left[\frac{\partial \ln \gamma_i}{\partial n_j} \right]_{T,P} = 0 \tag{3.5}$$

It is often convenient to use conventions other than (3.1) when dealing with dilute solutes. The convention used for activity coefficient data available for amino acid solutions is given by the following:

$$\gamma_i^* = \frac{a_i}{m_i} \quad , \; \gamma_i^* \rightarrow 1 \;\; as \;\; m_i \rightarrow 0 \tag{3.6}$$

where m_i is molality (moles of solute per kg of water solvent). Here the activity is defined as:

$$a_i = \frac{f_i}{f_i^*} \tag{3.7}$$

where f_i^* is the fugacity of i in a "hypothetical" ideal unit molal solution at mixture temperature and pressure. The standard state fugacity f_i^* has to be regarded as a correlating parameter in phase equilibrium calculations.

The activity coefficients in the two alternative conventions are related in a simple way. Let γ_i^∞ be the limiting value of γ_i in the convention of (3.1) at infinite dilution (x_i and $m_i \rightarrow 0$). Then

$$\frac{\gamma}{\gamma_i^{\infty}} = \frac{\gamma_i^*}{(1 - x_i)} \tag{3.8}$$

Equation (3.8) assumes that i is the solute in a binary mixture.

The relationships between activity coefficients in various standard states are discussed, for instance, by Denbigh (1971). Equation (3.8) can be derived by noting that the chemical potential of the solute must be the same however the standard state and activity coefficient are defined. Then

$$\mu_i = R\,T \ln(\gamma_i{}^* m) + \mu_{i_o}{}^* = R\,T \ln(\gamma_i x_i) + \mu_{i_o} \tag{3.9}$$

and therefore

$$\ln\left[\frac{\gamma_i{}^* m_i}{\gamma_i x_i}\right] = \frac{\mu_{i\,o} - \mu_{i_o}{}^*}{R\,T} \tag{3.10}$$

In these equations, μ_{i_o} and $\mu_{i_o}{}^*$ are the standard state chemical potentials.

The quantity on the right is independent of composition and the value can be determined by taking the limit as mole fraction and molality simultaneously go to zero. As $x \rightarrow 0$, $\gamma_i \rightarrow \gamma_i^{\infty}$ and $\gamma_i^* \rightarrow 1$. The ratio of molality to mole fraction in a binary system is,

$$\frac{m_i}{x_i} = \frac{m_i}{m_i / [\,m_i + 1000/M_W\,]} \tag{3.11}$$

where $1000/M_W$ is the number is the number of moles of water in 1000 grams. The limit of m_i/x_i as $m_i \rightarrow 0$ is therefore $(M_W/1000)$. Combining these limit results gives

$$\ln\left[\frac{M_W}{1000\,\gamma_i{}^{\infty}}\right] = \frac{\mu_{i\,o} - \mu_{i_o}{}^*}{R\,T} \tag{3.12}$$

and inserting this result in Equation (3.10) leads to

$$\gamma_i^{*} m_i = \frac{\gamma_i x_i M_W}{1000 \gamma_i^{\infty}} \tag{3.13}$$

But

$$\frac{x_i M_W}{m_i 1000} = x_{H2O} = 1 - \gamma_i \tag{3.14}$$

Therefore,

$$\gamma_i/\gamma_i^{\infty} = \gamma_i^{*}/(1 - x_i) \tag{3.15}$$

3.1.2 Excess Gibbs Free Energy Models

There are several commonly used functions for excess Gibbs free energy in the convention of (3.1). These include the Margules, Van Laar, Wilson, T-K-Wilson, NRTL, UNIQUAC, Scatchard-Hildebrand and UNIFAC models. These models are described in standard texts, e.g. Walas (1985).

The Margules equation is one of the early equations with superior performance. The expression for the excess Gibbs free energy of a binary system is given by:

$$\frac{G^E}{RT} = x_1 x_2 \left[A_{21} x_1 + A_{12} x_2 \right] \tag{3.16}$$

A_{21} and A_{12} are two parameters to be regressed from experimental data. This equation is capable of exhibiting a minimum or maximum in the activity coefficient versus mole fraction plot.

The development of the van Laar equation was based on the van der Waals equation of state, but it is commonly treated in a purely empirical manner because the fit to activity coefficient data using van der Waals parameters is poor. The van Laar equation is:

$$\frac{G^E}{RT} = \frac{1}{\left[\frac{1}{A'_{12}\,x_1} + \frac{1}{A'_{21}\,x_2}\right]} \tag{3.17}$$

A'_{12} and A'_{21} are two van Laar parameters and they are regressed from experimental data. To be able to represent the activity coefficient data over the full range of concentration, the signs of the two parameters must be identical.

Wilson's equation is based on the concept that interactions between molecules depend upon local mole fractions. The local mole fractions are defined in terms of probabilities using a Boltzman distribution. The Wilson's equation is given as:

$$\frac{G^E}{RT} = -x_1 \ln\left[x_1 + \Lambda_{12}\,x_2\right] - x_2 \ln\left[x_1\,\Lambda_{21} + x_2\right] \tag{3.18}$$

Λ_{12} and Λ_{21} are two interaction parameters used. The performance of this equation is much superior to the other equations discussed so far. The equation can also be used to represent the multi-component behavior with only binary interaction parameters. The drawback of Wilson's equation is that it cannot represent liquid-liquid immiscibility.

The NRTL (Renon) equation is based on a two cell theory. In the theory it is assumed that the liquid has structure made up of two types of cells those surrounding molecules of type 1 and those surrounding molecules of type 2. The NRTL (non-random two liquid) model is expressed as:

$$\frac{G^E}{RT} = x_1\,x_2\left[\frac{\tau_{21}\,G_{21}}{x_1 + x_2\,G_{21}} + \frac{\tau_{12}\,G_{12}}{G_{12}\,x_1 + x_2}\right] \tag{3.19}$$

where

$$G_{12} = \exp\left[- \alpha_{12}\, \tau_{12} \right] \tag{3.20}$$

$$G_{21} = \exp\left[- \alpha_{12}\, \tau_{21} \right] \tag{3.21}$$

The NRTL equation takes three parameters τ_{12}, τ_{21}, and α_{12}. It represents the binary equilibrium data well with these three parameters. It gives performance superior to the Margules and van Laar equations. NRTL is also better than Wilson's equation in the sense that it can represent liquid-liquid equilibria. The equation is also extended to multi-component mixtures with only binary interaction parameters. The main handicap of this model is to use 3 parameters as compared to two in the Wilson or UNIQUAC expressions.

The UNIQUAC (Universal Quasi-Chemical) equation is also a two liquid model based on the local composition concept. The excess Gibbs free energy is assumed to be made of two contributions; i.e. those due to differences in the sizes and shapes of the molecules, and those due to interaction energy between unlike molecules. UNIQUAC is one of the most popular models to predict activity coefficients. It can be applied to multicomponent mixtures using binary parameters only. It is also capable of predicting liquid-liquid equilibria and its temperature dependency is valid over a moderate range. UNIQUAC gives superior representation of the activity coefficients of mixtures of widely different molecules. The UNIQUAC model is given by:

$$G^E = G^E_{combinatorial} + G^E_{residual} \tag{3.22}$$

where

$$\frac{G^E_{combinatorial}}{R\,T} = \sum_i x_i \ln \frac{\phi_i}{x_i} + (z/2) \sum_i q_i\, x_i \ln \frac{\theta_i}{\phi_i} \tag{3.23}$$

$$\frac{G^{E}_{residual}}{R\,T} = -\sum_i q_i\, x_i \ln\left[\sum_j \theta_j\, \tau_{ji}\right] \tag{3.24}$$

and

$$\theta_i = \frac{q_i\, x_i}{\sum_j q_i\, x_i} \tag{3.25}$$

$$\phi_i = \frac{r_i\, x_i}{\sum_j r_j\, x_j} \tag{3.26}$$

$$\tau_{ji} = \exp\left[\frac{-u_{ji}}{R\,T}\right] \tag{3.27}$$

q_i and r_i are surface area and volume parameters, u_{ji} are interaction energy parameters, and z is the co-ordination number. This equation is algebraically complex and often its performance is not better than simpler equations.

3.1.3 UNIFAC Group-Contribution Model

In the UNIFAC (UNIQUAC Functional Group Activity Coefficient) model, the activity coefficient is assumed to be made of two contribution; as in the UNIQUAC model i.e. combinatorial and residual terms. The combinatorial part is taken from UNIQUAC and residual part from the ASOG model. The UNIFAC group-contribution activity coefficient model is given by:

$$\ln \gamma_i = \ln \gamma_i^{\,c} + \ln \gamma_i^{\,r} \tag{3.28}$$

$$\ln \gamma_i^{\,r} = \ln\left(\frac{\phi_i}{x_i}\right) + 1 - \left(\frac{\phi_i}{x_i}\right) - (z/2)\, q_i \left[\ln\left(\frac{\phi_i}{\theta_i}\right) + 1 - \left(\frac{\phi_i}{\theta_i}\right)\right] \tag{3.29}$$

$$\ln \gamma_i^{\,r} = \sum_k \nu_{ki}\left[\ln \Gamma_k - \ln \Gamma_k^{i}\right] \tag{3.30}$$

where ν_{ki} is the number of groups of type k in molecule i, Γ_k is the activity coefficient of group k at mixture composition, and $\Gamma_k{}^i$ is the activity coefficient of group k at a group composition corresponding to pure component i. Γ_k and $\Gamma_k{}^i$ are given by :

$$\ln \Gamma_k = (z/2)\, Q \left[-[\ln(\textstyle\sum_m \theta_m \tau_{mk})] + 1 - \sum_i \frac{\theta_i \tau_{ki}}{\sum_j \theta_j \tau_{ji}} \right] \tag{3.31}$$

$$\phi_i = \frac{x_i r_i}{\sum_i x_i r_j} \tag{3.32}$$

$$r_i = \textstyle\sum_k \nu_{ki} R_k \tag{3.33}$$

$$\theta_k = \frac{n_k (z/2) Q_k}{\sum_m n_m (z/2) Q_m} \tag{3.34}$$

$$\tau_{mk} = \exp\left[\frac{-a_{mk}}{T} \right] \tag{3.35}$$

R_k and Q_k are volume and surface area parameters, which are obtained from van der Waals volume (V_w) and the van der Waals area of a group (A_w), as suggested by Abrahams and Prausnitz (1975):

$$R = \frac{V_w}{15.17} \qquad (V_w \text{ in } cm^3/mol) \tag{3.36}$$

$$(\frac{z}{2})\, Q = \frac{A_w}{\left[2.5 \times 10^9\right]} \qquad (A_w \text{ in } cm^2/mol) \tag{3.37}$$

The non-symmetric group interaction parameters, a_{mk}, are regressed from experimental activity coefficients or phase equilibrium data. Group interaction parameters are assumed to be independent of temperature.

The UNIFAC model applies for the multi-component, non-electrolyte, non-polymeric mixtures in non-critical conditions. Parameters for a large number of groups are available, hence UNIFAC could be used for many systems. A large amount of development effort has been given to UNIFAC, hence it is the most popular activity coefficient model. One of the main reason for the popularity of a group-contribution model is that, only few groups can represent many compounds. Usually, a much smaller number of parameters is required in a group contribution model than in other models.

3.1.4 Modified UNIFAC Group-Contribution Model

Larsen, Rasmussen, and Fredenslund (1987) have introduced some modifications in the UNIFAC model. These modifications result in better prediction of vapor-liquid equilibria, much better predictions of excess enthalpies, and the same quality of prediction of liquid-liquid equilibria (as compared to the UNIFAC with the LLE parameters). Modifications are:

1. Interaction parameters have been made temperature dependent:

$$a_{ji} = a_{ji,1} + a_{ji,2}\,(T - T_0) + a_{ji,3}\left[T \ln \frac{T_0}{T} + T - T_0\right] \qquad (3.38)$$

where T_0 is an arbitrary reference temperature, chosen to be 298.15 K. The interaction parameters $a_{ji,1}$, $a_{ji,2}$, and $a_{ji,3}$ are regressed from experimental equilibrium data.

2. The combinatorial term has been modified:

The Stavermann-Guggenheim correction has been dropped because the contribution

from this term was negligible and sometimes it produced a negative value of the combinatorial term, which is unrealistic. The volume fraction has been modified as suggested by Kikic et al. (1980):

$$\ln \gamma_i^{\,r} = \ln (\omega_i/x_i) + 1 - (\omega_i/x_i) \tag{3.39}$$

where

$$\omega_i = \frac{x_i\, r_i^{\,2/3}}{\sum_j x_j\, r_j^{\,2/3}} \tag{3.40}$$

The modification in the volume fraction term gives much better prediction of VLE in alkane mixtures, especially when the difference in the size of the components is large. Modified UNIFAC has also been applied for predictions at high temperature and pressure with good results, by Gupte(1986).

Group size parameters (R_k and (z/2)Q_k), and group interaction parameters ($a_{ij,1}$, $a_{ij,2}$, and $a_{ij,3}$) for 21 main groups and 45 subgroups have been published (Larsen et al. 1987), which covers a wide variety of components.

The modified UNIFAC group-contribution model is used in this work to predict activity coefficients of amino acids and antibiotics. Some new groups are introduced. Size and interaction parameters are estimated for the new groups. For the sake of simplicity and due to insufficient experimental data, the interaction parameters $a_{ij,2}$ and $a_{ij,3}$ are taken to be zero for new groups: i.e.,

$$a_{ij} = a_{ij,1} \tag{3.41}$$

3.2 New Groups for Antibiotics and Amino Acids

3.2.1 Amino Acid Groups

To be able to use the Modified UNIFAC for activity coefficient predictions of eight amino acids (alanine, amino-butyric acid, glycine, hydroxy-proline, proline, serine, threonine, and valine), two main groups (or four subgroups) have been defined. These are:

1. *Glycine* group
2. *Proline* group

These groups represent both $d-$ and $l-$ optical isomers, and activity coefficients of $d-$ and $l-$ optical isomers are assumed to be identical in this work. The chemical structures of above groups are listed in the Table 5.1 . Note that UNIFAC is unable to distinguish between optical isomers. All eight amino acids studied are composed of the two new groups and the other standard UNIFAC groups, CH_2, CH_3 ...etc. To use this model it is necessary to regress interaction energies a_{ji} between the new groups and other standard groups.

3.2.2 Antibiotic Groups

For six antibiotics (anisomycin, carbomycin A, chloramphenicol, chloramphenicol A, griseofulvin, and hygromycin A), five new groups are defined. These new groups are:

1. *Anisomycin* group
2. *Carbomycin A* group
3. *Chloramphenicol* group
4. *Griseofulvin* group
5. *Hygromycin A* group

These five groups are large and with a variety of chemical structures.

Anisomycin, *carbomycin A*, *griseofulvin*, and *hygromycin A* groups are the same as the respective antibiotic molecules. It would be possible to break these groups into smaller groups, but due to limited experimental data this was not done. One may argue that, if one molecule is taken as a group, then why worry about a group contribution model. But the group contribution approach is still applied to the solvents, as the solvent molecules are divided into small groups, which still aids in reducing the total number of parameters required. The model will have predictive capabilities of solvents with the same structural groups as those solvents used in regressing parameters.

3.3 Solid - Liquid Equilibria

Equilibrium between a pure solid and the some species in a solution requires equal fugacities; i.e.

$$f_{iL} = f_{iS}{}^{o} \tag{3.42}$$

The fugacity of substance i in solution can be calculated from an activity coefficient model and a standard state fugacity, so that at saturation (when $x_i = x_i{}^{S}$ and $\gamma_i = \gamma_i{}^{S}$),

$$\gamma_i{}^{S} x_i{}^{S} f_{iL}{}^{o} = f_{iS}{}^{o} \tag{3.43}$$

and therefore

$$\gamma_i{}^{S} x_i{}^{S} = \frac{f_{iS}{}^{o}}{f_{iL}{}^{o}} \tag{3.44}$$

3.3.1 Antibiotic Solubility

Assuming that the right hand side of the Equation (3.44) is known, the

equation can be solved for $\gamma_i{}^S$. The UNIFAC energy interaction parameters for the antibiotic groups with standard UNIFAC groups have been evaluated from solubility data for a given antibiotic in several solvents with similar chemical make-up.

The ratio $(f_{iS}{}^o/f_{iL}{}^o)$ for the antibiotics has been evaluated from melting point data as described in section 4.5 .

3.3.2 Amino Acid Solubility

The amino acids are almost completely converted to the zwitterion form and/or to one of the ions with a net charge when dissolved in water. In treating the amino acids it has been assumed that activity coefficients of all the ionic species are equal and can be evaluated from Modified UNIFAC using the mole fraction in the UNIFAC as if no ionization occurred at all. However, an accurate representation of solubility variation with pH requires attention to the ionization reactions.

Using x_A for undissociated amino acid actually present in a saturated solution, Equation (3.43) is:

$$x_A \gamma_A f_{AL}{}^o = f_{AS}{}^o \tag{3.45}$$

The zwitterion is in chemical equilibrium with undissociated A. Hence from the definition of the dissociation equilibrium constant and assumed equal activity coefficients of all the amino acid ions,

$$K_D = \frac{m_{A\pm}}{m_A} = \frac{x_{A\pm}}{x_A} \tag{3.46}$$

Therefore, at equilibrium,

$$x_{A\pm}\,\gamma_A\left[\frac{f_{A_L}{}^{o}}{K_D}\right] = f_{A_S}{}^{o} \tag{3.47}$$

The amino acids decompose without melting and $f_{A_L}{}^{o}$ always refers to a hypothetical liquid state. It is convenient to think of $(f_{A_L}{}^{o}/K_D)$ as being $f_{A_{\pm L}}{}^{o}$, an equally hypothetical liquid state for the pure zwitterion. Then

$$x_{A\pm}\,\gamma_A = \frac{f_{A_S}{}^{o}}{f_{A_{\pm L}}{}^{o}} \tag{3.48}$$

Because the actual mole fraction of the zwitterion depends on the solution pH, the solubility of amino acids in water will vary as pH is varied.

If $x_A{}^{o}$ is the mole fraction of the amino acid calculated as if there were no ionization.

$$x_A{}^{o} = x_{A\pm} + x_{A+} + x_{A-} \tag{3.49}$$

(The mole fraction of the undissociated amino acid is assumed negligible.) Using the equilibrium constant K_1 and K_2 of Equations (2.7) and (2.8), yields

$$x_{A+} = \frac{[H^+]\,x_{A\pm}}{K_1} \tag{3.50}$$

and

$$x_{A-} = \frac{K_2\,x_{A\pm}}{[H^+]} \tag{3.51}$$

Then

$$x_A{}^{o} = x_{A\pm}\left[1 + \frac{[H^+]}{K_1} + \frac{K_2}{[H^+]}\right] \tag{3.52}$$

The equation for the solid–solution equilibrium is then

$$x_A{}^{o}\gamma_A = \left[\frac{f_{A_S}{}^{o}}{f_{A_{\pm_L}}{}^{o}}\right]\left[1 + \frac{[H^+]}{K_1} + \frac{K_2}{[H^+]}\right] \tag{3.53}$$

When γ_A is known as a function of amino acid mole fraction (on an unionized basis) and when $(f_{A_S}{}^{o}/f_{A_{\pm_L}}{}^{o})$ is known as function of temperature, Equation (3.53) can be used to determine the solubility of the amino acid in solutions of varying pH.

In this study, γ_A data from osmotic coefficient measurements have been used to determine the Modified UNIFAC a_{ij} energy parameters. Solubility information for the amino acids in neutral water at various temperatures have been used to correlate $(f_{A_S}{}^{o}/f_{A_{\pm_L}}{}^{o})$ in the form

$$\ln\left[\frac{f_{A_S}{}^{o}}{f_{A_{\pm_L}}{}^{o}}\right] = A' - \frac{B'}{T} \tag{3.54}$$

by using Equation (3.53) at $[H^+]$ calculated from Equation (2.13).

The nature of available data to permit the correlation is discussed in the next chapter.

Chapter 4.

Experimental Data

In this work an activity coefficient model for amino acids dissolved in water is derived from osmotic coefficient data. The standard state fugacity ratios of amino acids are derived from solubility in neutral water and dissociation constants are required to see the effect of pH on the solubility. Solubilities of antibiotics in various solvents and the antibiotic fusion temperature are required to construct the antibiotic activity coefficient model.

4.1 Osmotic Coefficients of Amino Acids

The original measurement of osmotic coefficient data and their conversion to activity coefficient data was done by Smith et al. (1937, 1940a, 1940b), Hutchens et al. (1963), and Ellerton et al. (1964). All these measurements were done at 25 ^{o}C. The osmotic coefficients were measured by the isopiestic method as suggested by Robinson and Sinclair (1934) using aqueous sucrose solutions as reference. In the isopiestic method, two different solutions are brought into thermodynamic equilibrium by bringing their vapors into physical contact. The two samples are allowed to exchange vapors of the solvent until the vapor pressure of the solvent equalizes in the two samples. Final concentrations of the samples are measured. One of the samples is a reference sample in which the osmotic coefficient of the solvent is known as a function of solute molality. At equilibrium, water has the same activity in both solutions and the water activity is known in the sucrose solution.

The osmotic coefficient of the sucrose solution is ϕ_S and is defined by

$$\phi_S = \frac{-\ln a_W}{(x_S/x_W)} \tag{4.1}$$

or

$$\phi_S = \frac{-\ln a_W}{m_S\left[\frac{M_W}{1000}\right]} \tag{4.2}$$

where x_S and x_W are sucrose and water mole fractions and m_S is the sucrose molality. Similar equations define the osmotic coefficient of the amino acid solution. Equal water activities at equilibrium therefore imply that

$$\phi_A = \phi_S\left[\frac{m_S}{m_A}\right] \tag{4.3}$$

Smith and Smith (1937, 1939) correlated ϕ_A against amino acid molality with

$$\phi_A = 1 + a\,m_A + b\,m_A{}^2 + c\,m_A{}^3 + d\,m_A{}^4 \tag{4.4}$$

The activity coefficient for the amino acid in the solution is calculated by integrating the Gibbs-Duhem equation as described in Lewis and Randall (1961), page 265. The equation is

$$\ln \gamma_A{}^m = \phi_A - 1 + \int_0^{m_A}\left[\frac{\phi_A - 1}{m_A}\right] dm_A \tag{4.5}$$

which yields

$$\ln \gamma_A{}^m = 2\,a\,m_A + (3/2)\,b\,m_A{}^2 + (4/3)\,c\,m_A{}^3 + (5/4)\,d\,m_A{}^4 \tag{4.6}$$

A point of subtlety is that $\gamma_A{}^m$ given by Equation (4.5) and (4.6) is in the convention that $\gamma_A{}^m \rightarrow 1$ as $m_A \rightarrow 0$. For use with Modified UNIFAC, the data given for the activity coefficients in the cited reference must be adjusted to the UNIFAC standard state;

Table 4.1a Molal Activity Coefficients (*-Log* 10)

for Amino Acids at 25 ^{o}C, (Fasman, 1976).

Molality	Alanine	α-amino n-butyric acid	Glycine	Hydroxy-proline
0.2	–0.002	–0.0046	0.0176	0.000
0.3	–0.003	–0.0070	0.0232	0.000
0.5	–0.005	–0.0122	0.0421	–0.001
0.7	–0.007	–0.0177	0.0489	–0.001
1.0	–0.010	–0.0275	0.0579	–0.003
1.5	–0.015	–0.0421	0.0868	–0.006
1.86	–0.019	-	-	-
2.0	-	–0.0664	0.1041	–0.011
2.5	-	-	0.1181	-
3.0	-	-	0.1297	-
3.114	-	-	0.1321	-

Table 4.1b Molal Activity Coefficients ($-Log\ 10$)

for Amino Acids at 25 ^{o}C, (Fasman, 1976).

Molality	Proline	Serine	Threonine	Valine
0.2	–0.008	0.022	0.005	–0.013
0.3	–0.012	0.032	0.007	–0.019
0.5	–0.020	0.052	0.011	–0.032
0.7	–0.029	0.070	0.015	-
1.0	–0.040	0.094	0.018	-
1.5	–0.060	0.127	0.022	-
2.0	–0.081	0.152	0.025	-
2.5	–0.103	0.174	-	-
3.0	–0.126	0.193	-	-
3.5	–0.148	0.207	-	-
4.0	–0.174	0.220	-	-
5.0	–0.224	-	-	-
6.0	–0.262	-	-	-
7.0	–0.296	-	-	-
7.3	–0.302	-	-	-

$$\left[\frac{\gamma_A}{\gamma_A^{\infty}}\right] = \frac{\gamma_A{}^{m}}{(1 - x_A)} \tag{4.7}$$

The values used in fitting UNIFAC parameters were taken from a table in the CRC Handbook of Biochemistry and Molecular Biology, Fasman (1976). They appear in Tables 4.1a and 4.1b.

4.2 Solubilities of Amino Acids in Water

Solubilities of *dl*-alanine, glycine, and *dl*-valine in water at various temperatures are taken from Fasman(1976). The original measurement of these data was done by Dalton and Schmidt (1933); Hade (1962); and Dunn, Ross, and Read (1933). These data are listed in the Table 4.2 .

The experimental procedure to estimate the solubility of amino acids includes preparation of a saturated sample, evaporation of the solvent, and weighting the residue. Care has to be taken to avoid the problems of conversion of amino acid at high temperatures and supersaturation.

4.3 Dissociation Constants of Amino Acids

Dissociation reaction equilibrium constant pK_1 and pK_2 for *dl*-alanine, glycine, and *dl*-valine, at two temperatures were taken from Fasman(1976). The values were extrapolated to the required temperatures. These data and their extrapolation formulae are listed in Table 4.3 . The original measurements of these data were by Christensen, Izatt, and Hansen (1967), Christensen, Oscarson, and Izatt (1968), Christensen, Izatt, Wrathall, and Hensen (1969), and King (1957).

The pK values are experimentally determined by titration of the amino acid. It

Table 4.2 Solubilities of Amino Acids in Neutral Water at Various Temperatures.

No.	Temperature (Kelvin)	*dl*-Alanine (g/kg water)	Glycine (g/kg water)	*dl*-Valine (g/kg water)
1	273.15	121.1	141.8	59.6
2	283.15	137.8	180.4	63.3
3	293.15	156.7	225.2	68.1
4	303.15	178.3	275.9	74.2
5	313.15	202.9	331.6	81.7
6	323.15	230.9	391.0	91.1
7	333.15	262.7	452.6	102.8
8	343.15	299.0	513.9	117.4
9	353.15	340.1	572.7	135.8
10	363.15	387.0	626.2	158.9
11	373.15	440.0	671.7	188.1

Table 4.3 Dissociation constants of Amino Acids.

(Temperature, in bracket)

pK	*dl*-Alanine	Glycine	*dl*-Valine
pK1	2.348 (298.15 K) 2.328 (313.15 K) 1.930 + 124.488/T	2.351 (298.15 K) 2.327 (313.15 K) 1.850 + 149.385/T	2.286 (298.15 K) 2.310 (323.15 K) 2.596 – 92.493/T
pK2	9.870 (298.15 K) 9.510 (313.15 K) 2.354 + 2240.776/T	9.777 (298.15 K) 9.460 (313.15 K) 3.159 + 1973.128/T	9.719 (298.15 K) 9.124 (323.15 K) 2.028 + 2293.063/T

is very simple to extract this information from the titration curve of pH versus equivalent OH^- concentration. pK_1 is equal to the pH at the point where the concentration of the anion equals the concentration of the amino acid; i.e., where the acid is exactly half neutralized. pK_2 is equal to the pH at the point where the concentration of the cation is equal to the concentration of the amino acid in the solution.

4.4 Solubility of Antibiotics

The solubility data for antibiotics, that were used in this work were measured by Weiss, Andrew and Wright (1957), Andrew and Weiss (1959), and by Marsh and Weiss (1967). Many of these data were also compiled by Tomlinson (1985) in the IUPAC Solubility Data Series. The solubility data are listed in the Tables 4.4a and 4.4b .

The same experimental procedure to estimate the solubility was followed by the above three groups of workers. 200 mg. of the antibiotic is added to 10 mL of the solvent in a 15 mL. glass-stoppered test tube. The tube is shaken by hand for two minutes at room temperature. The solubility is considered to be greater than 20 g/L if all the antibiotic appeared to be in the solution. If there was visible insoluble material, the suspension was centrifuged within an hour of the operation. The clear part from the centrifuge was filtered by vacuum through a medium-porosity sintered glass funnel. Two mL of the clear filtrate was added to a weighing bottle and evaporated by heating at 100 ^oC. The residue was further dried for three hours at 60 ^oC. After cooling, the bottle was reweighed and the solubility in g/L was calculated. The precision in the measurement of the solubility is $\pm$ 0.05 g/L for large solubilities and $\pm$ 0.0025 g/L for the low solubilities of antibiotics.

Table 4.4a Experimental Solubilities of Antibiotics

Solute	Solvent	Temp. (K)	Solubility g/L	Reference
Anisomycin	Cylcohexane	301.15	0.117	Andrew et al.(1959)
Anisomycin	Iso-Octane	301.15	0.010	"
Anisomycin	Benzene	301.15	3.39	"
Anisomycin	Toluene	301.15	1.52	"
Carbomycin A	Cyclohexane	301.15	0.44	Weiss et al.(1957)
Carbomycin A	Iso-Octane	301.15	0.065	"
Carbomycin A	Benzene	301.15	18.6	"
Carbomycin A	Toluene	301.15	3.78	"
Carbomycin A	Iso-Propanol	301.15	4.65	"
Carbomycin A	Iso-Amyl alcohol	301.15	8.1	"
Chloramphenicol	Benzene	301.15	0.26	"
Chloramphenicol	Toluene	301.15	0.145	"
Chloramphenicol	Benzyl alcohol	301.15	11.6	"
Chloramphenicol	Iso-Amyl alcohol	301.15	17.3	"
Chloramphenicol Palmitate	Cyclohexane	301.15	0.335	"
Chloramphenicol Palmitate	Iso-Octane	301.15	0.085	"

Table 4.4b Experimental Solubilities of Antibiotics

Solute	Solvent	Temp. (K)	Solubility g/L	Reference
Chloramphenicol Palmitate	Benzene	301.15	10.7	Weiss et al.(1957)
Chloramphenicol Palmitate	Toluene	301.15	13.52	"
Griseofulvin	Cyclohexane	294.15	0.025	Marsh et al.(1967)
Griseofulvin	Ethanol	294.15	5.445	"
Griseofulvin	Iso-Propanol	294.15	1.32	"
Griseofulvin	Iso-Amyl alcohol	294.15	1.045	"
Griseofulvin	Ethyl acetate	294.15	10.645	"
Griseofulvin	Iso-Amyl acetate	294.15	2.890	"
Hygromycin A	Cyclohexane	301.15	0.037	Andrew et al.(1959)
Hygromycin A	Ethanol	301.15	16.10	"
Hygromycin A	Iso-Propanol	310.15	11.05	"
Hygromycin A	Benzene	301.15	0.03	"
Hygromycin A	Benzyl alcohol	301.15	9.00	"
Hygromycin A	Iso-Amyl alcohol	310.15	9.65	"
Hygromycin A	Ethyl acetate	301.15	0.30	"
Hygromycin A	Iso-Amyl acetate	310.15	0.40	"

4.5 Entropy of Fusion of the Antibiotics

The entropy of fusion of the antibiotics, is taken in this study to be a constant value of 13.5 *cal/mol K*. Yalkowsky (1979) reported that the entropy of fusion of many drugs and rigid molecules of intermediate size can be estimated as 13.5 *cal/mol K*. Walden (1908) observed that the entropy of fusion of most organic compounds falls in a fairly narrow range around 13 *cal/mol K*. Experimental data by Tsonopoulos and Prausnitz (1971) are in good agreement with the observations of Walden (1908), that the entropy of fusion tends to be nearly constant, but 13.5 appears to be a better average value.

The entropy of fusion, ΔS_f, could be divided into four parts i.e. expansional, positional, rotational, and internal:

$$\Delta S_f = \Delta S_{exp.} + \Delta S_{pos.} + \Delta S_{rot.} + \Delta S_{int.} \qquad (4.8)$$

1. expansional : due to increase in the molecular distance on melting, which is evident by the increase in the volume.
2. positional : due to change from an ordered molecular center of gravity to the random positions in the liquid.
3. rotational : due to change in the ordered arrangement of major axes to randomly oriented arrangement in the liquid.
4. internal : due to change in the uniform conformation of a flexible molecule of the crystal into the random conformation of molecules in the liquid. Since most drug molecules are rigid, the internal contribution to the entropy is not applicable.

The most likely values for the expansional, positional, and rotational entropies are 2, 2.5, and 9.0 *cal/mol K* respectively. The total entropy of fusion can be estimated as:

$$\Delta S_f = 2.0 + 2.5 + 9.0 = 13.5 \quad cal/mol\ K \tag{4.9}$$

The entropy of fusion and the melting temperature are needed to evaluate the activity coefficients of the antibiotics in saturated solutions.

According to the Scatchard-Hildebrand equation, the solubility of a solid in a liquid solution is given by:

$$\ln (x_i{}^{s} \gamma_i{}^{s}) = \frac{-\Delta H_f}{R} \left[\frac{1}{T} - \frac{1}{T_m} \right] \tag{4.10}$$

where T is the solution temperature, T_m is the melting point of the solid solute, $x_i{}^{s}$ is the mole fraction of the solute at saturation, $\gamma_i{}^{s}$ is the activity coefficient of the solute at saturation and ΔH_f is the heat of fusion of the solute. Equation (4.10) is based on the identity the

$$\ln \left[\frac{f_S{}^{o}}{f_L{}^{o}} \right] = \int_{T_m}^{T} \frac{\Delta H_f}{R\, T^2}\, dT \tag{4.11}$$

and on assumption that ΔH_f is independent of temperature.

At the melting temperature T_m, the solid and liquid solute are at equilibrium, the Gibb's free energy of fusion (ΔG_f) of the solute equals to zero.

$$\Delta G_f = 0 = \Delta H_f - T_m \Delta S_f \tag{4.12}$$

which implies that

$$\Delta H_f = T_m \Delta S_f \tag{4.13}$$

from equation (4.12) and (4.13):

$$\ln (x_i{}^{s} \gamma_i{}^{s}) = \frac{-\Delta S_f}{R} \left[\frac{T_m}{T} - 1 \right] \tag{4.14}$$

but

$$\Delta S_f = 13.5 \; cal/mol\,K \tag{4.15}$$

hence

$$\gamma_i{}^s = \frac{1}{x_i{}^s} \exp\left[-6.7942 \left[\frac{T_m}{T} - 1 \right] \right] \tag{4.16}$$

From Equation (4.16) the activity coefficients of antibiotics at saturation ($\gamma_i{}^s$) are calculated using solubility and melting point information. For each antibiotic in the study, $\gamma_i{}^s$ is calculated for many organic solvents at a given temperature. These values are listed in Tables 6.2a and 6.2b. Melting point temperatures are given in Table 4.5 . These data were taken from the Merck Index (1970).

4.6 Chemical Structures

The chemical structures of amino acids are taken from Zubay(1988) and Fasman (1976). The chemical structure, molecular weight, and melting point temperature of antibiotics are taken from "The Merck Index" edited by Windholz (1976). The structure of the antibiotics were also rechecked from other publications. These properties are listed in Table 2.1, and Figure 2.1 .

Chapter 5.

Parameter Estimation

The Modified UNIFAC group contribution model has been used to correlate the activity coefficients of amino acids and antibiotics. The required size and area parameters were determined from molecular structure and tabulated parameters for the constituent groups. The interaction energy parameters between new groups and standard groups within UNIFAC have been estimated.

5.1 Size Parameters

Volume (R) and surface area with contact number ((z/2) Q) parameters are obtained from the molecular structure:

$$R = \frac{V_W}{15.17} \tag{5.1}$$

$$(\frac{z}{2})\,Q = \frac{A_W}{2.5\times10^9} \tag{5.2}$$

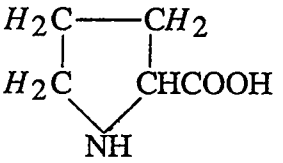
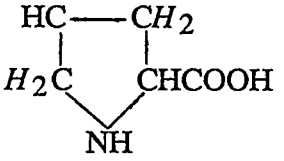
where V_W is the van der Waals volume in cm^3/mol and A_W is the van der Waals surface area in cm^2/mol. The normalization factors 15.17 and 2.5×10^9 are those derived by Abrams and Prausnitz (1975). These van der Waals volume and surface area parameters are assumed to be additive in groups contribution models; i.e.:

$$V_W = \Sigma_i\ V_{W,i} \tag{5.3}$$

$$A_W = \Sigma_i\ A_{W,i} \tag{5.4}$$

Van der Waals area and volume parameters are taken from Bondi (1968). Complex antibiotic molecules are broken into groups. The size parameters for the

Table 5.1 Group Size Parameters (Amino Acids)

No.	Group Name	Subgroup Structure	Parameter R	Parameter (z/2) Q
1.	*Glycine*	$CH_2(NH_2)COOH$	2.671	2.914
		$CH(NH_2)COOH$	2.443	2.602
2.	*Proline*		4.167	3.878
			3.939	3.566

new groups in the amino acids are listed in Table 5.1 and those for the new antibiotic groups are listed in Table 5.2 . For some antibiotic groups the R and (z/2)Q parameters are large numbers, because they contain many standard groups.

5.2 Interaction Parameters

Group interaction parameters, a_{ji} are temperature dependent in Modified UNIFAC :

$$a_{ji} = a_{ji,1} + a_{ji,2}\,(T - T_0) + a_{ji,3}\left[\ln\frac{T_0}{T} + T - T_0\right] \qquad (5.5)$$

For the new groups of amino acids and antibiotics, parameters $a_{ji,2}$ and $a_{ji,3}$ are taken to be zero, hence parameter a_{ji} is taken to be temperature independent:

$$a_{ji} = a_{ji,1} \qquad (5.6)$$

This is done arbitrarily to reduce the total number of interaction parameters to be regressed. Interaction parameters are estimated at temperature 298.15 K (T_0), hence activity coefficient prediction at this temperature would be same as with the temperature dependent parameters. In fact, for practical situations we only need to know activity coefficients of amino acids and antibiotics near room temperature, because the extraction and purification processes for these biochemicals are designed near room temperature and drug design considerations are also at human/animal body temperatures. Parameters a_{ji} are estimated by minimizing an objective function. Parameters are listed in Tables 5.3, 5.4a and 5.4b .

Table 5.2 Group Size Parameters (Antibiotics)

No.	Group Name	Chemical Formula	R	(z/2) Q
1.	*Anisomycin*	$C_{14}H_{19}NO_4$	10.1258	7.828
2.	*Carbomycin A*	$C_{42}H_{67}NO_{16}$	31.6568	25.595
3.	*Chloramphenicol*	$C_{11}H_{11}Cl_2N_2O_4$	9.8957	7.776
4.	*Griseofulvin*	$C_{17}H_{17}ClO_6$	11.4753	10.932
5.	*Hygromycin A*	$C_{23}H_{29}NO_{12}$	19.5745	16.588

Table 5.3 Group Interaction Parameters (Amino Acids)

Group k	$a_{Glycine-k}$	$a_{k-Glycine}$	$a_{Proline-k}$	$a_{k-Proline}$
CH_2	2281.651	1916.836	-	-
OH	6769.627	–336.169	–278.471	123.096
H_2O	740.876	–13.046	346.396	–346.399

Table 5.4a Group Interaction Parameters (Antibiotics)

k	Group k	$a_{k,CH2}$	$a_{CH2,k}$	$a_{k,ACH}$	$a_{ACH,k}$
1.	*Anisomycin*	–581.995	2152.658	–491.233	2691.239
2.	*Carbomycin A*	409.186	–134.375	451.642	–183.849
3.	*Chloramphenicol*	129.660	143.830	4638.009	–137.623
4.	*Griseofulvin*	7141.996	–92.613	-	-
5.	*Hygromycin A*	640.062	–69.383	243.936	42.194

Table 5.4b Group Interaction Parameters (Antibiotics)

k	Group k	$a_{k,OH}$	$a_{OH,k}$	$a_{k,CCOO}$	$a_{CCOO,k}$
1.	*Anisomycin*	-	-	-	-
2.	*Carbomycin A*	7181.870	–204.200	-	-
3.	*Chloramphenicol*	718.806	–212.671	–1321.783	4780.022
5.	*Griseofulvin*	353.474	–145.354	–3.977	68.440
4.	*Hygromycin A*	204.110	75.549	76.706	240.547

5.2.1 Objective Function for Amino Acids

The objective function, O.F., for amino acids is the sum of squares of differences of natural logarithms of experimental and calculated activity coefficient ratios. The word activity coefficient ratio, here, stands for the ratio of the activity coefficient to the infinite dilution activity coefficient. Such ratio is taken in the calculation only because the experimental data were available in this form. As described in Chapter 4, the osmotic coefficient was used to measure γ for the non-volatile amino acids.

$$O.F. = \sum_{i=1}^{n} \left[\ln \left(\frac{\gamma_{i,exp.}}{\gamma^{\infty}_{i,exp.}} \right) - \ln \left(\frac{\gamma_{i,Mod.UNIFAC}}{\gamma^{\infty}_{i,Mod.UNIFAC}} \right) \right]^2 \qquad (5.7)$$

5.2.2 Objective Function for Antibiotics

The objective function, O.F., is the sum of the squares of difference of experimental and calculated activity coefficients of the antibiotic in the saturated solutions. The experimental activity coefficients have been calculated only from saturation data in various solvents, hence this form of the objective function is adopted.

$$O.F. = \sum_{i=1}^{n} \left[\ln \left(\gamma_i^{s,exp.} \right) - \ln \left(\gamma_i^{s,Mod.UNIFAC} \right) \right]^2 \qquad (5.8)$$

Fredenslund et al. (1977) reported that by using the difference of logarithms in the objective function, in general, one gets a good fit to the experimental activity coefficients.

5.3 Optimization Algorithm

The objective functions have been minimized by means of an extended simplex method. The simplex method does not need complex coding, involve matrix

operations and doesn't require derivatives. The simplex method always converges.

The program used in the calculation is listed in Fredenslund et al. (1977). The program and the associated subroutines for calculating the activity coefficient had to be modified slightly to accommodate the differences between the original UNIFAC equations and Modified UNIFAC.

The basic idea behind the simplex method is to make a simplex of (n+1) dimensional space for n parameters to be estimated. For a two parameter case,it can be pictured as movement of an amoeba that slides over the surface of the objective function, trying to find the deepest place to rest. For a two parameter search, the simplex is a triangle, and for fitting four parameters the simplex is a pentagon. Each vertex of the simplex has an associated response value equal to the objective function at that point.

The simplex algorithm optimizes the objective function by moving in the "downhill" direction, by a series of reflections, expansions and contractions. At each iteration the value of the worst vertex gets improved. At convergence, all the vertices are at their minimum values. The initial simplex is made by changing initial parameters one at a time by 10%. Reflection, expansion and contraction coefficients used are 1, 0.5, and 2 respectively.

Before accepting the final set of parameters, several remote initial guesses were tried, to make sure that we had not reached a false convergence at a point other than the minimum. In fact, different initial guesses of parameters yielded the same set of final parameters. A typical set of initial guesses could be (500, –500) and (–500, 500).

5.4 Fugacity Ratio Parameters

The fugacity ratios (${f_{iS}}^{o}/{f_{i\pm_L}}^{o}$) for solid and liquid amino acids were correlated as a function of temperature by:

$$\ln\left[\frac{{f_{iS}}^{o}}{{f_{i\pm_L}}^{o}}\right] = A'_i - \frac{B'_i}{T} \qquad (5.9)$$

For each amino acid, solubility of the solid in neutral water permitted calculation of the resulting pH. The mole fraction at saturation permitted calculation of the Modified UNIFAC activity coefficient that was correlated from osmotic coefficient data. This combination of information gave values for ln (${f_{iS}}^{o}/{f_{i\pm_L}}^{o}$) at several temperatures.

Parameters A'_i and B'_i were calculated by fitting an line to the curve ln(${f_{iS}}^{o}/{f_{i\pm_L}}^{o}$) versus (1/T). The sum of square deviations in ln(${f_{iS}}^{o}/{f_{i\pm_L}}^{o}$) values was minimized. The program used was taken from the listing in "Numerical Recipes" by Press et al. (1986). This program provides the 95% confidence limits for the regressed parameters. The parameters and their confidence limits are reported in Table 5.5 .

Table 5.5 ($f_{A_S}{}^{o} / f_{A_{\pm L}}{}^{o}$), T Correlation Constants.

$$Ln\left[\frac{f_{A_S}{}^{o}}{f_{A_{\pm L}}{}^{o}}\right] = A' - \frac{B'}{T}$$

(95% confidence limits in the brackets)

No.	Amino Acid	A′	B′ (Kelvin)
1.	*dl*-Alanine	2.060 (±0.125)	1110.971 (±39.956)
2.	Glycine	2.042 (±0.384)	885.689 (±121.870)
3.	*dl*-Valine	2.305 (±0.116)	1217.946 (±37.095)

Chapter 6.

Results and Discussion

Modified UNIFAC size parameters and interaction parameters for new groups of amino acids and antibiotics are presented in Tables 5.1, 5.2, 5.3, 5.4a and 5.4b. The results of correlating energy interaction parameters and the solubility of amino acids and antibiotics are presented here.

6.1 Activity Coefficient Ratios of Amino Acids

Activity coefficient ratios (γ/γ^{∞}) of the five amino acids alanine, glycine, hydroxy-proline, proline, and serine have been used to regress energy interaction parameters. Predictions are compared with the experimental results. The overall summary of these results is in Table 6.1 .

Seventeen data points of γ/γ^{∞} of alanine and glycine in aqueous solutions have been used to estimate four Modified UNIFAC interaction parameters $a_{glycine-H2O}$, $a_{H2O-glycine}$, $a_{glycine-CH2}$, and $a_{CH2-glycine}$. In correlations of the activity coefficient ratios (γ/γ^{∞}) of alanine and glycine, the root mean square error is 8.97% and 4.20% respectively. The root mean square error used here is (for any value X)

$$\%\ rms\ error \quad = \quad \frac{100}{N^{1/2}} \left[\sum \left[1 - \frac{X_{pred.}}{X_{exp.}} \right]_i^2 \right]^{1/2} \qquad (6.1)$$

On the addition of the CH_2 groups to the glycine, first γ^{∞} decreases and then increase on addition of more CH_2 groups. Chemical structure of the glycine, alan-

Table 6.1 Overall Representation of Experimental γ Data for Amino Acids with Modified UNIFAC

No.	Amino Acid	No. of Data points	% Root Mean Square Error in Prediction	Predicted γ^{∞} at 298.15 K	No. of parameters regressed
1	Glycine	10	4.20	9.20	}4
2	Alanine	7	8.97	6.43	
3	Amino-butyric Acid	7	17.34	10.00	0
4	Valine	3	12.15	17.90	0
5	Serine	11	3.32	4.88	2
6	Threonine	7	13.78	5.79	0
7	Proline	15	3.01	0.08	2
8	Hydroxy-Proline	7	0.06	0.18	2
	Overall	67	8.52		10

ine, amino-butyric acid, and valine are in the order of increasing CH_2 groups. This interesting behavior of γ^{∞} may be explained on the fact that interaction between CH_2 and *glycine* results in increased γ, but interaction between CH_2 and H_2O tries to decrease it.

Eleven data points for (γ/γ^{∞}) of serine in aqueous solution were used to regress two interaction parameters $a_{glycine-OH}$ and $a_{OH-glycine}$. The r.m.s. error in correlating the activity coefficient ratio is 3.32%. Serine is an addition of an OH group to alanine, but its γ^{∞} is lower than that of alanine. This represent, that the interaction between *OH* and *glycine*, decreases the activity coefficient.

Interaction parameters between *proline* and H_2O (two parameters) were regressed from the fifteen data points of (γ/γ^{∞}) for proline in water, with a root mean square error in the prediction of 3.01%. Similarly, 7 data points for hydroxy-proline were used to predict interaction parameters between *proline* and OH ($a_{proline-OH}$, and $a_{OH-proline}$) with an excellent fit showing an r.m.s. error of 0.06%.

The activity coefficient ratio of amino acids versus mole fraction are plotted in Figures 6.1, 6.2, 6.3, 6.4, and 6.5 . The trend of the activity coefficient ratio versus mole fraction agrees with the experimental behavior for glycine, hydroxy-proline, proline, and serine, but for alanine (Figure 6.2) it is opposite. In the case of alanine, since the qualitative predictions are not good, care should be taken in using Modified UNIFAC for higher concentrations, where the gap between the experimental and predicted activity coefficient ratio may be significant.

Because the amino acids are not volatile γ^{∞} data cannot be determined directly but have to be inferred. Also the UNIFAC model implies a significant value of γ^{∞} for mixtures of large and small molecules, like amino acid and water, even

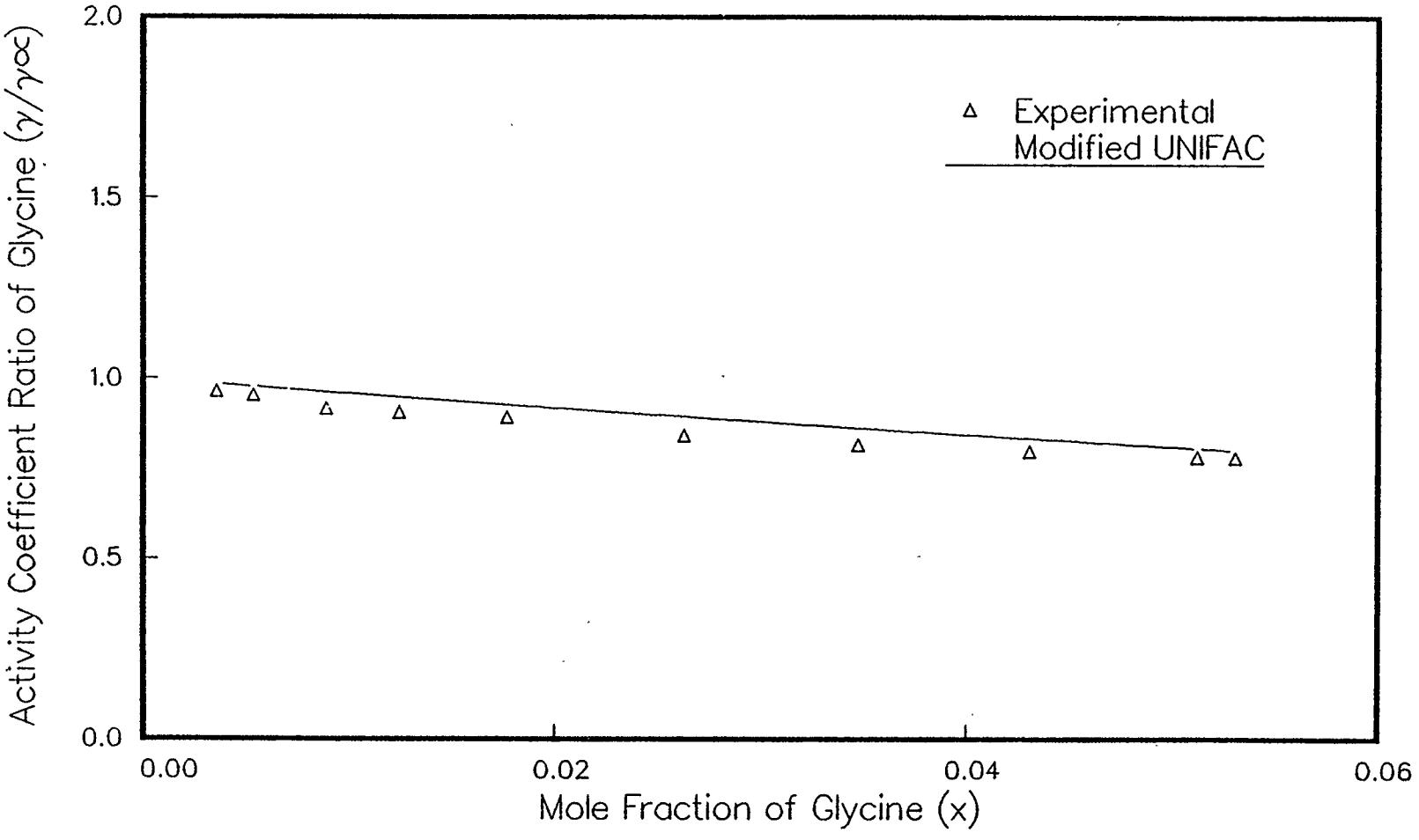


Figure 6.1 Experimental and predicted values for the activity coefficient ratio of glycine in water at 298.15 K. Experimental data from Fasman (1976).

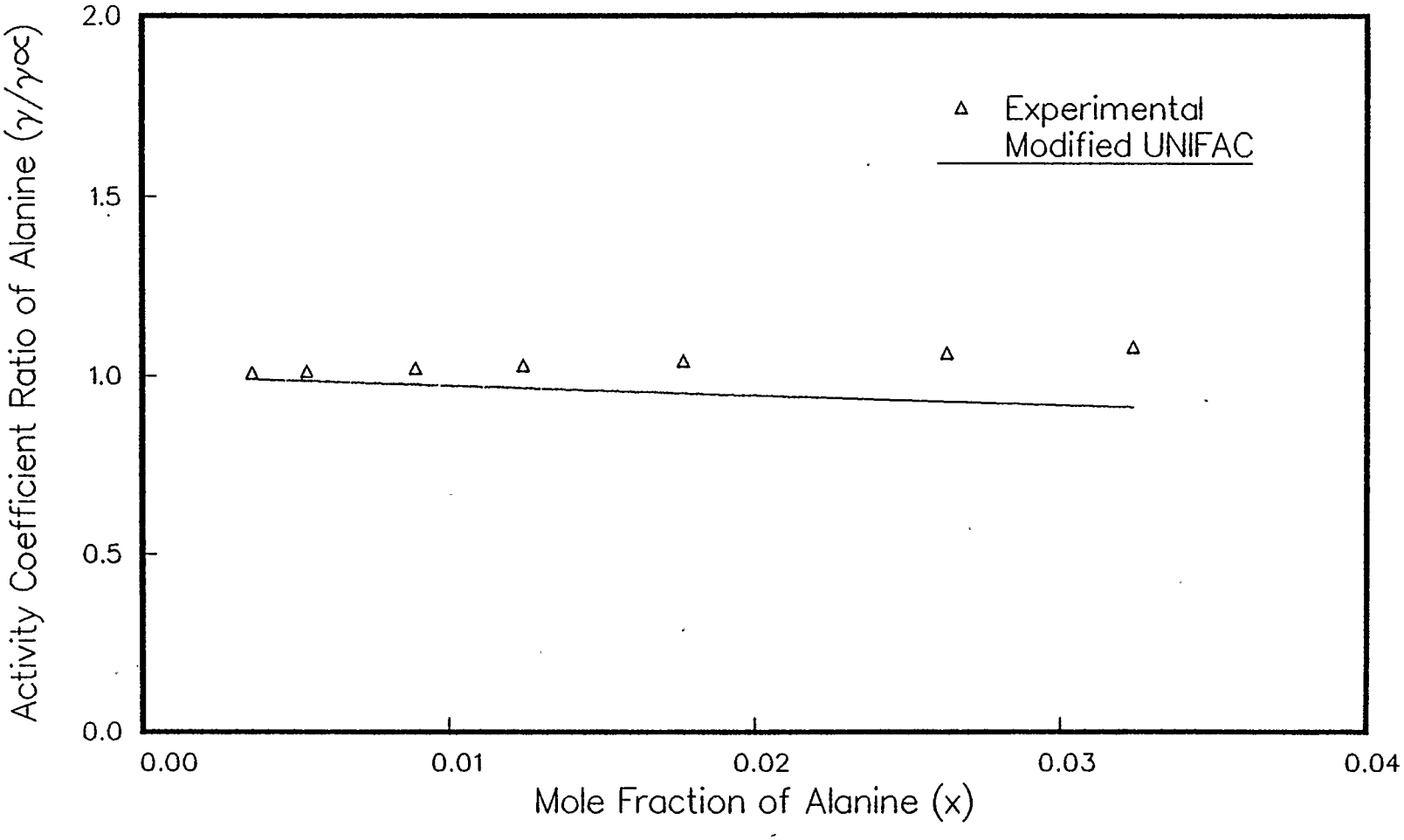


Figure 6.2 Experimental and predicted values for the activity coefficient ratio of alanine in water at 298.15 K. Experimental data from Fasman (1976).

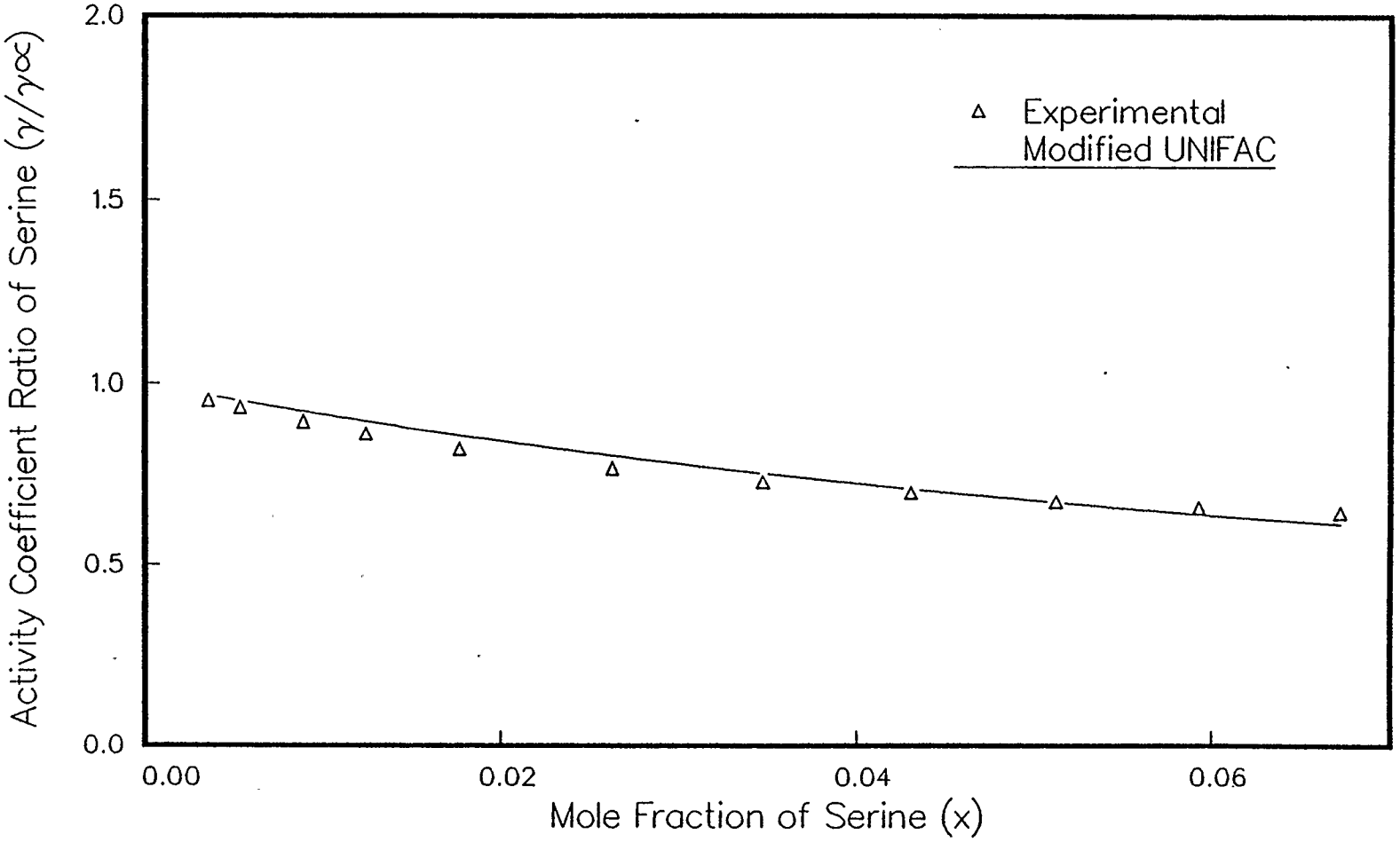


Figure 6.3 Experimental and predicted values for the activity coefficient ratio of serine in water at 298.15 K. Experimental data from Fasman (1976).

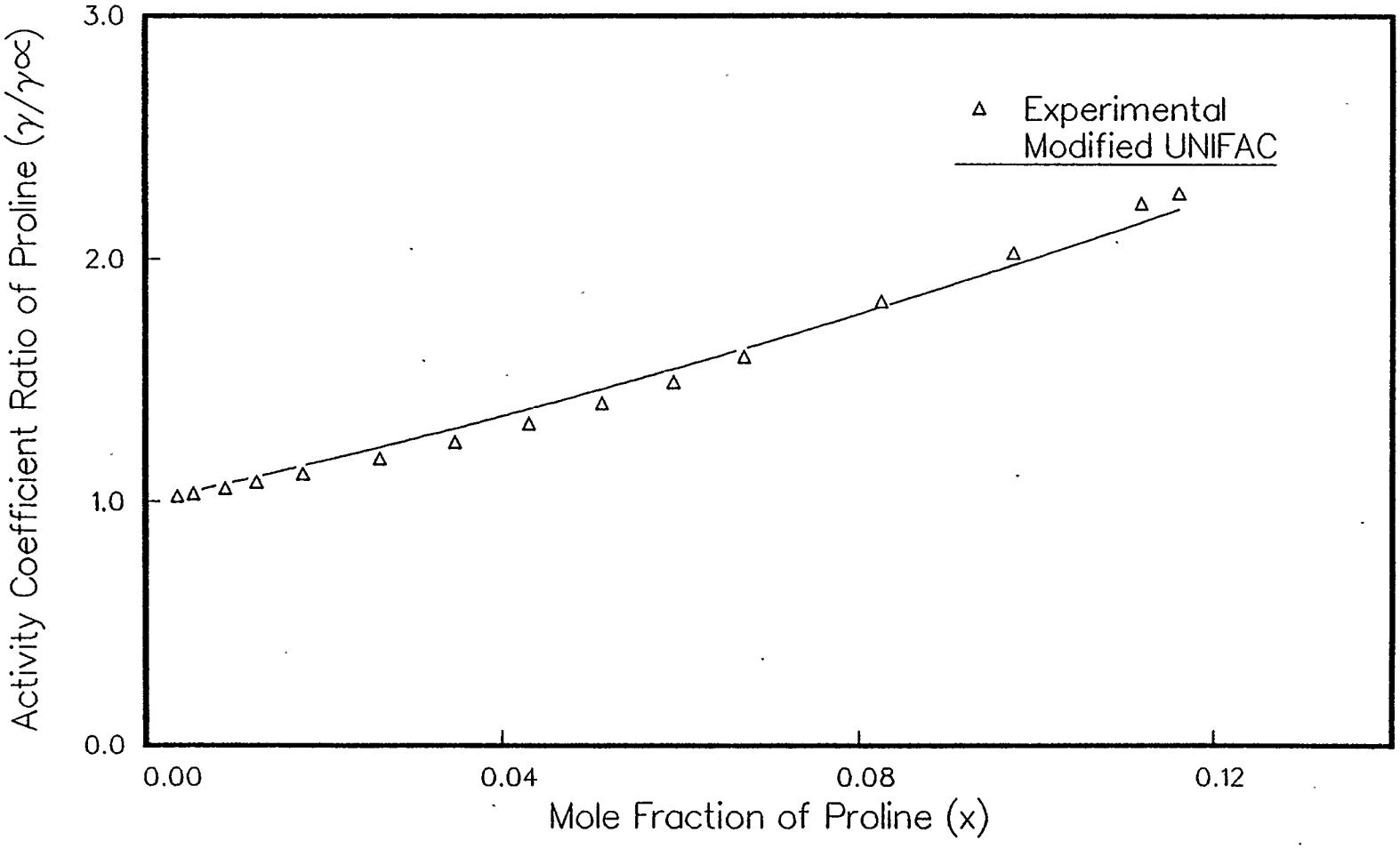


Figure 6.4 Experimental and predicted values for the activity coefficient ratio of proline in water at 298.15 K. Experimental data from Fasman (1976).

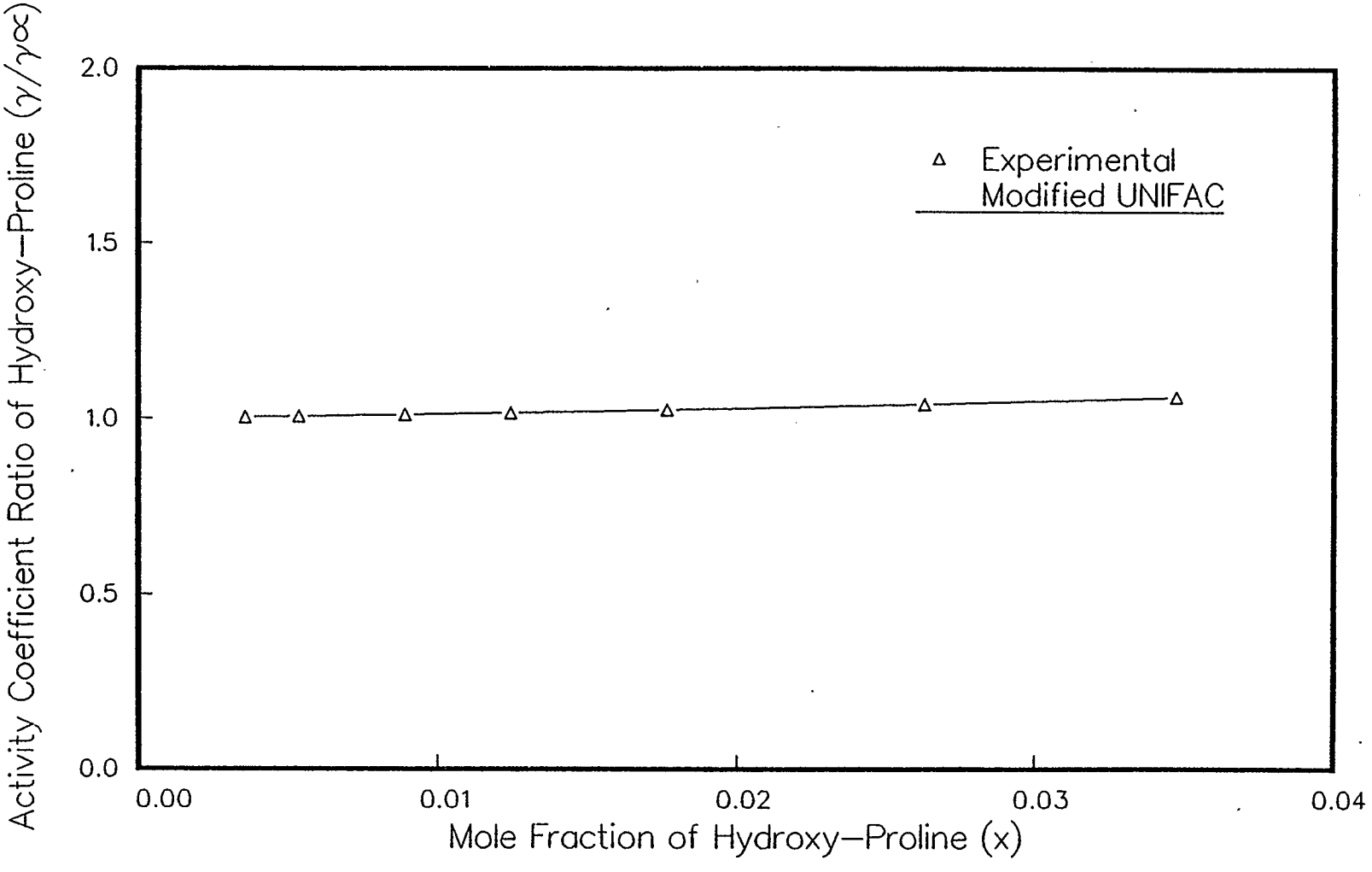


Figure 6.5 Experimental and predicted values for the activity coefficient ratio of hydroxy–proline in water at 298.15 K. Experimetal data from Fasman (1976).

when the regressible interaction energy parameters are given zero values. Even though the activity coefficient ratio in H_2O is near 1.0 for three of the five amino acids in Figures 6.1 to 6.5, the solutions are far from ideal as indicated by the large values found for γ^{∞}.

The increase of γ with mole fraction shown by alanine, proline and hydroxy-proline is difficult to correlate and the success of Modified UNIFAC appears significant.

The overall root mean square error in prediction of the activity coefficient ratios of alanine, glycine, hydroxy-proline, proline, and serine is 4.44% with 10 interaction parameters being regressed from 50 data points. These predictions are better than similar predictions for hydrocarbons by the Modified UNIFAC group-contribution model.

Predictive capability of the model are shown in Table 6.1 where the r.m.s. errors in γ/γ^{∞} for amino-butyric acid, valine and threonine are presented. Data for these three amino acids were not used in fitting interaction parameters. The results are discussed further in the next chapter

6.2 Activity Coefficient at Saturation of Antibiotics

The activity coefficient at saturation in various organic solvents of the aniso-mycin, carbomycin A, chloramphenicol, chloramphenicol palmitate, griseofulvin, and hygromycin A were calculated from the entropy of fusion and melting point data, using the Scatchard-Hildebrand equation, as discussed in the preceding chapters. The organic solvents are cyclohexane,iso-octane, benzene, toluene, iso-propanol, iso-amyl alcohol, ethyl acetate, and iso-amyl acetate.

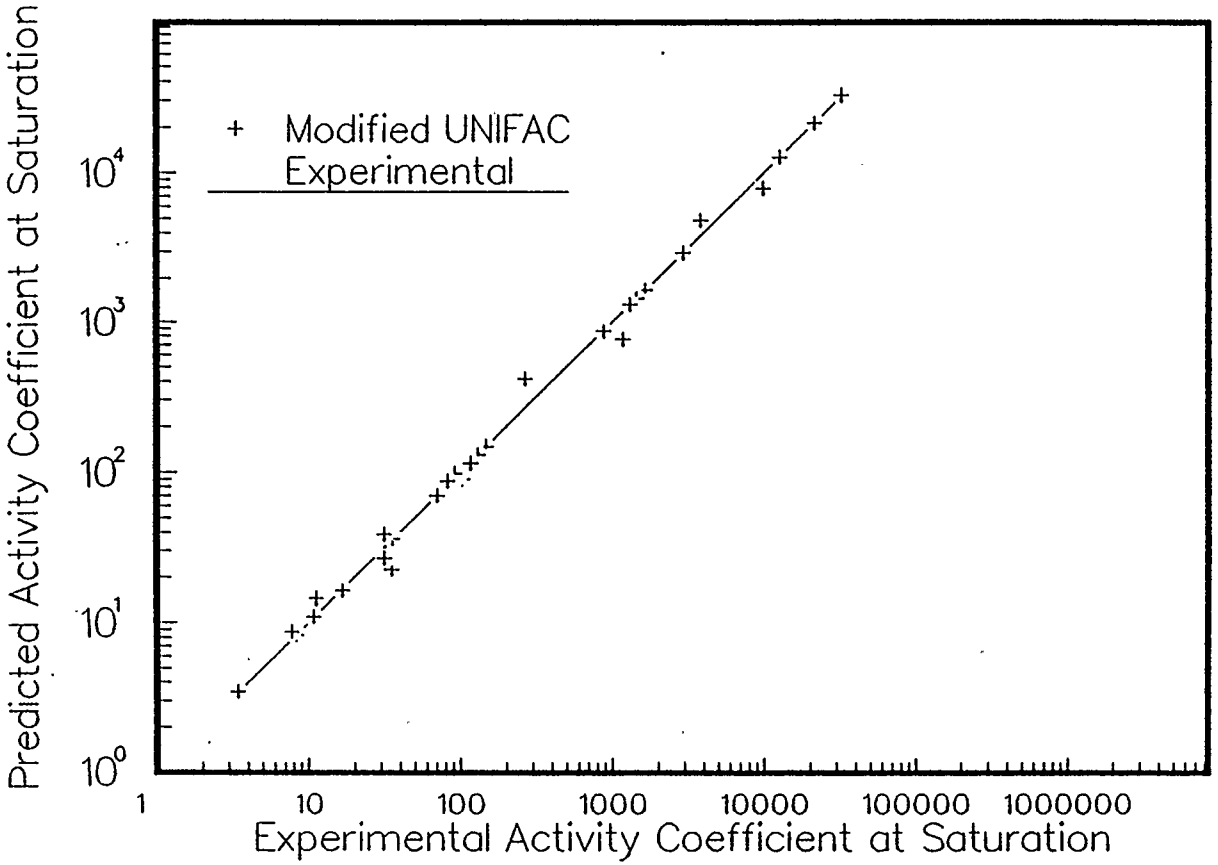


Figure 6.6 Experimental and predicted values for the activity coefficients at satuaration of anisomycin, carbomycin A, chloramphenicol, chlora-mphenicol palmitate, griseofulvin and hygromycin A. Temperature is 294.15 K for griseofulvin and 301.15 K for other five antibiotics.

A total of 32 interaction parameters were estimated from 32 data points. All 32 parameters were not regressed in one optimization, but they were estimated in many separate optimizations, a few parameters at a time. The absolute percent deviation in the predictions of γ^S from the experimental data varies from 0.0% to 55.2% and the root mean square deviation is 16.5%. These results are listed in Tables 6.2a and 6.2b.

The γ_S correlations for the individual antibiotics are discussed below.

Anisomycin

Solubility data were available for anisomycin in cyclohexane and iso-octane. These two data point were used to determine two CH_2, *anisomycin* energy interaction parameters. Similarly, the *ACH*, *anisomycin* parameters were found from benzene and toluene solubilities. The γ^S values are evaluated exactly in all four solvents.

The γ^S in the iso-octane is very high, which accounts for a very poor solubility of the anisomycin in iso-octane. The predictions of γ versus mole fraction profiles of anisomycin in some solvents, which are discussed later in this thesis, are not realistic.

Carbomycin A

Four energy interaction parameters between *carbomycin A* and the CH_2 and *OH* groups were obtained together from solubility data in cyclohexane and three alkanols. The two *carbomycin A* and *ACH* parameters were obtained from benzene and toluene solubility data.

It was impossible to find parameters that would give exactly any of the experimental solubilities. The parameter sets only provide non-zero minima of the objective functions in the two cases. These minimum points were reached in the simplex minimization procedure from several different initial guesses.

Predictions of γ^S for carbomycin A in the alkane solvents (cyclohexane and iso-octane) are not good; the deviations from the experimental results are 55.2% and -35.3%. Predictions in the alcohols (iso-propanol and iso-amyl alcohol) are very good and in aromatic solvents (benzene and toluene) are moderately good. Carbomycin A has very low solubility in the alkane solvents, which is accompanied by the poor predictability of γ^S by Modified UNIFAC.

Chloramphenicol and Chloramphenicol Palmitate

These two antibiotics involve only one new group but palmitate itself include the "acetate" group, *CCOO*. Eight solubility data were available for these two antibiotics in two alkanes, two acetates, one alkanol, benzene, toluene and benzyl alcohol. The eight required parameters were found by simultaneously fitting the eight data. None of the data were matched exactly.

γ^S predictions for chloramphenicol in aromatic solvents and in alcohols are very good, as the deviations from the experimental results range from 0.7% to –2.6%. In these solvents, γ^S is well represented by Modified UNIFAC.

In alkane solvents (cyclohexane and iso-octane) the deviations in predictions of γ^S for chloramphenicol palmitate are 26.1% and –20.3%, which emphasizes the poor representation of data by the model used. However for benzene and toluene the γ^S data are well represented.

Griseofulvin

Four *griseofulvin* energy interaction parameters with CH_2 and *OH* groups were obtained simultaneously from solubility data in three alkanols and in cyclohexane. Solubilities in two acetates were then used to determine the *CCOO* interaction

Table 6.2a Experimental and Calculated Activity Coefficients at Saturation, for Antibiotics

Solute	Solvent	Temp. (K)	$\gamma^{s,exp}$	$\gamma^{s,predicted}$	% Deviation
Anisomycin	Cylcohexane	301.15	1658.495	1658.495	0.000
Anisomycin	Iso-Octane	301.15	12699.571	12699.626	0.000
Anisomycin	Benzene	301.15	70.142	70.142	0.000
Anisomycin	Toluene	301.15	129.843	129.843	0.000
Carbomycin A	Cyclohexane	301.15	266.596	413.815	55.222
Carbomycin A	Iso-Octane	301.15	1181.083	764.398	–35.280
Carbomycin A	Benzene	301.15	7.734	8.780	13.520
Carbomycin A	Toluene	301.15	31.559	26.709	–15.367
Carbomycin A	Iso-Propanol	301.15	35.795	36.299	1.407
Carbomycin A	Iso-Amyl alcohol	301.15	116.893	114.772	–1.814
Chloramphenicol	Benzene	301.15	878.047	868.058	–1.138
Chloramphenicol	Toluene	301.15	1307.455	1316.466	0.689
Chloramphenicol	Benzyl alcohol	301.15	16.768	16.328	–2.623
Chloramphenicol	Iso-Amyl alcohol	301.15	10.804	10.905	0.935
Chloramphenicol Palmitate	Cyclohexane	301.15	3830.963	4830.163	26.082
Chloramphenicol Palmitate	Iso-Octane	301.15	9881.486	7876.901	–20.286

Table 6.2b Experimental and Calculated Activity Coefficients at Saturation, for Antibiotics

Solute	Solvent	Temp. (K)	$\gamma^{s,exp}$	$\gamma^{s,predicted}$	% Deviation
Chloramphenicol Palmitate	Benzene	301.15	147.054	147.944	0.605
Chloramphenicol Palmitate	Toluene	301.15	96.734	98.456	1.780
Griseofulvin	Cyclohexane	294.15	1317.544	1314.645	–0.221
Griseofulvin	Ethanol	294.15	11.203	14.423	28.741
Griseofulvin	Iso-Propanol	294.15	35.405	22.464	–36.552
Griseofulvin	Iso-Amyl alcohol	294.15	31.418	38.583	22.806
Griseofulvin	Ethyl acetate	294.15	3.429	3.429	0.000
Griseofulvin	Iso-Amyl acetate	294.15	8.296	8.296	0.000
Hygromycin A	Cyclohexane	301.15	21527.672	21506.504	–0.098
Hygromycin A	Ethanol	301.15	91.707	98.063	6.931
Hygromycin A	Iso-Propanol	310.15	102.413	90.193	–11.942
Hygromycin A	Benzene	301.15	32500.054	32500.072	0.000
Hygromycin A	Benzyl alcohol	301.15	92.132	92.132	0.000
Hygromycin A	Iso-Amyl alcohol	310.15	82.416	87.642	6.340
Hygromycin A	Ethyl acetate	301.15	2933.482	2933.482	0.000
Hygromycin A	Iso-Amyl acetate	310.15	1447.821	1447.820	0.000

parameters.

The alkane and alkanol data could not be fitted exactly but an exact match with the two acetate data was obtained. In the cyclohexane, γ^S is well predicted but in alkanols (ethanol, iso-propanol, and iso-amyl alcohol) predictions are not good. It seems that the interactions between the *griseofulvin* group and the *OH* group are not well represented.

Hygromycin A

Solubility in three alkanols and cyclohexane were used to fit four CH_2 and *OH* energy interaction parameters. The two *ACH* parameters were found subsequently from benzene and benzyl alcohol data. Ethyl acetate and iso-amyl acetate data were used, also separately, to evaluate the two *CCOO* parameters.

Parameters for the *OH* and CH_2 interactions would not match exactly the alkanol and cyclohexane data. However, exact matches with the other data pairs were possible. Overall the model gives good predictions of activity coefficient for hygromycin A.

It is apparent that the Modified UNIFAC group-contribution model gives good predictions of γ^S for the above six antibiotics in a variety of solvents. However, for solvents containing CH_2 groups there is some difficulty in the predictions.

6.3 Standard State Fugacity Ratio for Amino Acids

The amino acid activity coefficient parameters were evaluated from osmotic coefficient data. Additionally, solubility data are available. Equation (3.46) can be used with the solubility information of amino acids in neutral water to evaluate the

Table 6.3 Calculated ($f_{A_S}{}^{o}/f_{A_{\pm_L}}{}^{o}$)

Temperature, K	*dl*–Alanine	Glycine	*dl*–Valine
273.15	0.130	0.273	0.112
283.15	0.154	0.327	0.135
293.15	0.178	0.382	0.159
303.15	0.204	0.436	0.184
313.15	0.231	0.486	0.209
323.15	0.257	0.531	0.235
333.15	0.284	0.568	0.262
343.15	0.311	0.599	0.289
353.15	0.337	0.621	0.317
363.15	0.363	0.637	0.346
373.15	0.388	0.660	0.374

ratio ($f_{A_S}{}^{o}/f_{A_{\pm L}}{}^{o}$) for the amino acids. Since the solid amino acids decomposes before melting, no separate route is available to evaluate ($f_{A_S}{}^{o}/f_{A_{\pm L}}{}^{o}$) as could be done for some of other chemicals.

Calculated values of $\ln(f_{A_S}{}^{o}/f_{A_{\pm L}}{}^{o})$ are given in Table 6.3 .

It is reasonable to correlate these data by the equation

$$\ln\left[\frac{f_{A_S}{}^{o}}{f_{A_{\pm L}}{}^{o}}\right] = A' - \frac{B'}{T} \qquad (6.2)$$

The two parameters A′ and B′ were estimated by least squares regression from the solubility and dissociation constant data for amino acids. The 95% confidence limits for the parameters were also calculated. Parameters A′ and B′ are reported in Table 5.5 for *dl*-alanine, glycine, and *dl*-valine.

The parameter B′ can be thought as the heat of fusion of the amino acid divided by the gas constant. The molar heat of fusion has an usual trend of increasing with the increase in the molecular weight of the substance. The same effect is observered in the values of B′, as its value increases from glycine to *dl*-alanine to *dl*-valine.

The values obtained for ΔH_f is B′ = ΔH_f/R are at least of reasonable size relative to typical heats of fusion of solids.

Chapter 7.

Model Predictions

For some amino acids which were not used in determining Modified UNIFAC parameters, predictions of (γ/γ^{∞}) have been made and compared with the experimental data. Solubilities of antibiotics in mixed solvents, and the precipitation of an antibiotic from a solution by the addition of another solvent have also been predicted. However, due to data nonavailability these predictions cannot be compared with experimental data. The effect of pH and temperature on the solubility of the amino acids is also examined.

7.1 Activity Coefficients of Some Amino Acids

Activity coefficient ratios (γ/γ^{∞}) of amino-butyric acid, valine, and threonine have been calculated using the Modified UNIFAC parameters derived from data for simpler amino acids. The predictions can be compared with seventeen experimental data points. Predictions for the individual amino acids are discussed below.

Amino-Butyric Acid

Calculated activity coefficient ratios for amino-butyric acid is compared with 7 experimental points in Figure 7.1 . The r.m.s. error in the prediction is 17.12%, which is comparable with the reported predictions by Modified UNIFAC for many hydrocarbon systems. However, qualitatively the results are not good. The slope of (γ/γ^{∞}) versus mole fraction is positive for the experimental results, however it is negative for the predictions. The errors at the highest mole fractions are large.

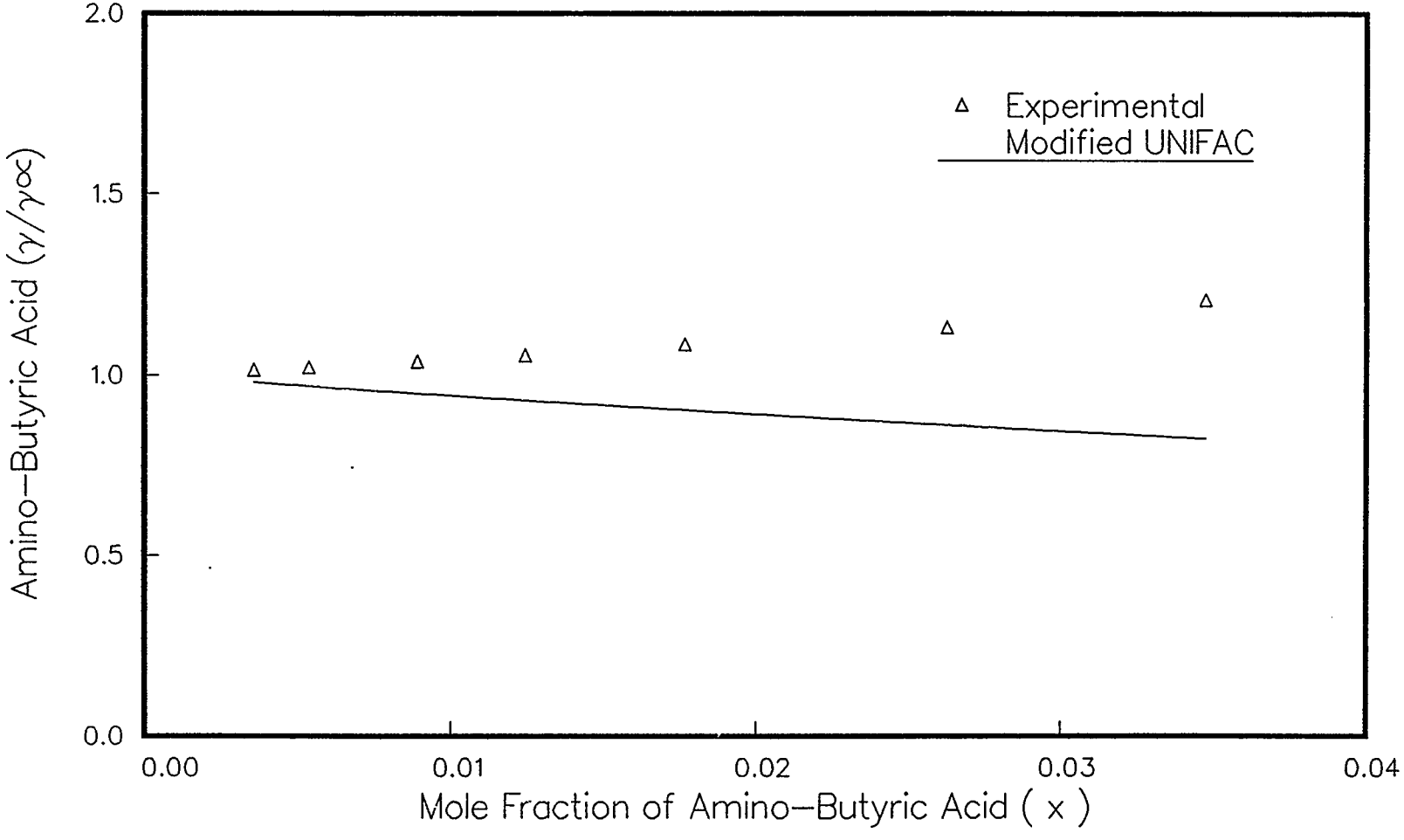


Figure 7.1 Experimental and predicted values for the activity coefficient ratio of amino–butyric acid in water at 298.15 K. Experimental data from Fasman (1976).

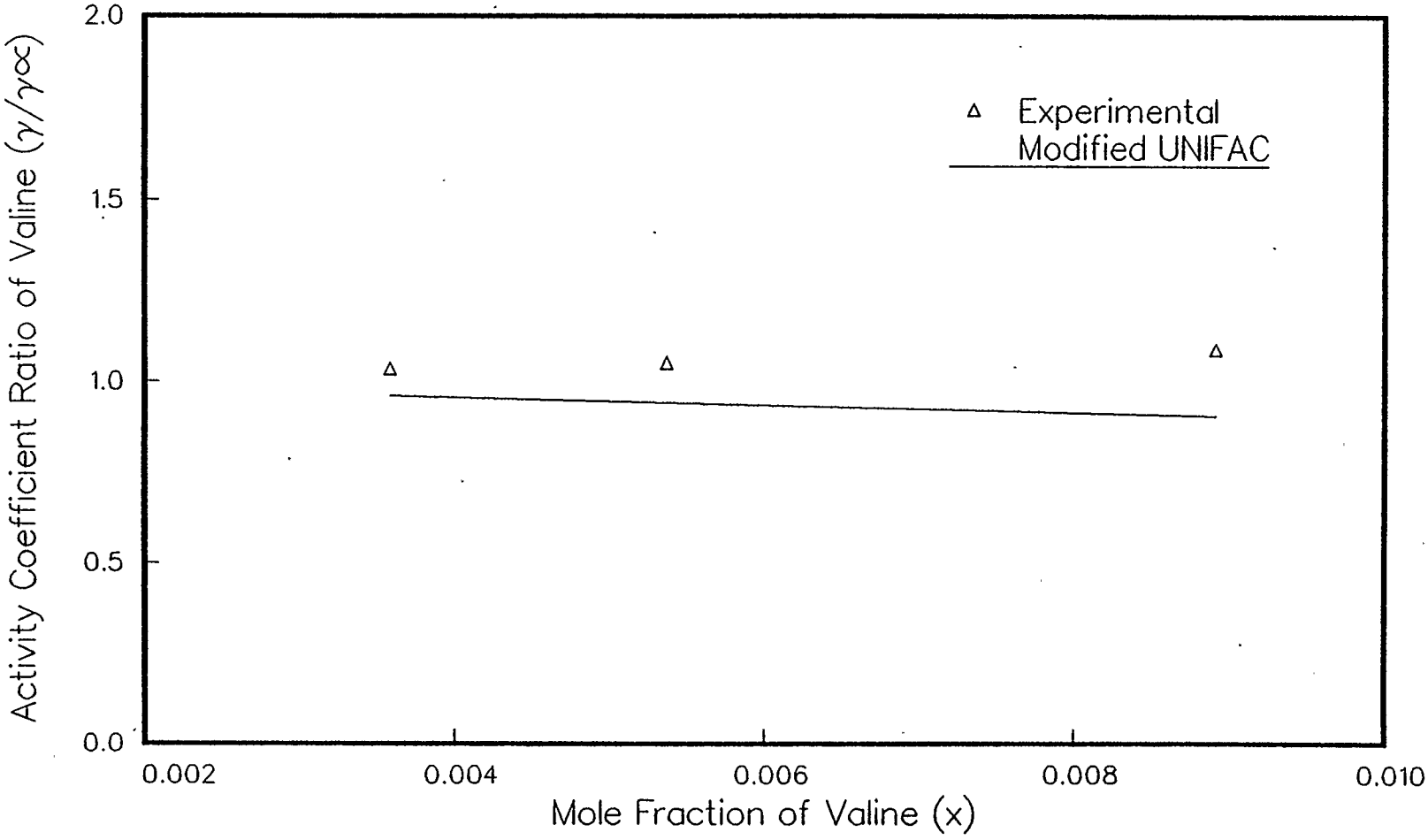


Figure 7.2 Experimental and predicted values for the activity coefficient ratio of valine in water at 298.15 K. Experimetal data from Fasman (1976).

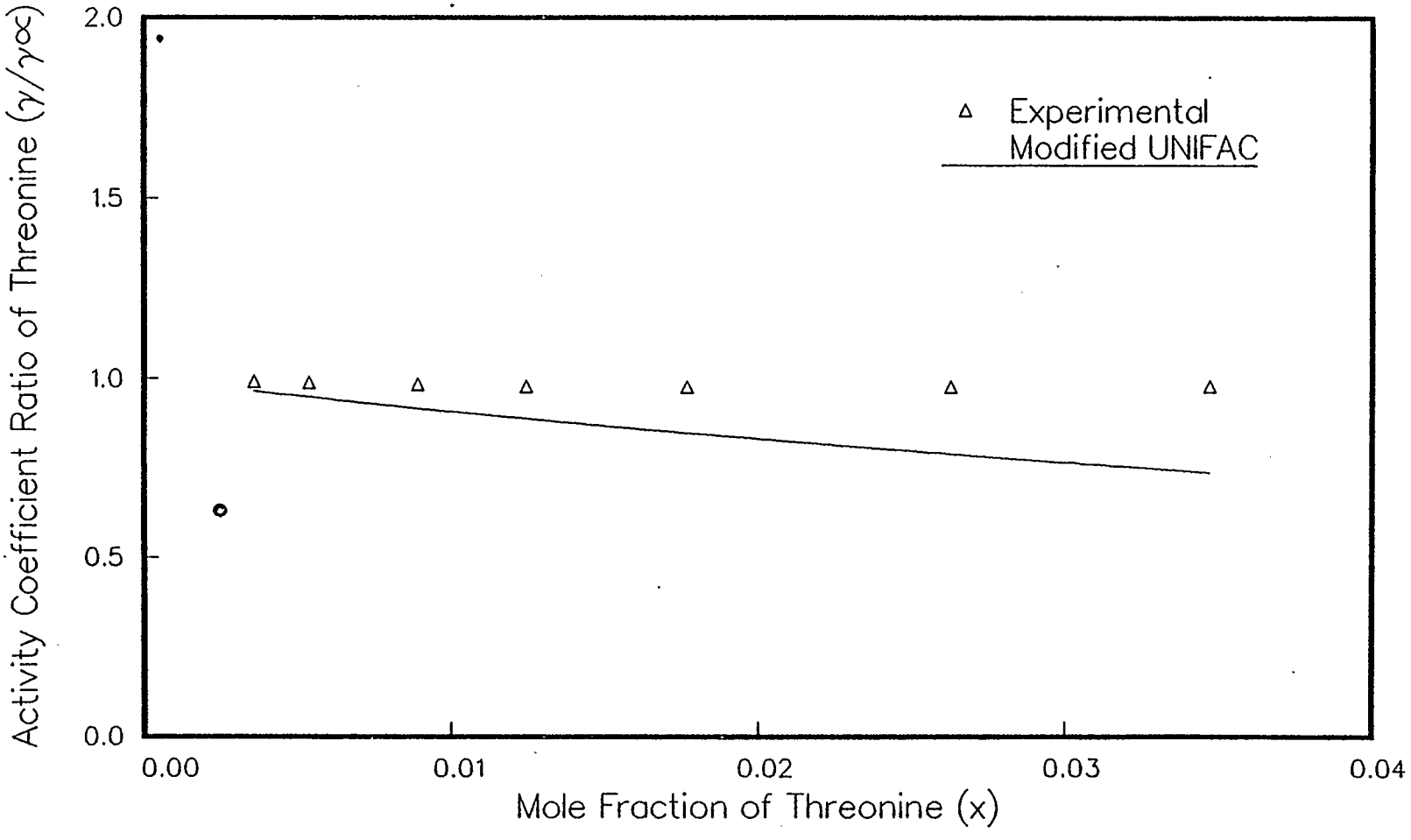


Figure 7.3 Experimental and predicted values for the activity coefficient ratio of threonine in water at 298.15 K. Experimental data from Fasman (1976).

Valine

Three experimental data of (γ/γ^{∞}) for valine in water were compared with the predicted values. The root mean square deviation is 12.08%. Qualitatively, the predictions have the same problem as in the case of amino-butyric acid. The results are shown in Figure 7.2 .

Threonine

Threonine has an OH group added to the amino-butyric acid structure. Interactions among *OH*, CH_2, H_2O, and *glycine* groups are involved. Seven experimental data points of (γ/γ^{∞}) for threonine in water are compared with the model predictions in Figure 7.3 . The r.m.s. deviation is 13.95% . The slope of the curve of (γ/γ^{∞}) versus mole fraction of threonine is negative for both the experimental and predicted results, however there is a difference between the magnitudes of the slopes.

Overall, the Modified UNIFAC group-contribution model, as used here for amino acids is disappointing. One has to be careful in using the model. The problem is probably traceable to the large contribution to the activity coefficients from the combinatorial term which contains no adjustable parameters.

7.2 Activity Coefficient Profile of Antibiotics

The variation in the activity coefficients of anisomycin, carbomycin A, chloramphenicol, chloramphenicol palmitate, griseofulvin and hygromycin A with mole fraction, in various organic solvents have been calculated. These profiles are in Figures 7.4 to figure 7.19 . The end point of these curves is the solubility limit at the specified temperature.

The solubilities of the antibiotics in any of the solvents is small. For most of

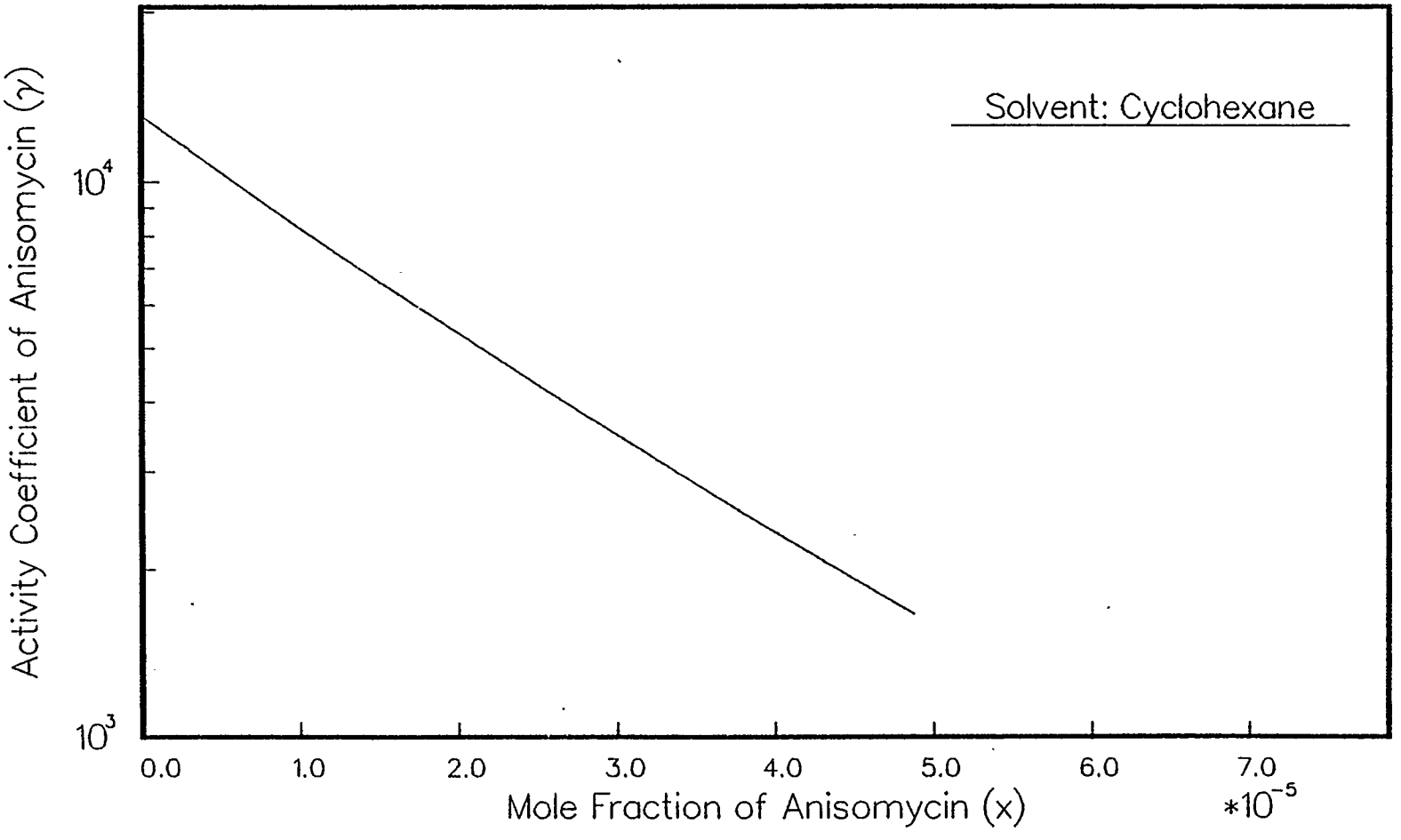


Figure 7.4 Activity coefficient predictions of anisomycin in cyclohexane at 301.15 K, using Modified UNIFAC Group–Contribution model.

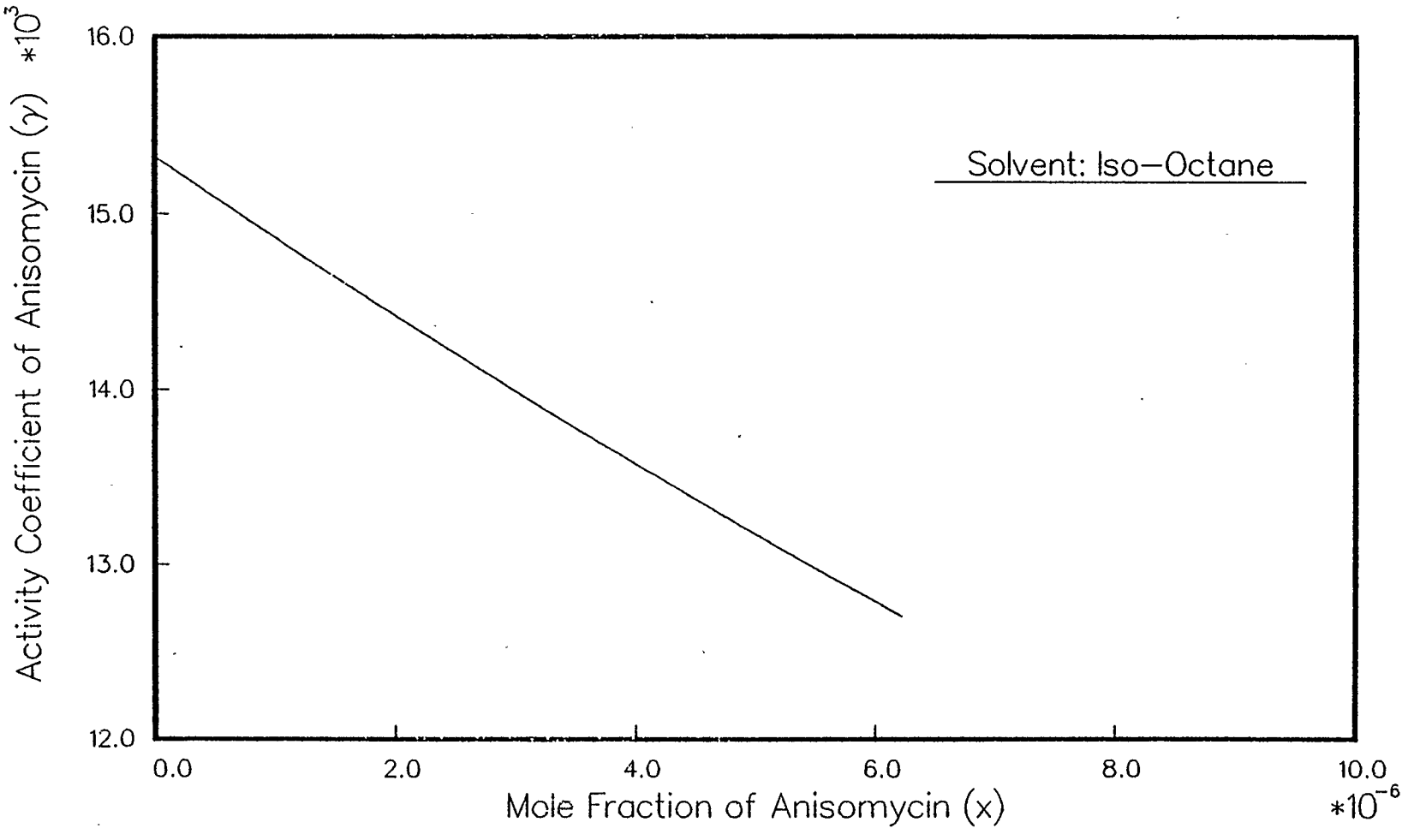


Figure 7.5 Activity coefficient predictions of anisomycin in iso–octane at 301.15 K, using Modified UNIFAC Group–Contribution model.

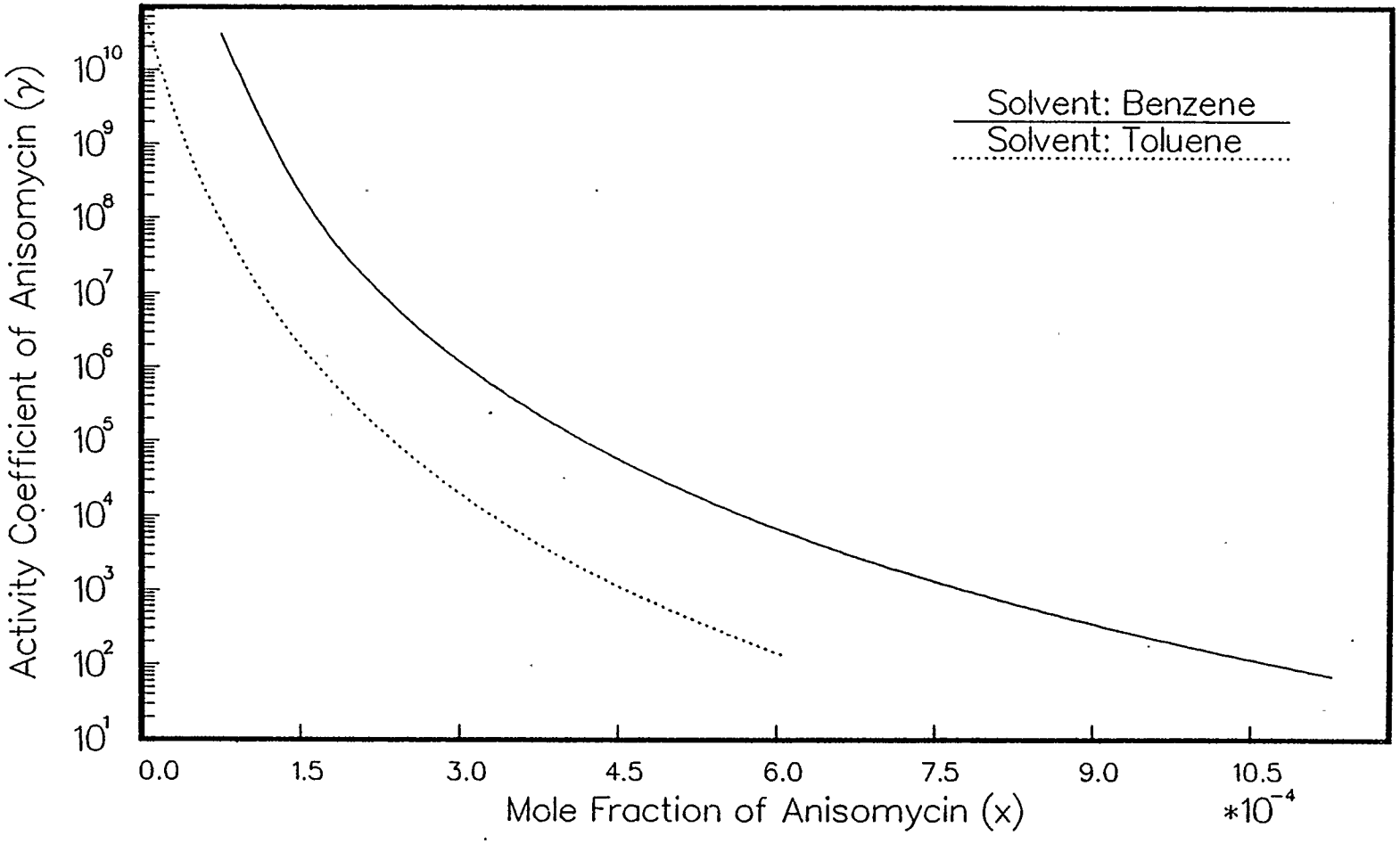


Figure 7.6 Activity coefficient predictions of anisomycin in benzene and toluene at 301.15 K, using Modified UNIFAC Group–Contribution model.

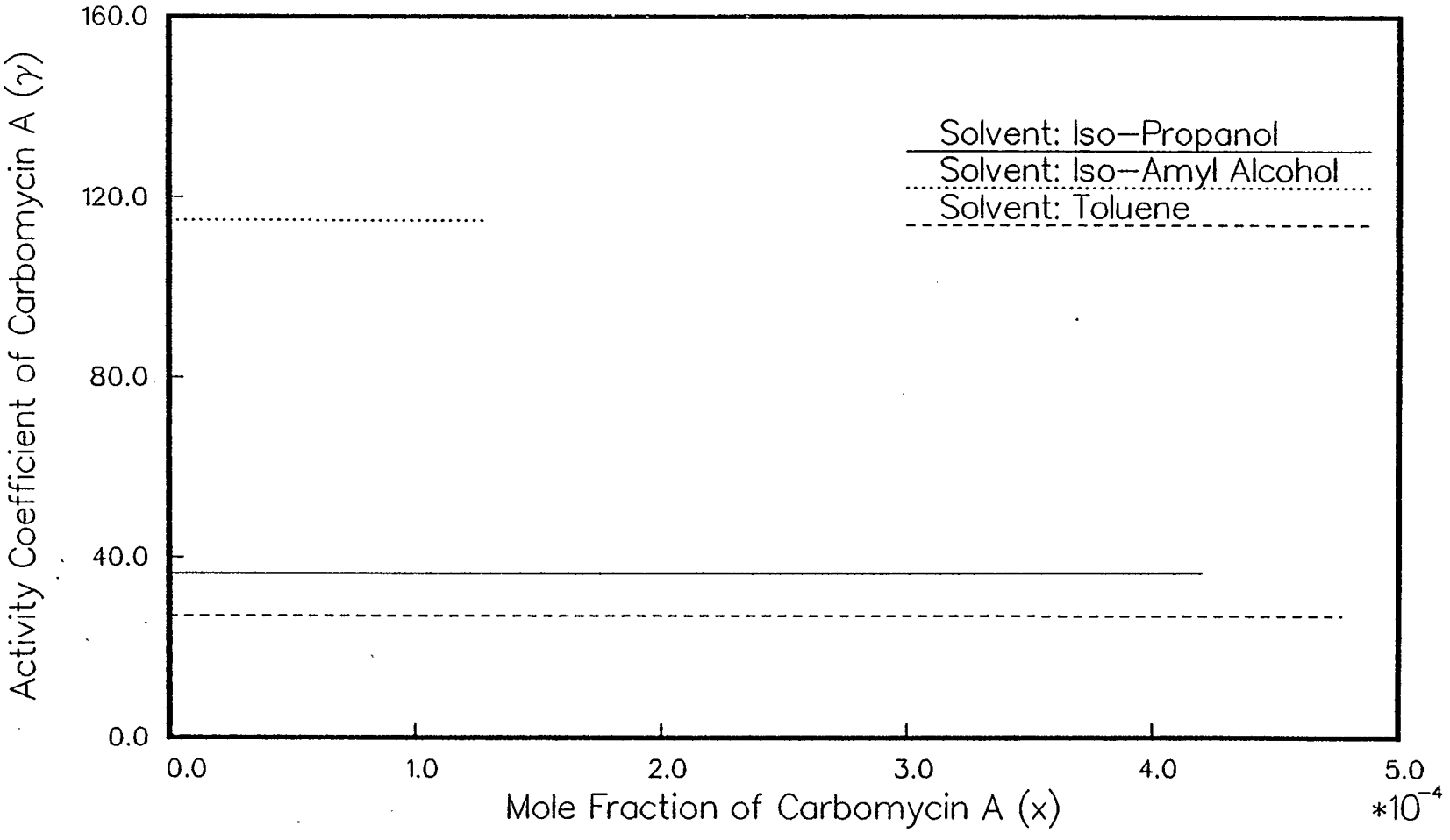


Figure 7.7 Activity coefficient predictions of carbomycin A in iso–propanol, iso–amyl alcohol and toluene at 301.15 K, using Modified UNIFAC Group–Contribution model.

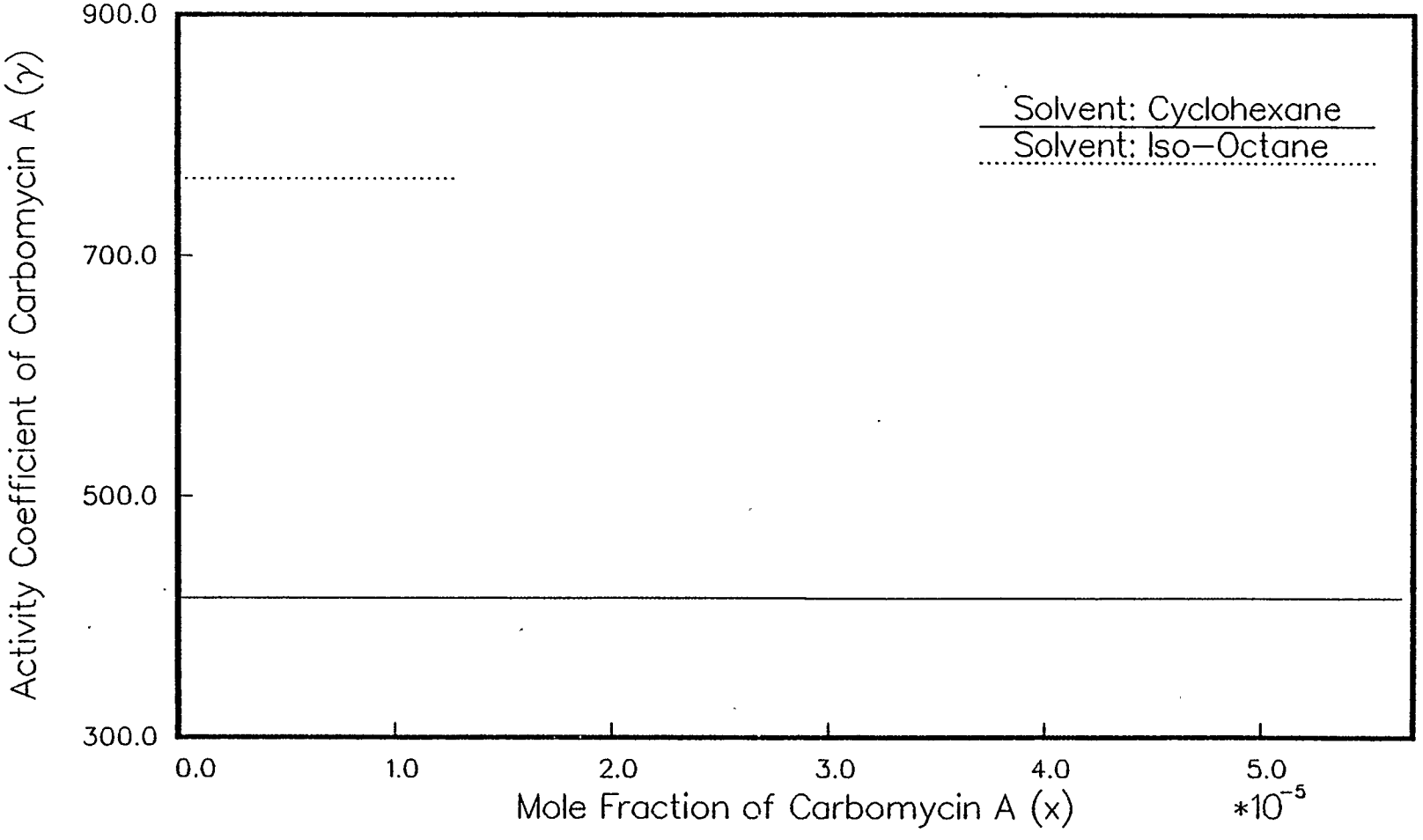


Figure 7.8 Activity coefficient predictions of carbomycin A in cyclohexane and iso–octane at 301.15 K, using Modified UNIFAC Group Contribution model.

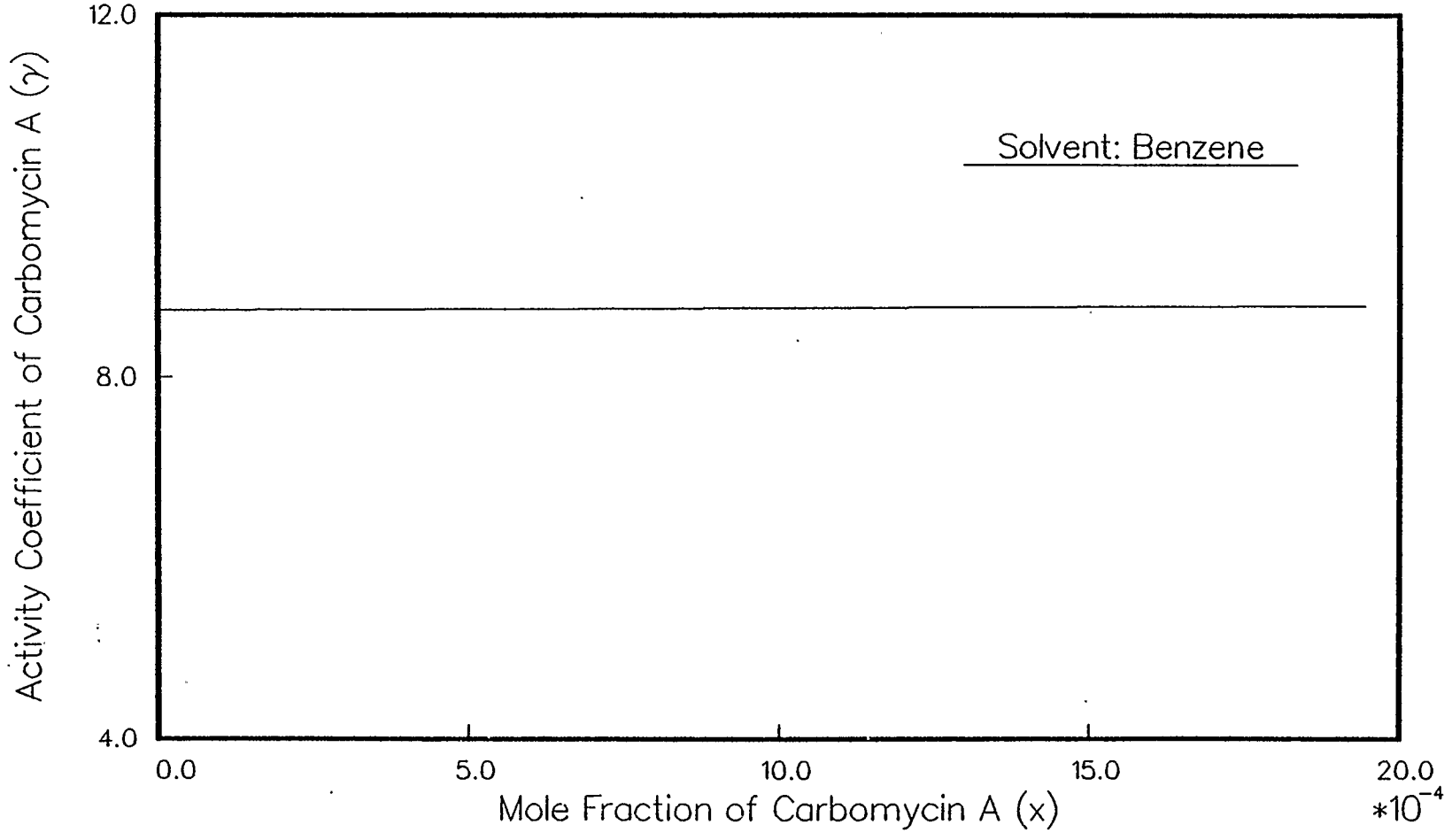


Figure 7.9 Activity coefficient predictions of carbomycin A in benzene at 301.15 K, using Modified UNIFAC Group–Contribution model.

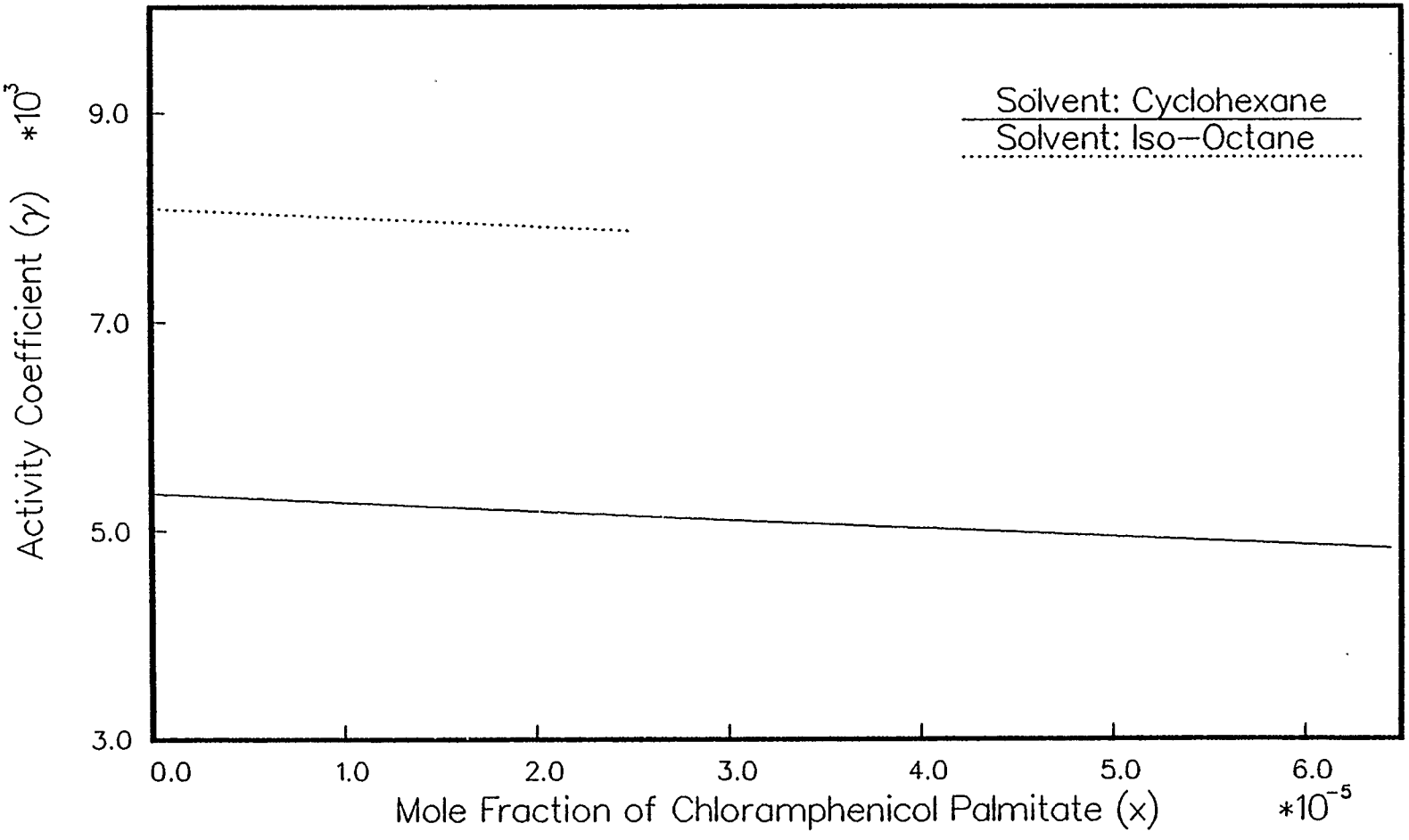


Figure 7.10 Activity coefficient predictions of chloramphenicol palmitate in cyclohexane and iso-octane at 301.15 K, using Modified UNIFAC Group-Contribution model.

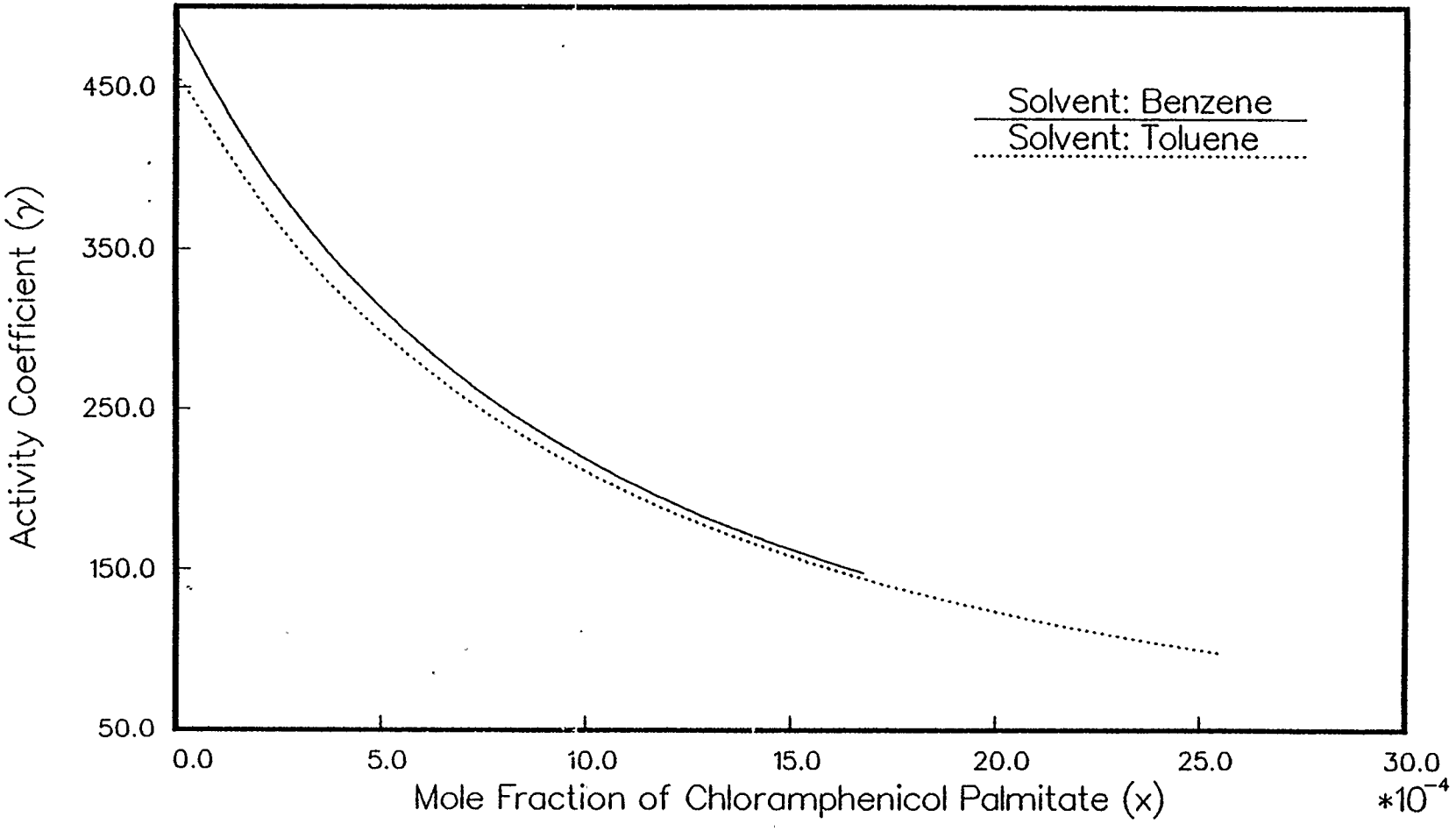


Figure 7.11 Activity coefficient predictions of chloramphenicol palmitate in benzene and toluene 301.15 K, using Modified UNIFAC Group–Contribution model.

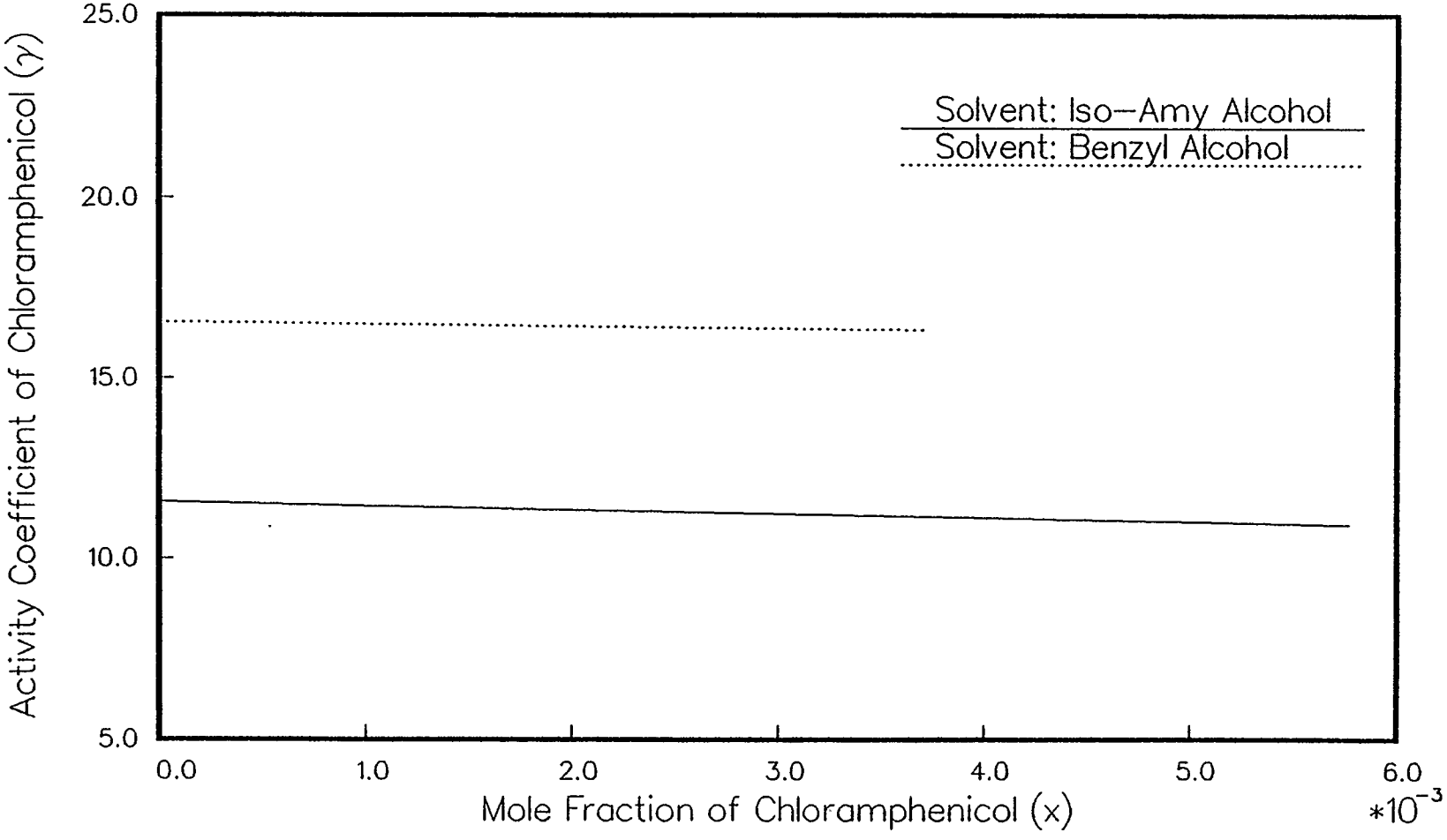


Figure 7.12 Activity coefficient predictions of chloramphenicol in iso–amyl alcohol and benzyl alcohol at 301.15 K, using Modified UNIFAC Group–Contribution model.

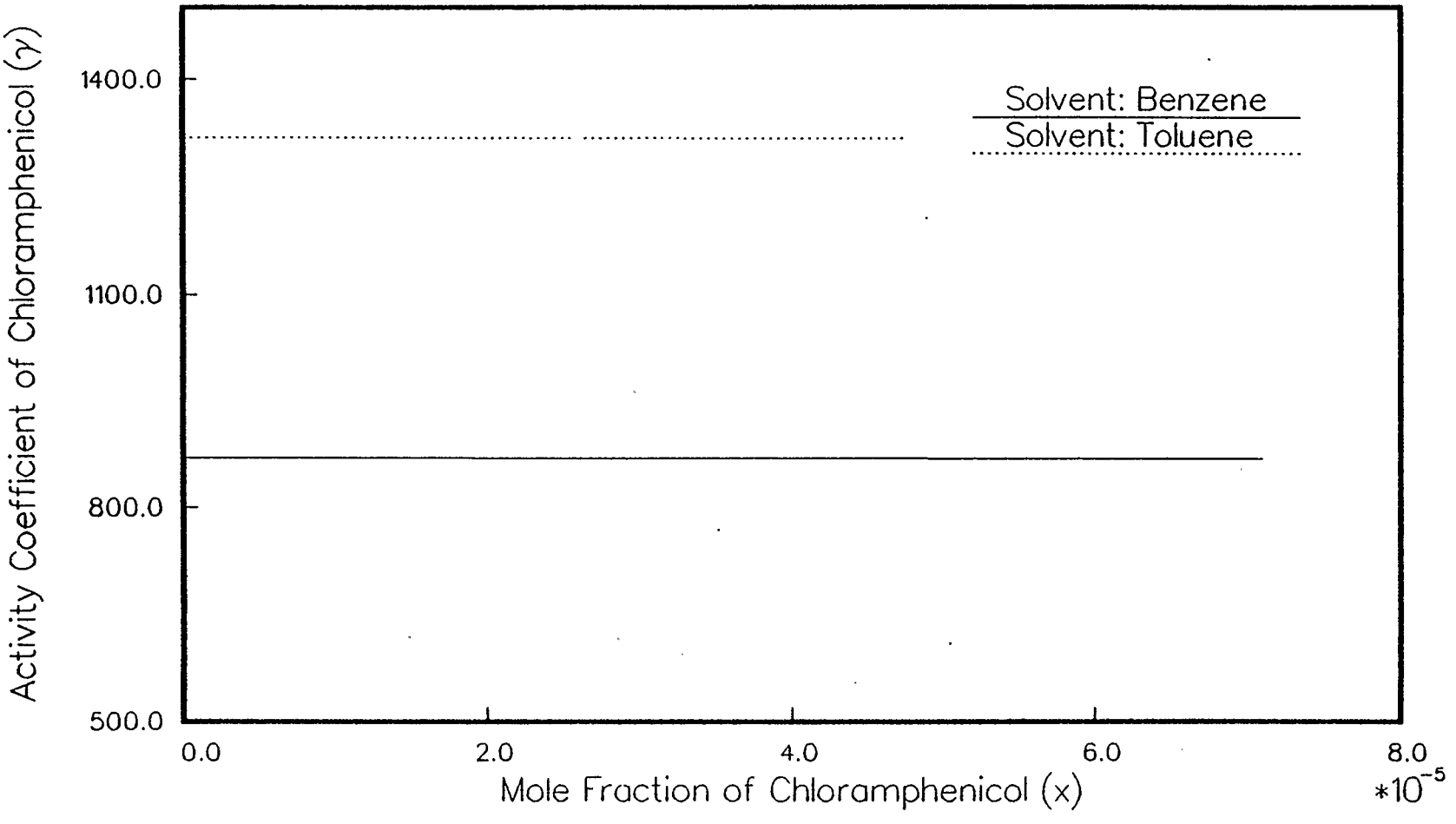


Figure 7.13 Activity coefficient predictions of chloramphenicol in benzene and toluene at 301.15 K, using Modified UNIFAC Group–Contribution model.

the antibiotic/solvent combinations, γ changes very little from the γ^{∞} value over the possible range of antibiotic mole fraction. This is reasonable behavior. There are a few exceptions that should be noted.

The activity coefficient profiles of anisomycin in cyclohexane, benzene and toluene show a very unusual behavior. The slope of the activity with mole fraction of anisomycin is negative at some or all points in these solvents. This represents liquid instability at concentrations below the saturation point, which is totally unrealistic. Hence, Modified UNIFAC fails in these cases. It has been observed, in above cases, that if the surface area parameter can be reduced, the qualitative results will improve. One may adjust the size parameters to fix this problem of unusual γ-profile for anisomycin.

Activity coefficients of carbomycin in iso-propanol, iso-amyl alcohol, toluene, cyclohexane, iso-octane, and benzene show a flat profile. It seems that the γ^{∞} remains almost equal to γ^{S} in alcohols, alkane, and aromatic solvents.

Chloramphenicol palmitate in cyclohexane and iso-octane shows γ versus x plot of negative slope of small magnitude, however in the benzene and toluene this is of variable large magnitude, negative slope. This variation of γ may play an important role in design of extraction units for chloramphenicol palmitate, especially when using an aromatic solvents.

The activity coefficient of chloramphenicol in alcohols and aromatic solvents slowly decreases with increase in its concentration. Solutions of chloramphenicol in iso-amyl alcohol, benzene or toluene are highly non-ideal with positive deviations from ideality.

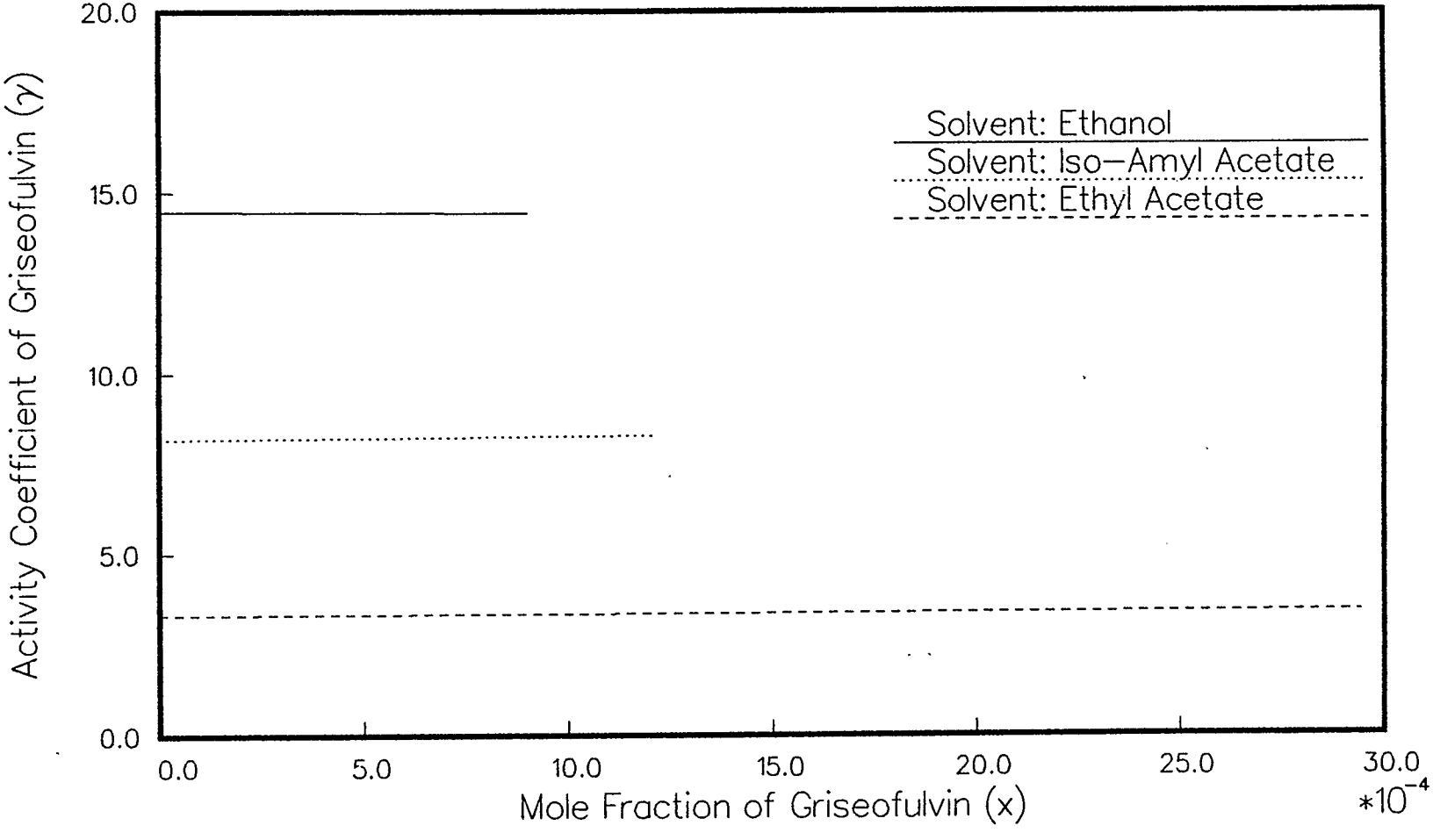


Figure 7.14 Activity coefficient predictions of griseofulvin in ethanol, iso–amyl acetate and ethyl acetate at 294.15 K, using Modified UNIFAC Group–Contribution model.

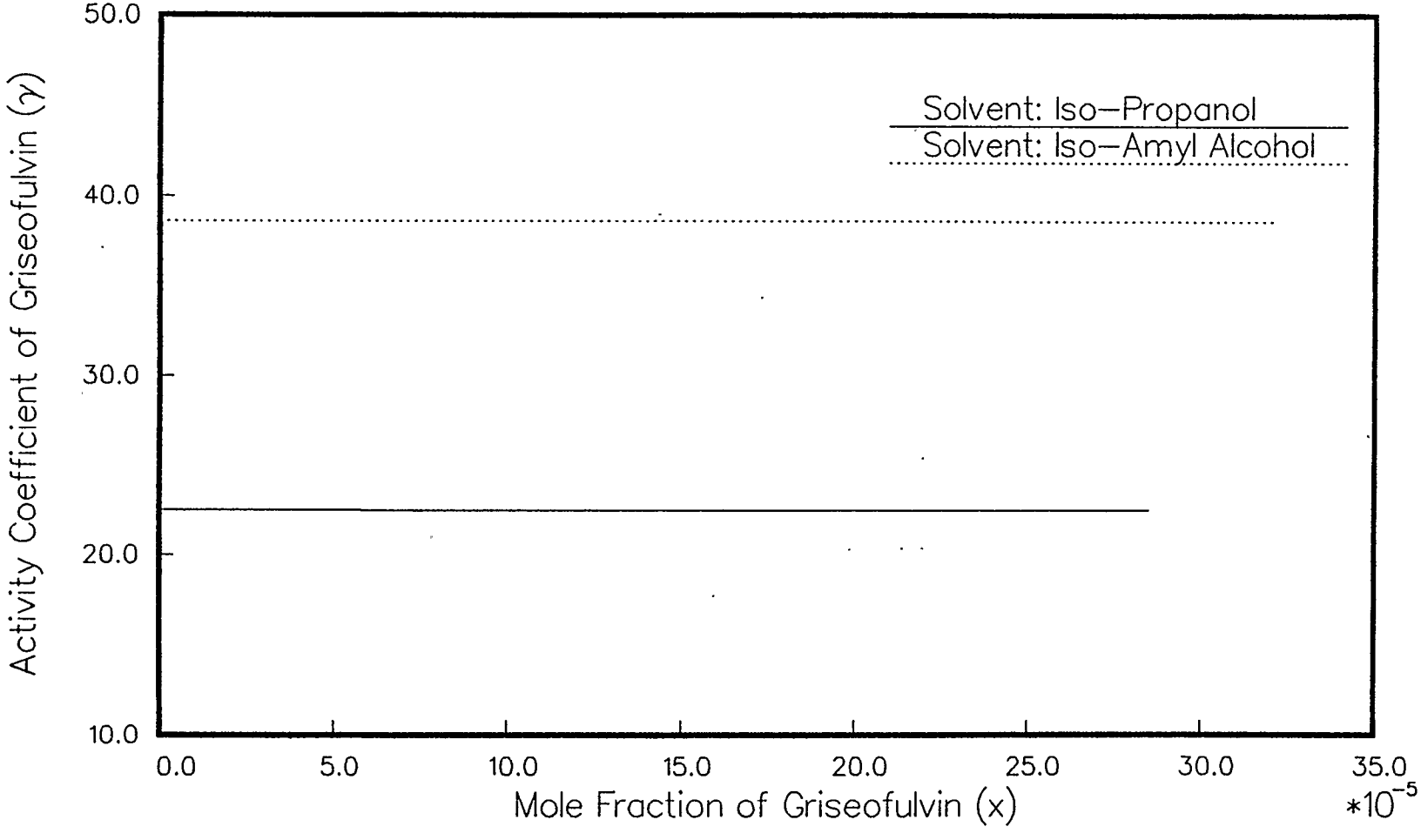


Figure 7.15 Activity coefficient predictions of griseofulvin in iso–propanol and iso–amyl alcohol at 294.15 K, using Modified UNIFAC Group–Contribution model.

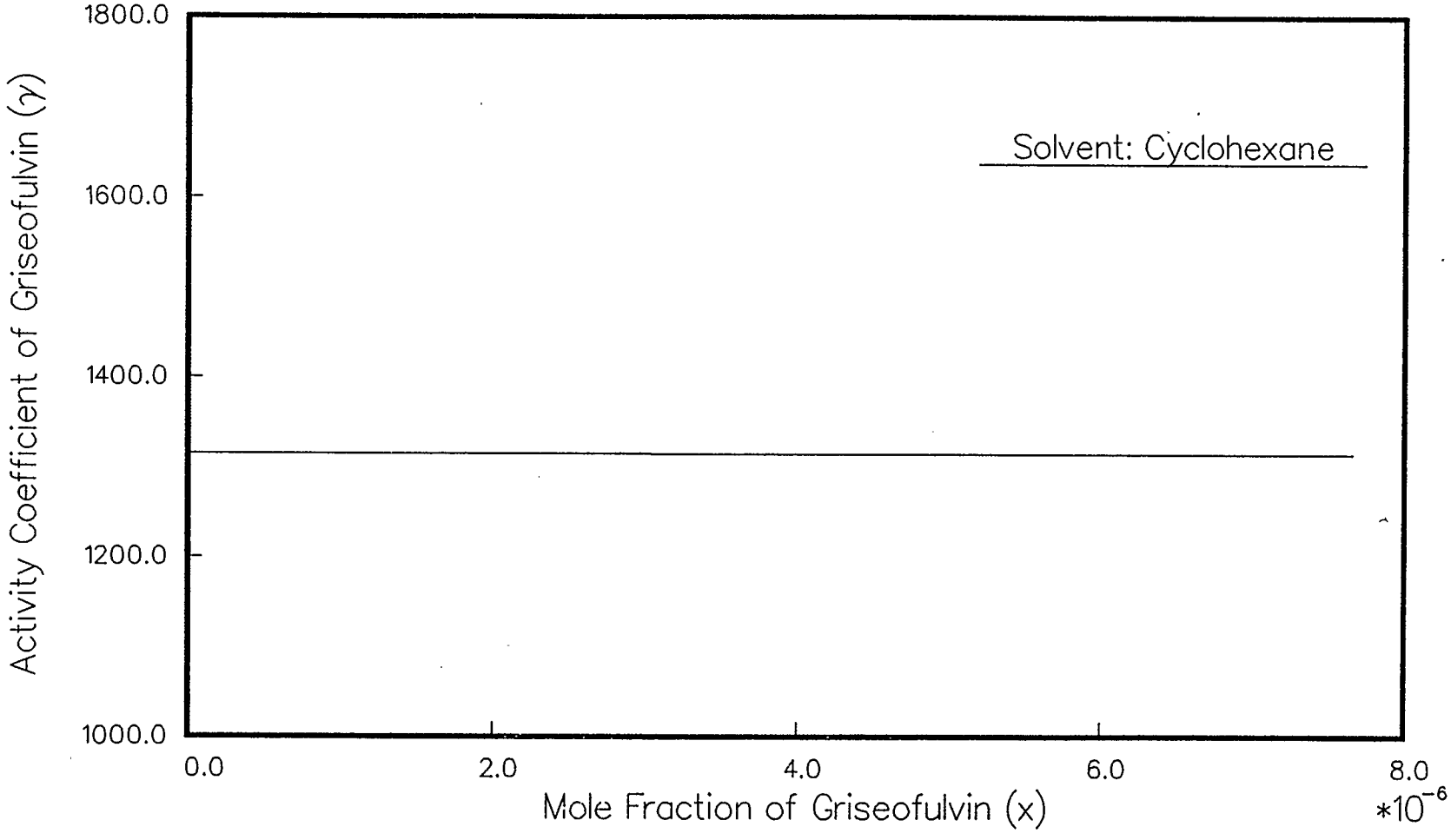


Figure 7.16 Activity coefficient predictions of griseofulvin in cyclohexane at 294.15 K, using Modified UNIFAC Group Contribution model.

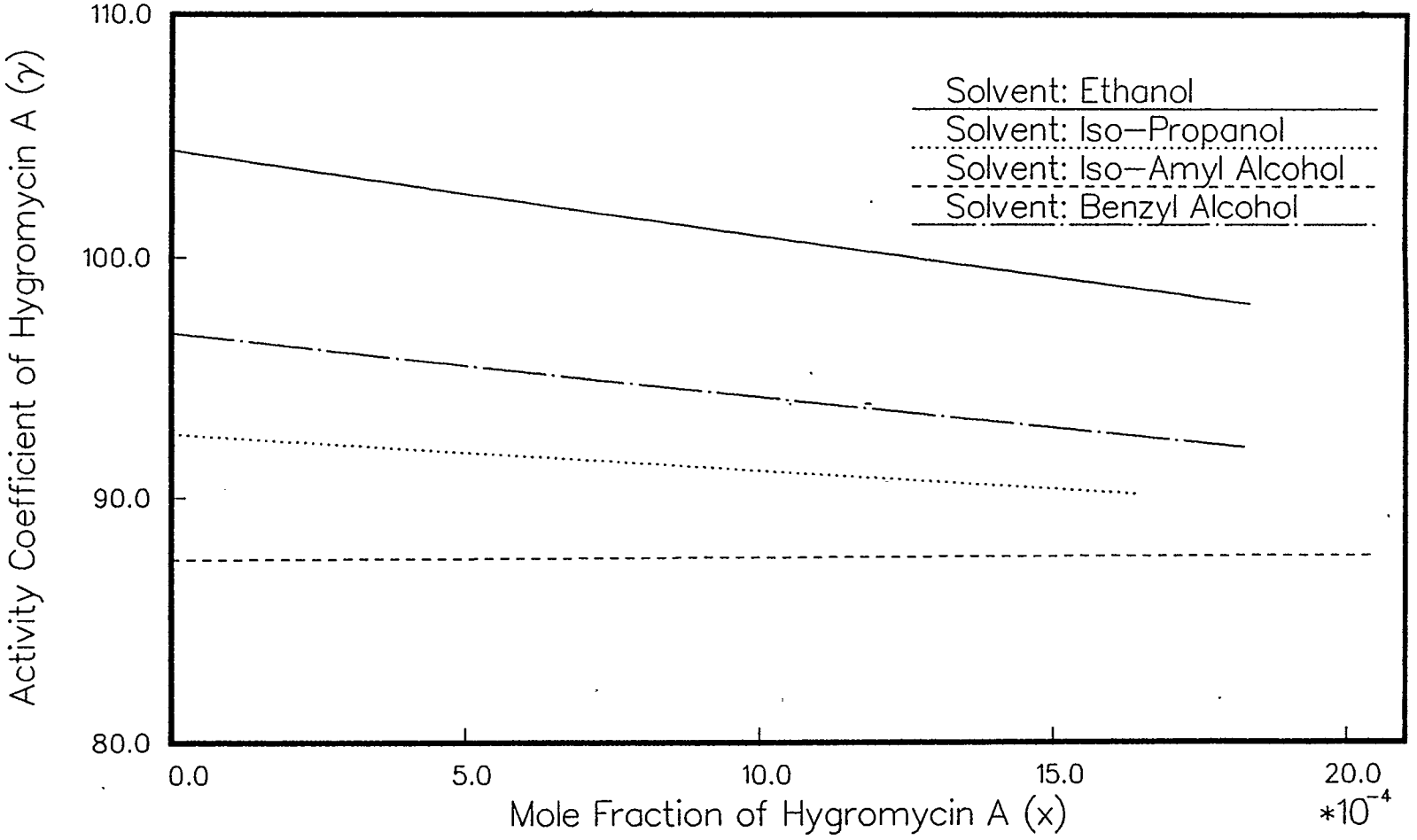


Figure 7.17 Activity coefficient predictions of hygromycin A in ethanol, iso–propanol, iso–amyl alcohol and benzyl alcohol at 301.15 K, using Modified UNIFAC Group Contribution model.

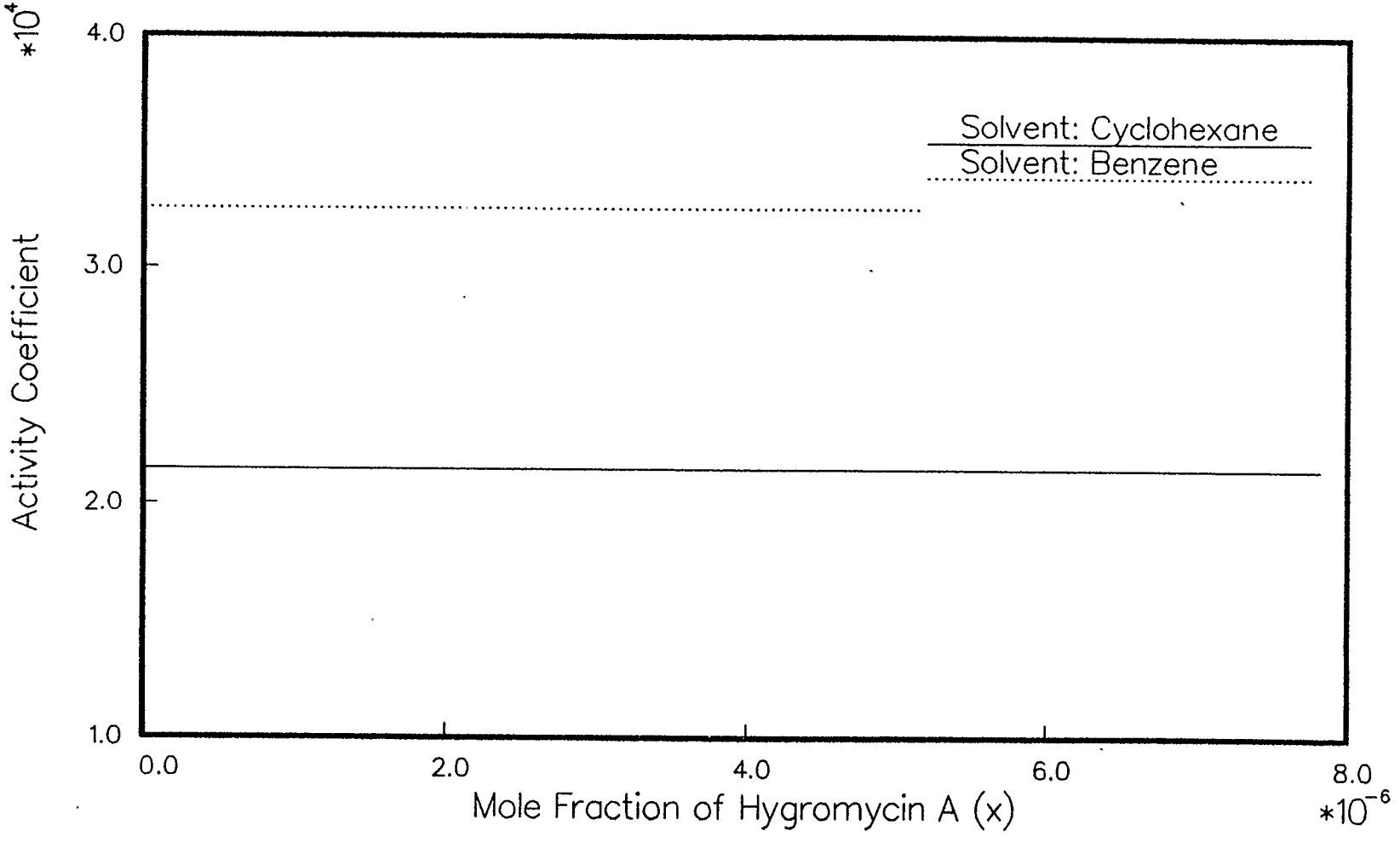


Figure 7.18 Activity coefficient predictions of hygromycin A in cyclohexane and benzene at 301.15 K, using Modified UNIFAC Group Contribution model.

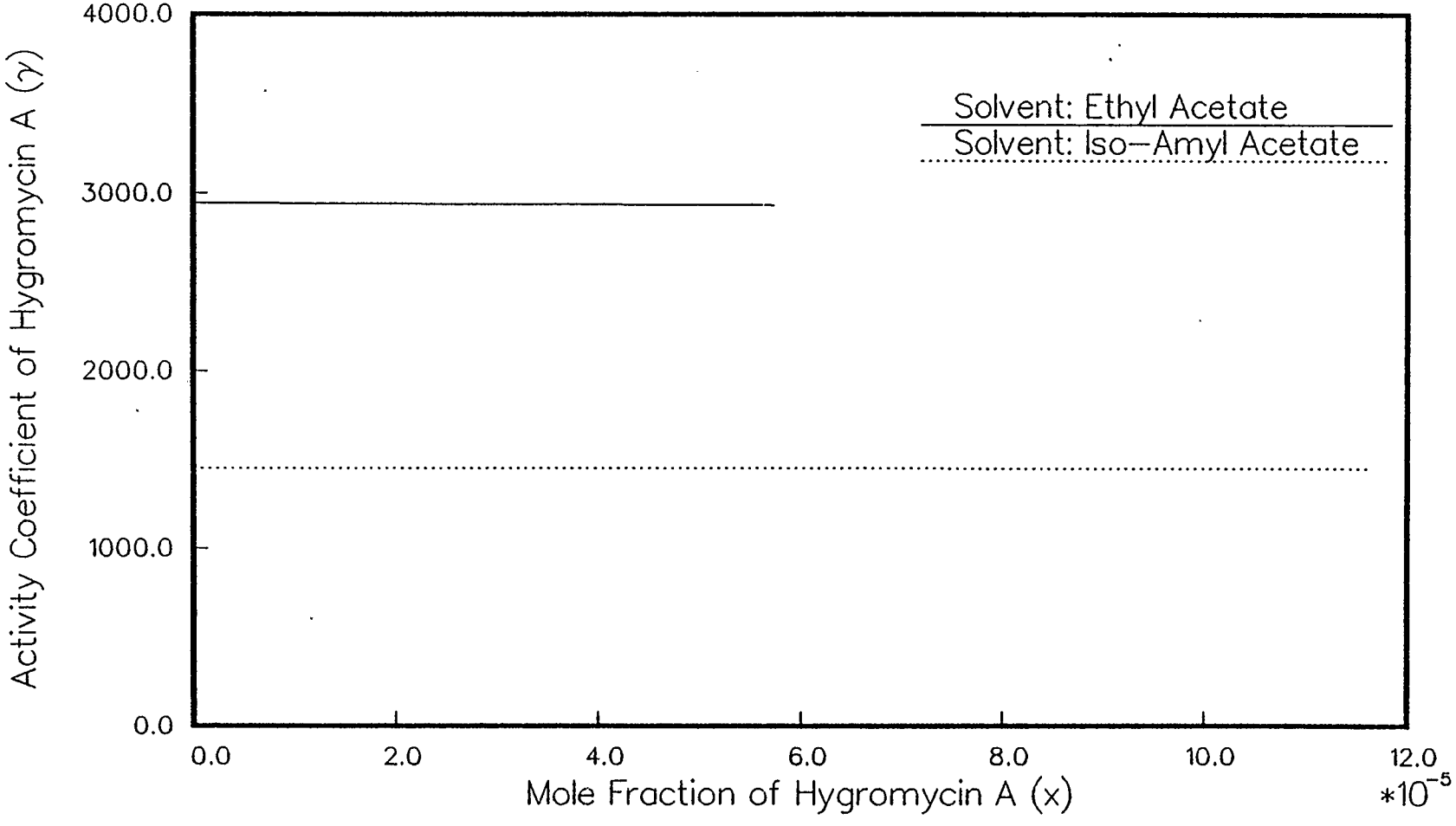


Figure 7.19 Activity coefficient predictions of hygromycin A in ethyl acetate and iso–amyl acetate at 301.15 K, using Modified UNIFAC Group Contribution model.

The activity coefficient of griseofulvin in alcohols, acetates, and in cyclohexane is almost constant up to the solubility limit. The value of γ varies for different solvents; for the cyclohexane it is the highest among the six solvents studied.

Hygromycin A shows a nearly constant value of the activity coefficient in acetates, benzene and in cyclohexane, but in the case of alcohols γ decreases significantly with increase in the concentration except in the case of benzyl alcohol. The γ behavior in benzyl alcohol is close to that in benzene.

7.3 Recovery of Antibiotics by Mixing Solvents

Modified UNIFAC group interaction parameters from binary data can also be used for multicomponent mixtures. This has been demonstrated by calculating the activity coefficients of carbomycin A in mixtures of toluene and cyclohexane. The solubilities of carbomycin A in the mixed solvents of various compositions have been calculated. These solubility results are plotted in Figure 7.20 . Similar calculations can be made for the desired combinations of antibiotics and solvents, for the available group parameters.

Figure 7.20 shows that the solubility of carbomycin A in the mixed solvent cyclohexane-toluene varies non linearly with the mole fraction of cyclohexane (on an antibiotic free basis). The prediction of the solubility at the end points (pure solvents) can be compared with the available experimental data. The error in the solubility in pure toluene is 15% which is of the expected magnitude from the error in the prediction of γ^S (Table 6.2a).

An interesting phenomenon has been observed : by addition of a particular solvent to a solution of the antibiotic, the activity coefficient of the antibiotic can be

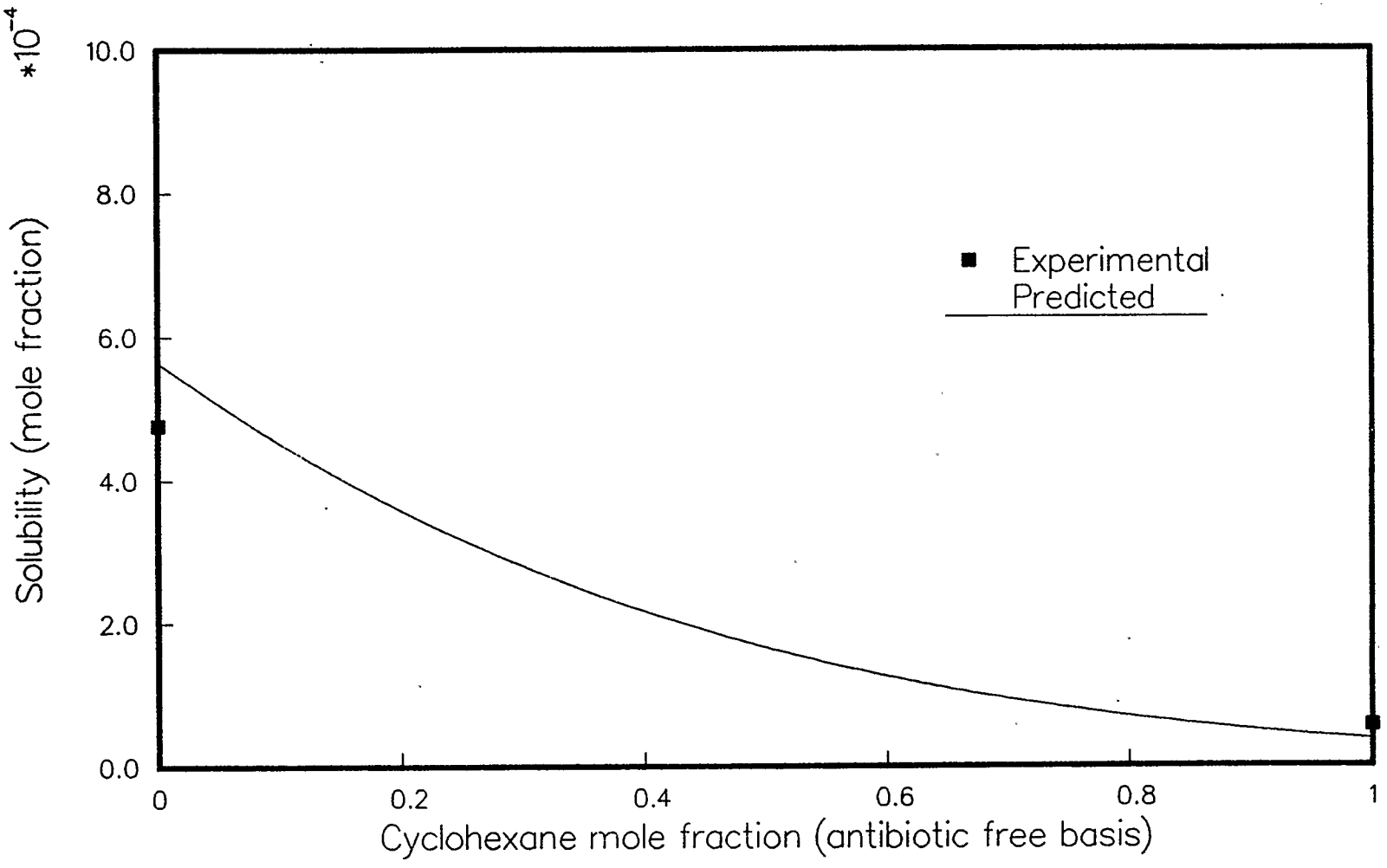


Figure 7.20 Solubility of Carbomycin A in cyclohexane–toluene at 301.15 K. Experimental data of pure solvent by Andrew and Weiss (1959).

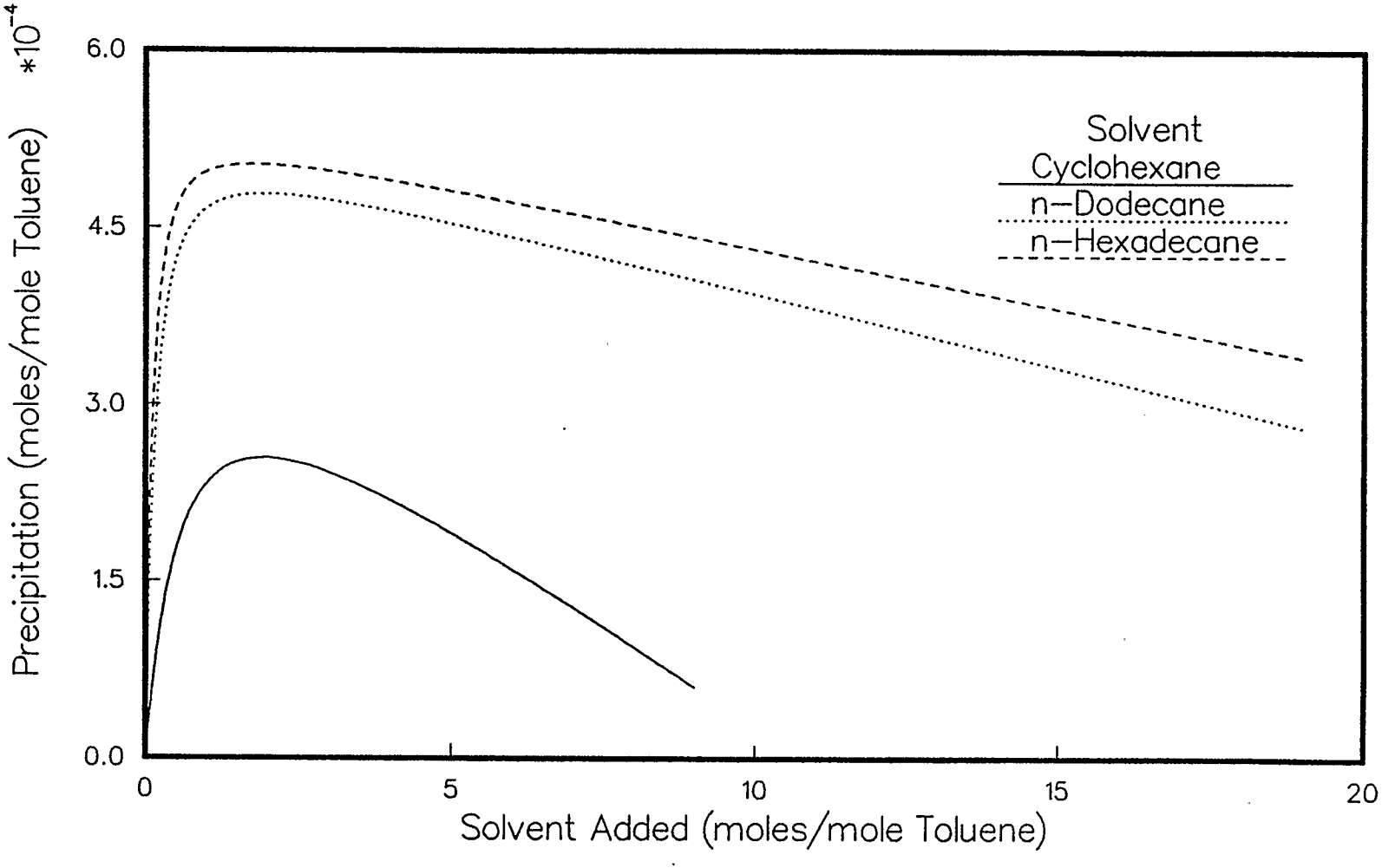


Figure 7.21 Prediction of precipitation of carbomycin A from its saturated solution in toluene at 301.15 K, by adding cyclohexane, n–dodecane and n–hexadecane in the solution.

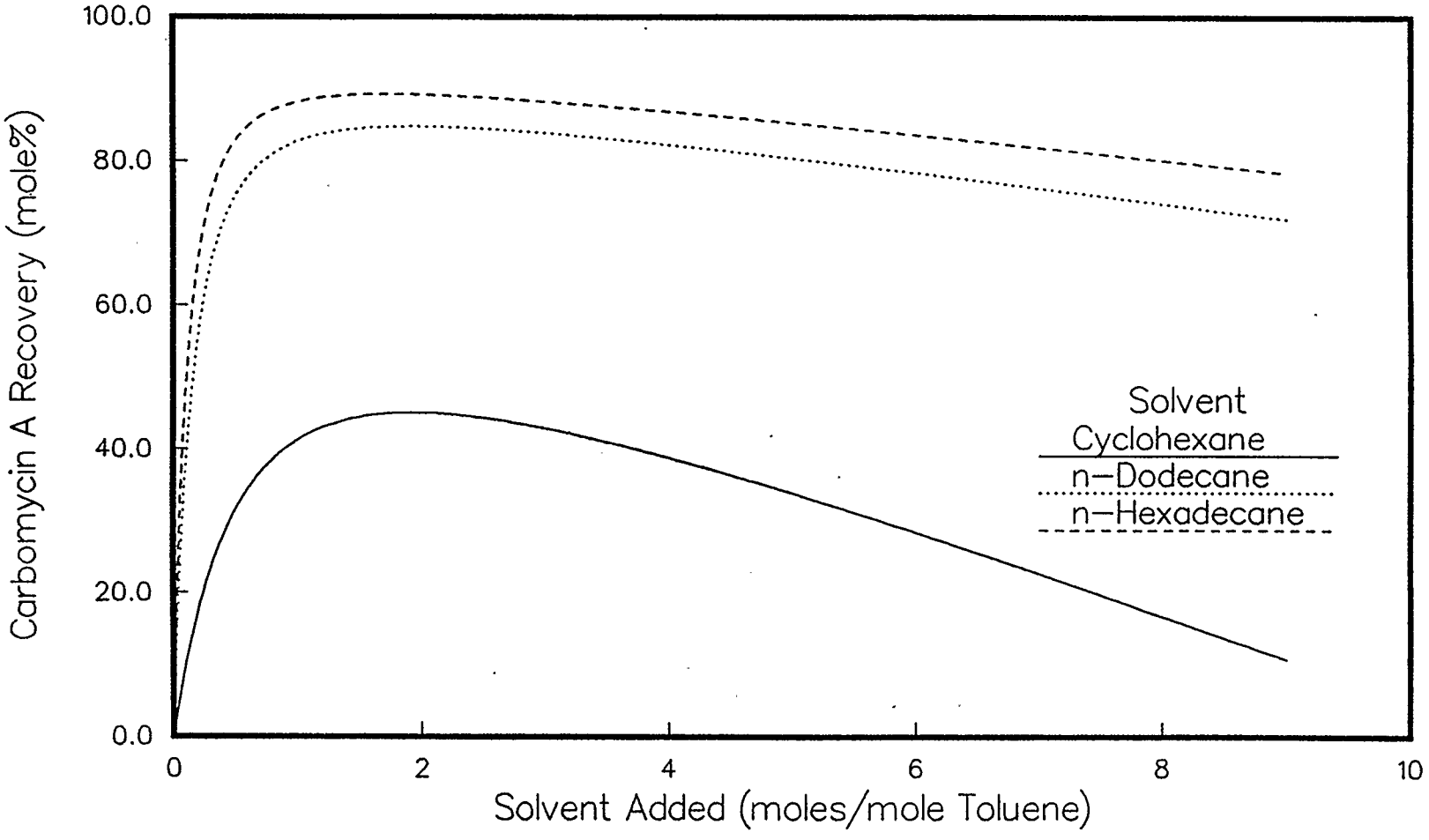


Figure 7.22 Recovery of carbomycin A by precipitation from its saturated solution in toluene at 301.15 K, by adding cyclohexane, n–dodecane and n–hexadecane in the solution.

increased. At the point when the activity of the antibiotic goes above the saturation activity, precipitation of the antibiotic occurs. This phenomenon can be used as a technique for the recovery of the antibiotics.

Calculations have been made to predict the precipitation of carbomycin A from a saturated solution in toluene. On addition of cyclohexane, n-dodecane, or n-hexadecane, precipitation of the carbomycin A occurs. The amount of precipitation first increases, then goes to a maximum, followed by a decrease in the precipitation, as more of the second solvent is added. The amounts of precipitation with the number of moles added of a second solvent are plotted in Figure 7.21 . The mole fraction recovery of the carbomycin A has been plotted in Figure 7.22 . Three solvents cyclohexane, n-dodecane, and n-hexadecane are compared. The maximum recovery was calculated as 45%, 85%, and 89% with the three solvents, respectively. The maximum % recovery increases with the n-alkane chain length of the second solvent.

The calculations for carbomycin A demonstrate the potential value of the group contribution method in the design of separation processes for antibiotics. It is possible with the model to examine the usefulness of a variety of solvents as precipitating agent for a given antibiotic, so long as the energy interaction parameters between the antibiotic and the constituent groups in the solvent are know. In the present study, interaction parameters with CH_2, OH, ACH and $CCOO$ groups have been fitted for some of the antibiotics. The model could be used to predict solubility behavior in any solvent composed of these groups.

7.4 Temperature, pH effects on the Solubility of Amino Acids

The pH of the aqueous solution has an interesting effect on the solubility of

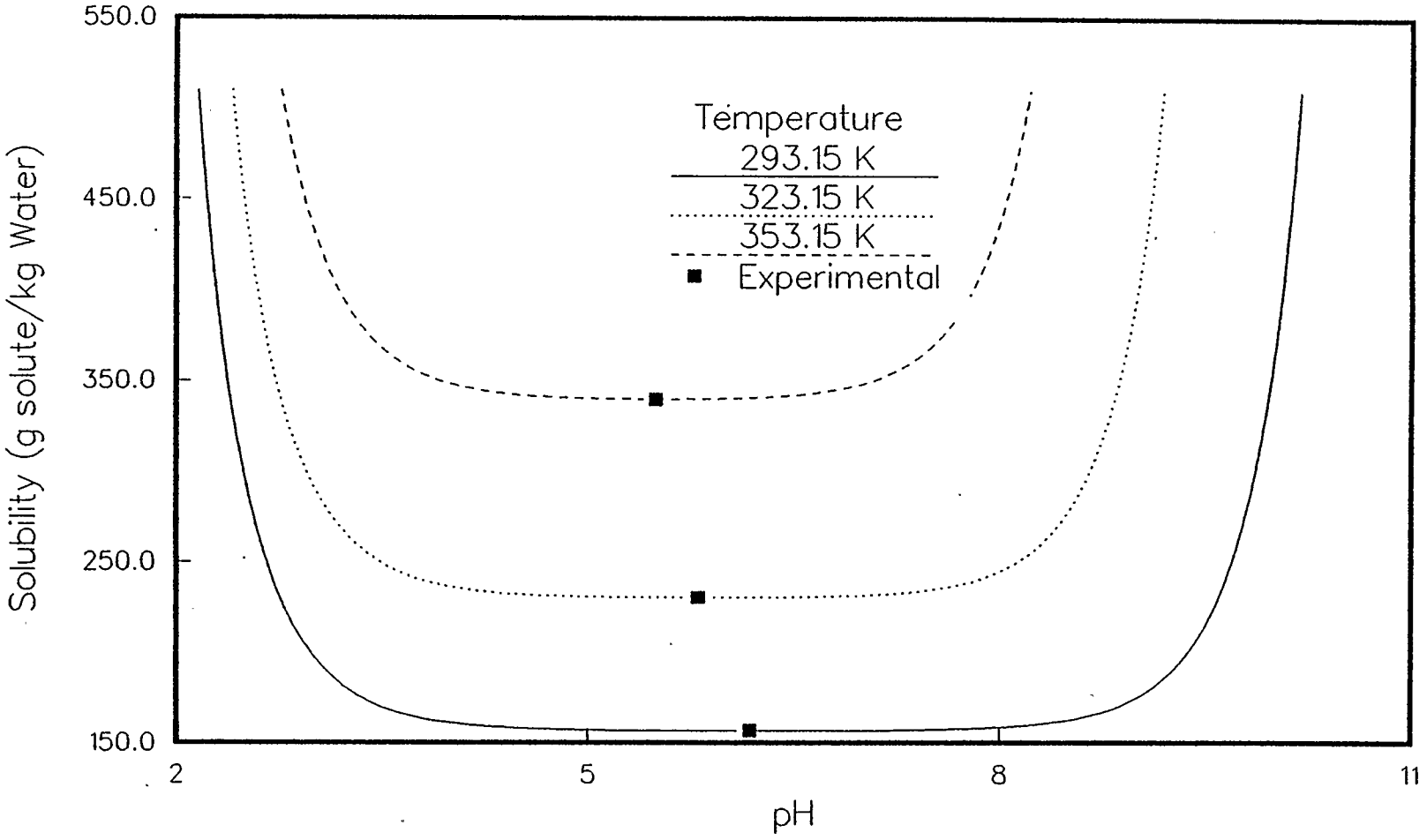


Figure 7.23 Calculated values for the solubility of dl–alanine in water. Experimental data from Fasman (1976).

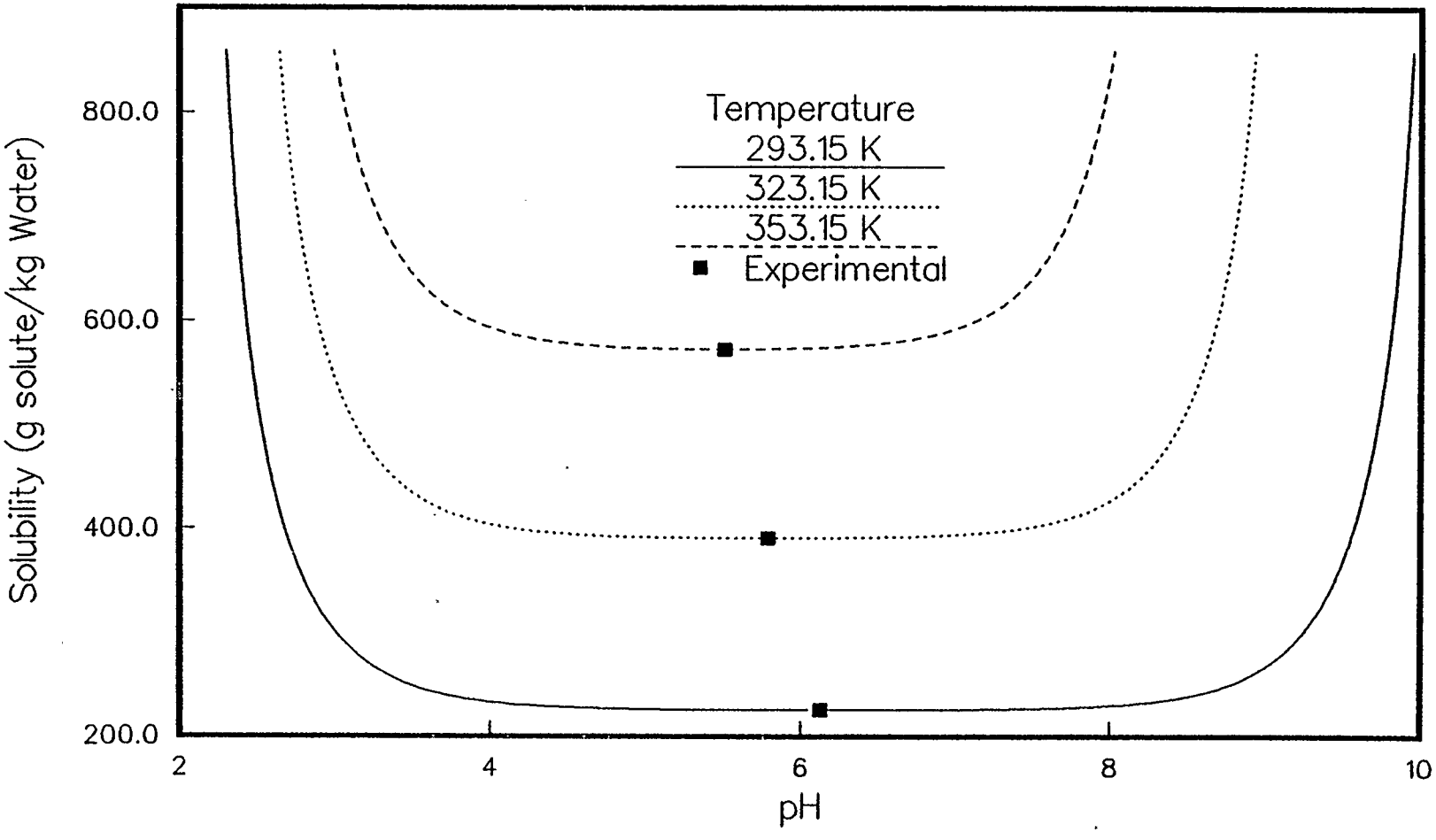


Figure 7.24 Predicted values for the solubility of glycine in water. Experimental data from Fasman (1976).

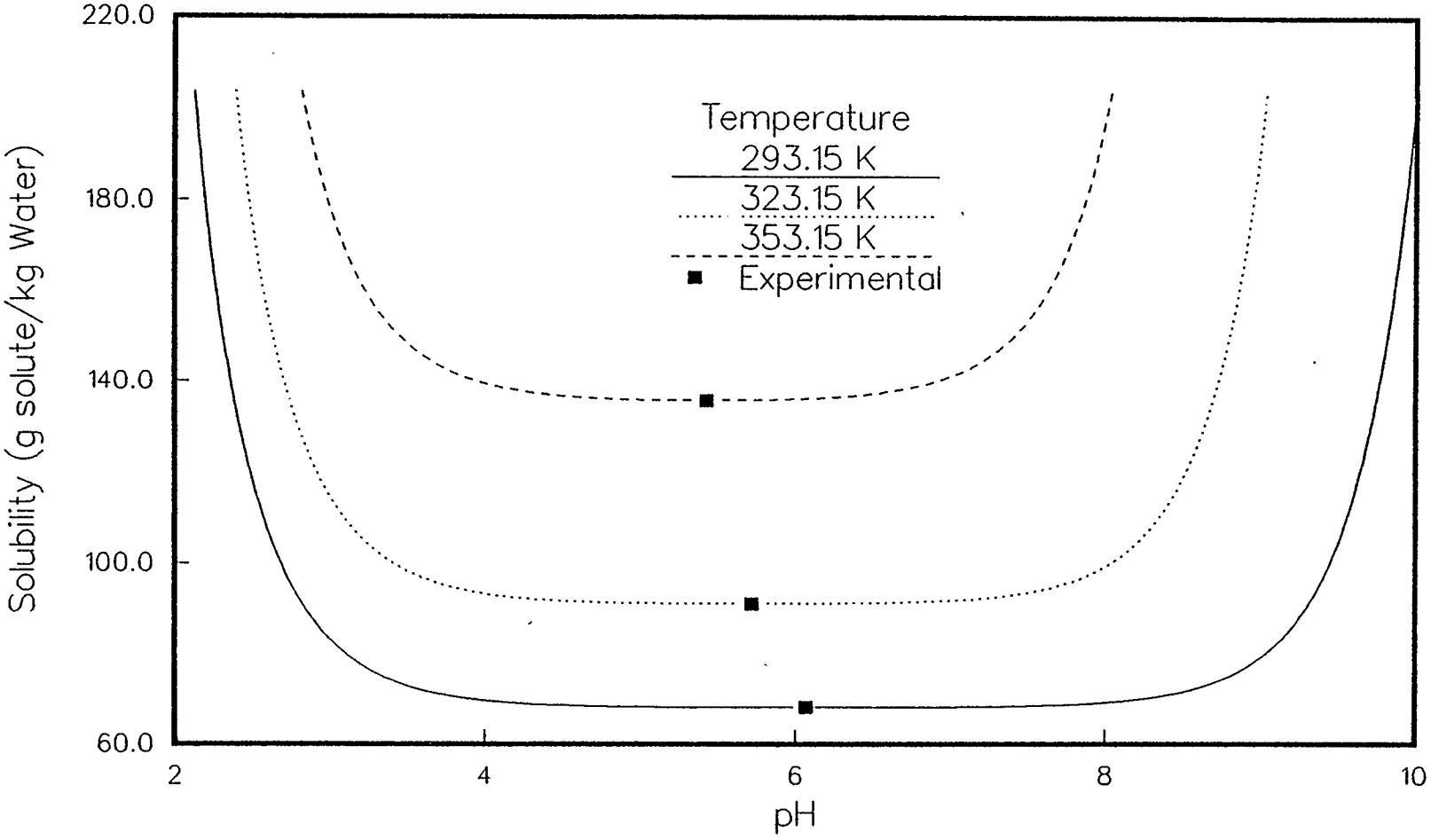


Figure 7.25 Calculated values for the solubility of dl–valine in water.
Experimental data from Fasman (1976).

amino acids. At low and high pH (i.e., acidic and basic solutions), solubility is remarkably high, but at middle pH it goes through a minimum for an extended range of pH. This interesting behavior is due to the zwitterion nature of the amino acids. Amino acids are weak acids, hence the isoelectric point (i.e., the pH where the solubility is a minimum) remains on the slightly acidic side of the pH scale.

A higher temperature gives higher solubility of the amino acids. This is evident from the solubility data given in Table 4.2 .

The effect of pH and temperature on the solubility of *dl*-alanine, glycine, and *dl*-valine has been studied by using Equations (3.46), and (3.47) simultaneously with Modified UNIFAC for the activity coefficients. These results are plotted in Figures 7.23, 7.24 and 7.25 .

Qualitatively, the predictions are good and fully support the physico-chemical reasoning. Nass (1988) has produced a model for tryptosine and di-iodotryptosine that fits experimental data for those two acids. Quantitatively, the results are still to be compared with the experimental data. However, the calculated results are certainly correct at the iso-electric pH since solubility at this point (the minimum for the given amino acid) was used in fitting the model parameter $f_{A_S}{}^{o}/f_{A_{\pm_L}}{}^{o}$.

The activity coefficient correlations affect the numerical value obtained for $f_{A_S}/f_{A_{\pm_L}}{}^{o}$ and, if some other activity coefficient model were used, this parameter would have to have to be re-evaluated. However, the nature of the amino acid solubility curve will probably not be much affected by any re-correlation. In the present case, the rather disappointing quality of the activity coefficient model for some of the amino acids is not expected to affect the calculated solubility curves very seriously.

Chapter 8.

Conclusions

The main emphasis in this research was to characterize the thermodynamic liquid phase behavior of biochemicals. Attempts have been made to predict activity coefficients of antibiotics and amino acids and their solubility. The following conclusions can be drawn:

1. The Modified UNIFAC group-contribution model has been applied to predict the activity coefficients of the amino acids in aqueous solutions. Overall predictions are not especially good and use of the model for higher amino acids must be with caution.

2. A model to predict the effect of pH and temperature on the solubility of amino acids is presented. Qualitative predictions are good and the model gives the correct solubility of the amino acids studied in neutral water.

3. A procedure to predict the activity coefficients of antibiotics, from melting point and solubility data is presented. The model can be used for some antibiotics in mixed solvents made up from CH_2, OH, ACH and $CCOO$ groups. Success of the model is mixed with some failures to give physically meaningful activity coefficient behavior, especially for anisomycin.

4. The use of Modified UNIFAC to predict precipitation of solid antibiotic in mixed solvent has been demonstrated. The modeling technique proposed has potential for use in design of antibiotic purification processes.

5. Quantitative predictions of amino acids solubility variation with pH and of the solubility of antibiotics in mixed solvents cannot be verified from data currently available in the literature. Even a few solubility measurements for antibiotics in mixed solvents would be very useful in refining the UNIFAC models presented here.

Nomenclature *

Symbols	Description
a	group interaction parameter; activity
A	amino acid; a parameter
f	fugacity
g	grams
G	Gibbs free energy
H	enthalpy
K	dissociation constant
L	liter
m	molality
n	moles of amino acid
N	number of point
O.F.	objective function
p	$-log\ 10$
Q	surface area parameter
R	volume parameter; gas constant
S	entropy
T	temperature
w	water
x	mole fraction
z	contact number parameter

* other symbols are defined in the local text.

Subscripts

A	amino acid
exp.	experimental
D	dissociation to zwitterion
E	excess
i	component i
j	component j
k	group k
L	liquid
m	group m; melting
Mod.UNIFAC	Modified UNIFAC group contribution model
n	group n
opt.	optimum
pred.	predicted
S	solid; sucrose
+	cation
±	zwitterion
-	anion
α	tetrahedral

Superscripts

c	combinatorial
m	molal
r	residual
s	saturation

S	sucrose
∞	infinite dilution

Greek Symbols

Γ	residual group activity coefficient
γ	activity coefficient
Δ	difference
θ	surface area fraction
Σ	sum
τ	Boltzman factor
ε	reaction extent
ϕ	osmotic coefficient
ω	volume fraction, modified
ν	number of groups in a molecule
μ	chemical potential

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