



SEPARATION AND PURIFICATION OF AMINO ACIDS

A DISSERTATION PRESENTED TO THE
FACULDADE DE ENGENHARIA DA UNIVERSIDADE DO PORTO
FOR THE DEGREE OF DOCTOR IN BIOLOGICAL AND CHEMICAL ENGINEERING

by

Luisa Alexandre Rodrigues da Fonseca Ferreira

SUPERVISOR

Professora Doutora Maria Eugénia Rebello de Almeida Macedo

CO-SUPERVISOR

Professor Doutor Simão Pedro de Almeida Pinho



Department of Chemical Engineering

Faculty of Engineering

University of Porto

Porto, Portugal

SEPTEMBER 2008

RESUMO

Este trabalho tem como principal objectivo o estudo do equilíbrio sólido-líquido em sistemas contendo aminoácidos, nomeadamente em água pura e em soluções aquosas com álcoois ou sais.

Inicialmente é focada a importância da correlação e previsão das propriedades dos aminoácidos em soluções aquosas com álcoois ou sais. Em seguida, efectua-se uma revisão crítica da informação experimental disponível e dos diferentes modelos usados para correlacionar e/ou prever as propriedades termodinâmicas deste tipo de misturas.

No sentido de contribuir para o aumento do conhecimento científico relativo às solubilidades de aminoácidos, em diferentes solventes, foi implementado um programa experimental para medir a solubilidade dos aminoácidos glicina, DL-alanina, L-serina, L-treonina and L-isoleucina em solventes puros, e em soluções aquosas contendo álcoois (etanol, 1-propanol, 2-propanol) ou electrólitos [KCl ou $(\text{NH}_4)_2\text{SO}_4$], numa gama de temperaturas entre 298.15 e 333.15 K. Os métodos gravimétrico, e espectrofotométrico de reacção com a ninhidrina, foram as técnicas analíticas escolhidas para realizar as medições. Para o desenvolvimento e validação de modelos termodinâmicos utilizou-se uma extensa base de dados, que inclui a informação experimental já disponível na literatura, referente a diferentes propriedades termodinâmicas de soluções aquosas contendo aminoácidos, e os novos dados experimentais medidos neste trabalho.

Aplicaram-se as equações de Pitzer-Simonson-Clegg na correlação e previsão das solubilidades dos aminoácidos em soluções aquosas de electrólitos bem como na correlação dos coeficientes de actividade. Este modelo é expresso na escala de concentração em fracção molar incluindo um termo de Debye-Hückel, para as interacções de longo alcance, e uma expansão de Margules, para as interacções de curto alcance. A qualidade dos resultados obtidos é bastante satisfatória.

Em relação à modelação da solubilidade de aminoácidos em soluções água-álcool, duas metodologias são propostas: i) definição de solubilidade em excesso combinada com os modelos NRTL, NRTL modificado, UNIQUAC modificado, e o modelo proposto por Gude e colaboradores (1996a,b); ii) a aplicação da equação de estado recentemente desenvolvida, Perturbed-Chain SAFT. As potencialidades destes modelos são apresentadas e discutidas. Os resultados obtidos indicam que os dois procedimentos são satisfatórios. Contudo, combinando a solubilidade em excesso como o modelo NRTL modificado é possível correlacionar e prever a solubilidade dos aminoácidos nos diferentes sistemas aquosos com álcoois, no intervalo de temperatura estudado, com precisão mais elevada. Consequentemente, esta metodologia é a recomendada para aplicação em simulação de processos de separação.

ABSTRACT

The main objective of this thesis is the study of solid-liquid equilibrium in amino acid systems, namely in pure water, and in aqueous alcohol or electrolyte solutions.

The importance of the correlation and prediction of properties for aqueous amino acid solutions with alcohols or electrolytes is initially focused. The available experimental data and the different models to correlate or/and predict the thermodynamic properties for this kind of mixtures are critically reviewed.

A systematic experimental program is implemented to measure the solubilities of the amino acids glycine, DL-alanine, L-serine, L-threonine and L-isoleucine in pure solvents, in aqueous alcohol (ethanol, 1-propanol or 2-propanol) and electrolyte [KCl or $(\text{NH}_4)_2\text{SO}_4$] solutions in the temperature range between 298.15 and 333.15 K. The gravimetric and the spectrophotometric ninhydrin methods are the analytical techniques chosen to perform the measurements. A reliable and consistent database, that includes both the available experimental information from the open literature and the new data measured in this work is established, and used for the development of consistent thermodynamic models.

The Pitzer-Simonson-Clegg equations are applied in the correlation of the amino acids solubilities in the aqueous electrolyte solutions at different temperatures simultaneously with activity coefficient data. The equations are expressed on a mole fraction basis and include a Debye-Hückel term, for the long-range forces, and a Margules expansion, for the short-range interactions. Very satisfactory correlation and prediction results are obtained.

Concerning modelling amino acid solubility in aqueous-alkanol solutions two different approaches are proposed: i) the excess solubility approach is applied with the NRTL, modified NRTL, modified UNIQUAC and the model presented by Gude et al. (1996a,b); ii) the recently developed Perturbed-Chain SAFT equation of state is used. The potentialities of these models are presented and discussed. The results achieved indicate that both procedures give satisfactory results. Nevertheless, the combination of the excess solubility approach with the modified NRTL model is the methodology that allows a more successful correlation and prediction of the amino acids solubilities in the different aqueous alcohol systems at the temperature range studied. Therefore, it is recommended for engineering purposes.

RÉSUMÉ

L'objectif principal de cette thèse est l'étude de l'équilibre solide-liquide chez des systèmes contenant des acides aminés, plus précisément soit à l'eau pure, soit pour des solutions alcooliques ou contenant des électrolytes.

Au début, l'importance de la corrélation et prévision des propriétés des solutions aqueuses des acides aminés comprenant des alcools ainsi que des électrolytes est discutée. Les données expérimentales disponibles et les différents modèles qui ajustent ou/et prévoient les propriétés thermodynamiques chez cette classe de mélanges sont révisées et critiquées.

Un programme expérimental systématique est mis à l'oeuvre afin de mesurer les solubilités des acides aminés glycine, DL-alanine, L-serine, L-thréonine et L-isoleucine dans des solvants purs, ainsi que dans des mélanges eau/alcools (éthanol, 1-propanol or 2-propanol) et des solutions contenant des électrolytes [KCl ou $(\text{NH}_4)_2\text{SO}_4$] dans le domaine de températures entre 298.15 et 333.15 K. Le dosage par gravimétrie et l'analyse spectrophotométrique à la ninhydrine sont les techniques d'analyse choisies pour mener à bout les estimations de composition. Une base de données consistante et digne de confiance, comprenant aussi bien les renseignements expérimentaux disponibles dans la littérature que les nouvelles données mesurées grâce à ce travail est établie, a été a servi au développement de modèles thermodynamiques consistants.

Les équations de Pitzer-Simonson-Clegg sont appliquées à la corrélation des solubilités des acides aminés dans des solutions aqueuses d'électrolytes à plusieurs températures en même temps que des données de coefficients d'activité. Les équations sont exprimées à l'aide de fractions molaires et comprennent un terme de Debye-Hückel, tenant compte des forces de longue portée, et une expansion de Margules, décrivant l'effet des interactions de courte portée. Une corrélation assez satisfaisante et de bons résultats pour les prévisions ont été obtenus.

En ce qui concerne la modélisation de la solubilité des acides aminés dans des solutions aqueuses d'alcools, deux différentes méthodes sont proposées: i) le concept d'excès de solubilité est appliqué en combinaison avec les modèles NRTL, NRTL modifié, UNIQUAC modifié et le modèle présenté par Gude et al. (1996a,b); ii) l'équation d'état de la chaîne perturbée SAFT est exploitée. Les potentialités de ces modèles sont présentées et discutées. Les résultats obtenus indiquent que les deux procédures mènent à des résultats satisfaisants. Néanmoins, la combinaison de la méthode de l'excès de solubilité avec le modèle NRTL modifié est celle qui permet les meilleures corrélations et prévisions des solubilités des acides aminés dans des solutions aqueuses contenant des alcools pour le domaine de température étudié. Par conséquent, elle est recommandée pour des applications de génie.

To my Parents

ACKNOWLEDGEMENTS

During this journey, that was worthwhile for my development as a student and as a person, I was encouraged and supported by many persons, to whom I wish to dedicate the following lines and my sincere gratitude.

First of all, I would like to thank to Professor Eugénia Macedo and Professor Simão Pinho for the kindness and concern with which they received me when I arrived to FEUP, for the encouragement to start my Ph.D. studies, for the interesting subject and for being available when I needed.

To Professor Eugénia Macedo I want to express my most felt acknowledgment for the friendship, care, attention, confidence, support, encouragement, teaching, and excellent supervision.

To Professor Simão Pinho, my sincere gratitude, for the friendship, awareness, dedication, excellent guidance, and helpful discussions.

To Professor Jørgen Møllerup I want to express my thanks for the friendship, enthusiasm, total dedication, teaching and interesting discussions.

I wish to thank my supervisors for giving me the opportunity to have the wonderful experience to study abroad.

To Laboratory of Separation and Reaction Engineering (LSRE), headed by Professor Alírio Rodrigues, I want to state my deepest gratitude for all the support, trust and facilities.

I also want to express my gratitude to everyone at IVC-SEP, especially to Professor Erling Stenby, for the wonderful time spent during my visits, for all the support and facilities given.

Special thanks to Professor José Miguel Loureiro, for the attention, constant availability and incentives during this journey.

My gratitude is also due to Dr. Martin Breil, for the friendship, availability and all the help during the development of my work at IVC-SEP.

I am grateful to the entire Thermodynamics group: Oscar Rodríguez, Ana Paula Tavares, António Queimada, Olga Ferreira, Pedro Madeira, Adriano Salgado, Raquel Cristóvão, Nuno Garrido, Fátima Mota, Sara Silvério, for your friendship, care and support whenever needed.

To all the other LSRE colleagues and friends, particularly to Sílvia Santos, Nuno Lourenço, Isabel Martins, Miriam Zabkova, who shared the office with me in the last years and Eduardo Silva, Mafalda Ribeiro and Manuela Vilarinho for all the companionship and for being anytime ready to help.

To all my IVC-SEP colleagues and new friends at Denmark, especially to Ioannis Tsvintzelis, who shared the office with me, for all the companionship.

To the Department's secretary and technician staff, especially to Susana Cruz for all the help with many different issues.

I acknowledge my Ph.D. scholarship (SFRH/BD/17897/2004) and all the financial support from Fundação para a Ciência e a Tecnologia (Portugal).

To my house-mate-friends Joana, Inês, Mónica and Carla I wish to thank all the encouragement, all good moments shared and the optimism that helped me to proceed forward.

To all the other dearest friends you were a source of joy, and help along this road, always available in good and bad moments of life.

To my Family I am forever grateful for your understanding, endless patience, and care and for sharing the cheerful moments. To my sweet godchild, Ana Carolina, for the happiness, joy, and warmth I want to express my affection.

Finally, to my beloved Parents for all the encouragement, affection, and unwavering belief in me, I want to express my love and special thanks.

And to all others, that I might have forgotten, my earnest gratitude.

To all and for all... Muito Obrigada

TABLE OF CONTENTS

	<i>Page</i>
List of Figures	v
List of Tables.....	xi
1. Introduction	1
1.1 Importance and Motivation.....	1
1.2 Objectives	2
2. Thermodynamics of Amino Acid Solutions	5
2.1 Introduction.....	5
2.2 Historic Preview	5
2.3 The use of Amino Acids – Market Overview and Prospects.....	7
2.4 The Chemistry of Amino Acids.....	8
2.4.1 Classification.....	8
2.4.2 Acid-Base Behaviour: Zwitterions.....	10
2.5 State of the Art.....	11
2.5.1 Experimental Methods for Solubility Measurements.....	11
2.5.2 Experimental Data.....	13
2.5.2.1 Water/Amino Acid.....	13
2.5.2.2 Water/Alcohol/Amino Acid.....	16
2.5.2.3 Water/Electrolyte/Amino Acid	17
2.5.3 Modelling	20
2.5.3.1 Water/Amino Acid.....	20
2.5.3.2 Water/Alcohol/Amino Acid.....	28
2.5.3.3 Water/Electrolyte/Amino Acid	30
2.6 Conclusions.....	35
3. Solid-Liquid Equilibrium: Experimental Studies	37
3.1 Introduction.....	37
3.2 Analytical Method	38
3.2.1 Chemicals	39

3.2.2	Apparatus Description	39
3.2.3	Procedure	41
3.2.3.1	Preparation of the Different Solutions	42
3.2.3.2	Experimental Procedure	42
3.2.3.3	Stirring Time	44
3.2.4	Method Reliability	46
3.3	Experimental Measured Data	47
3.3.1	Binary Systems: Water/Amino Acid.....	47
3.3.2	Ternary Systems: Water/Alcohol/Amino Acid	52
3.3.2.1	Water/Ethanol/Amino Acid Systems	52
3.3.2.2	Water/1-Propanol/Amino Acid Systems	55
3.3.2.3	Water/2-Propanol/Amino Acid Systems	59
3.3.2.4	Critical Analysis	62
3.3.3	Ternary Systems: Water/Electrolyte/Amino Acid.....	65
3.3.3.1	Water/KCl/Amino Acid Systems	65
3.3.3.2	Water/(NH ₄) ₂ SO ₄ /Amino Acid Systems	68
3.3.3.3	Critical Analysis	71
3.4	Conclusions	79
4.	Modelling Amino Acid Solubility in Electrolyte Solutions	81
4.1	Introduction	81
4.2	Theoretical Fundamentals	82
4.3	Solubility Prediction.....	87
4.3.1	Parameter Estimation.....	87
4.3.2	Results and Discussion	89
4.4	Thermodynamic Modelling.....	89
4.4.1	Parameter Estimation.....	90
4.4.2	Results and Discussion	93
4.5	Conclusions	100
	Nomenclature	101
5.	Modelling Amino Acid Solubility in Alkanol Solutions (g^E Models).....	105
5.1	Introduction	105

5.2	Excess Solubility Approach.....	106
5.2.1	NRTL Model	107
5.2.2	Modified NRTL Model	108
5.2.3	Modified UNIQUAC Model	109
5.2.4	Model by Gude et al. (1996)	110
5.3	Parameters Estimation	110
5.3.1	NRTL Model	112
5.3.2	Modified NRTL Model	113
5.3.3	Modified UNIQUAC Model	115
5.3.4	Model by Gude et al. (1996)	117
5.4	Results and Discussion	117
5.5	Conclusions.....	125
	Nomenclature.....	126
6.	Modelling Amino Acid Solubility in Alkanol Solutions (PC-SAFT EoS)	129
6.1	Introduction.....	129
6.2	Theoretical Background.....	130
6.3	Model Description	131
6.4	Estimation of Amino Acid PC-SAFT Parameters	132
6.5	Solubility and the Estimation of Hypothetical Melting Properties.....	136
6.6	Modelling Amino Acid Solubilities in Pure Alcohols.....	138
6.7	Modelling Amino Acid Solubilities in Mixed Solvents	140
6.8	Parameters Estimation – Discussion.....	147
6.9	PC-SAFT Parameters by Fuchs et al. (2006).....	152
6.9.1	Critical Analysis.....	153
6.9.2	Prediction of Amino Acid Solubilities in Mixed Solvents.....	156
6.10	PC-SAFT Parameters - A Comparison of the Results of This Work and Fuchs et al. (2006)	156
6.11	Equation of State versus g^E Models.....	162
6.12	Conclusions.....	163
	Nomenclature.....	165
7.	Conclusions	167

7.1	Main Conclusions.....	167
7.2	Suggestions for Future Work	171
References		173
Appendices		
A.	The Chemistry of Amino Acids	195
B.	Mechanism of the Reaction of Ninhydrin.....	199
C.	Calibration Curves	201
D.	Summary of Equations (Perturbed-Chain SAFT EoS).....	203

LIST OF FIGURES

	<i>Page</i>
<i>Figure 2.1</i> General structure of α -amino acids	9
<i>Figure 2.2</i> General structure of α -proline	9
<i>Figure 2.3</i> General acid-base equilibria for an amino acid	10
<i>Figure 3.1</i> Experimental apparatus	40
<i>Figure 3.2</i> Equilibrium jacketed glass cells	41
<i>Figure 3.3</i> Solubility of L-serine in water versus stirring time at two different temperatures.....	45
<i>Figure 3.4</i> Solubility of amino acids (S_{AA}) in water at different temperatures.....	48
<i>Figure 3.5</i> Relative solubilities of amino acids in water/ethanol solutions at 298.15 K.....	54
<i>Figure 3.6</i> Relative solubilities of amino acids in water/ethanol solutions at 313.15 K.....	54
<i>Figure 3.7</i> Relative solubilities of amino acids in water/ethanol solutions at 333.15 K.....	55
<i>Figure 3.8</i> Relative solubilities of amino acids in water/1-propanol solutions at 298.15 K	57
<i>Figure 3.9</i> Relative solubilities of amino acids in water/1-propanol solutions at 313.15 K	58
<i>Figure 3.10</i> Relative solubilities of amino acids in water/1-propanol solutions at 333.15 K	58
<i>Figure 3.11</i> Relative solubilities of amino acids in water/2-propanol solutions at 298.15 K	59
<i>Figure 3.12</i> Relative solubilities of amino acids in water/2-propanol solutions at 313.15 K	60
<i>Figure 3.13</i> Relative solubilities of amino acids in water/2-propanol solutions at 333.15 K	60
<i>Figure 3.14</i> Glycine relative solubilities in water/ethanol solutions at 298.15 K: comparison with the solubility data available in the literature	63
<i>Figure 3.15</i> Effect of the different alcohols on the solubilities of L-isoleucine and L-serine at different temperatures	64

<i>Figure 3.16</i> Relative solubilities of different amino acids in water/KCl solutions at 298.15 K.....	67
<i>Figure 3.17</i> Relative solubilities of different amino acids in water/KCl solutions at 323.15 K.....	68
<i>Figure 3.18</i> Relative solubilities of different amino acids in water/(NH ₄) ₂ SO ₄ solutions at 298.15 K.....	70
<i>Figure 3.19</i> Relative solubilities of different amino acids in water/(NH ₄) ₂ SO ₄ solutions at 323.15 K.....	70
<i>Figure 3.20</i> Relative solubilities of different amino acids in water/electrolyte solutions at 298.15 K: (a) KCl; (b) (NH ₄) ₂ SO ₄	72
<i>Figure 3.21</i> Relative solubilities of different amino acids in water/electrolyte solutions at 323.15 K: (a) KCl; (b) (NH ₄) ₂ SO ₄	72
<i>Figure 3.22</i> Relative solubilities of L-threonine and L-serine in water/electrolyte solution at 298.15 K: (a) KCl; (b) (NH ₄) ₂ SO ₄	73
<i>Figure 3.23</i> Relative solubilities of L-threonine and L-serine in water/electrolyte solution at 323.15 K: (a) KCl; (b) (NH ₄) ₂ SO ₄	73
<i>Figure 3.24</i> Relative solubilities of glycine and DL-alanine in water/electrolyte solution at 298.15 K: (b) (NH ₄) ₂ SO ₄ ; (c) Na ₂ SO ₄	74
<i>Figure 3.25</i> Comparison of solubility data of glycine in water/KCl solutions at 298.15 K.....	75
<i>Figure 3.26</i> Comparison of solubility data of DL-alanine in water/KCl solutions at 298.15 K.....	76
<i>Figure 3.27</i> Comparison of solubility data of glycine or DL-alanine in water/Na ₂ SO ₄ solutions at 298.15 K: ×, Islam and Wadi (2001); □, this work.....	77
<i>Figure 3.28</i> Relative solubilities of L-isoleucine in water/(NH ₄) ₂ SO ₄ solutions versus temperature: ● Givand et al. (2001) (m = 0.5), ■ Givand et al. (2001) (m = 1.08), ▲ Givand et al. (2001) (m = 2.67), ○ This work (m = 0.5), □ This work (m = 1.0), Δ This work (m = 2.0).....	78
<i>Figure 4.1</i> Experimental and calculated solubilities of glycine in water/KCl solutions at 298.15 K.....	94
<i>Figure 4.2</i> Experimental and calculated solubilities of DL-alanine in water/KCl solutions at 298.15 K.....	94

<i>Figure 4.3</i>	Ratio of the mean ionic activity coefficients of KCl in the presence to those in the absence of glycine at 298.15 K: comparison of the model performance with and without solubility data	95
<i>Figure 4.4</i>	Experimental and calculated ratio of the mean ionic activity coefficients of KCl in the presence to those in the absence of L-serine at 298.15 K.....	96
<i>Figure 4.5</i>	Solubilities of glycine or L-serine in water/KCl solutions: comparison between model correlation (—) and the experimental data measured in this work at different temperatures.....	96
<i>Figure 4.6</i>	Water activity in aqueous 1 <i>m</i> KCl solutions containing amino acids at 298.15 K: comparison between model results and the experimental data given by Pinho (2008)	98
<i>Figure 4.7</i>	Water activity in aqueous 3 <i>m</i> KCl solutions containing amino acids at 298.15 K: comparison between model results and the experimental data given by Pinho (2008)	99
<i>Figure 5.1</i>	Relative solubilities of amino acids in water/ethanol solutions at 298.15 K.....	118
<i>Figure 5.2</i>	Relative solubilities of amino acids in water/1-propanol solutions at 298.15 K	120
<i>Figure 5.3</i>	Relative solubilities of amino acids in water/2-propanol solutions at 298.15 K	120
<i>Figure 5.4</i>	Relative solubilities of L-isoleucine in water/1-propanol solutions at different temperatures.....	121
<i>Figure 5.5</i>	Relative solubilities of amino acids in water/1-butanol solutions at 298.15 K. Data from Gude et al. (1996b).....	122
<i>Figure 5.6</i>	Glycine relative solubilities in water/alcohol solutions at 298.15 K. Comparison with the model by Orella and Kirwan (1991)	122
<i>Figure 5.7</i>	Modified NRTL predictions of the relative solubilities of L-threonine in water/ethanol solutions. Data from Sapoundjiev et al. (2006)	123
<i>Figure 5.8</i>	Modified NRTL predictions of the relative solubilities of glycine and DL-alanine in water/ethanol solutions. Data from Dunn and Ross (1938)	124
<i>Figure 6.1</i>	Solubilities of glycine in water at different temperatures.....	137
<i>Figure 6.2</i>	Solubilities of DL-alanine in water at different temperatures	137

<i>Figure 6.3</i>	Solubilities of L-serine in water at different temperatures	138
<i>Figure 6.4</i>	Solubilities of L-threonine in water at different temperatures.....	138
<i>Figure 6.5</i>	Solubilities of L-isoleucine in water at different temperatures	138
<i>Figure 6.6</i>	Solubilities of glycine in different pure alcohols (k_{ij} adjusted to the pure alcohol).....	139
<i>Figure 6.7</i>	Solubilities of DL-alanine in different pure alcohols (k_{ij} adjusted to the pure alcohol).....	139
<i>Figure 6.8</i>	Solubilities of L-serine in different pure alcohols (k_{ij} adjusted to the pure alcohol).....	139
<i>Figure 6.9</i>	Solubilities of L-threonine in different pure alcohols (k_{ij} adjusted to the pure alcohol).....	139
<i>Figure 6.10</i>	Solubilities of L-isoleucine in different pure alcohols (k_{ij} adjusted to the pure alcohol).....	140
<i>Figure 6.11</i>	Solubilities of glycine in various alcohol-water mixtures: PC-SAFT (a) prediction, (b) correlation	142
<i>Figure 6.12</i>	Solubilities of DL-alanine in various alcohol-water mixtures: PC-SAFT (a) prediction, (b) correlation	143
<i>Figure 6.13</i>	Solubilities of L-serine in various alcohol-water mixtures: PC-SAFT (a) prediction, (b) correlation	144
<i>Figure 6.14</i>	Solubilities of L-threonine in various alcohol-water mixtures: PC-SAFT (a) prediction, (b) correlation	145
<i>Figure 6.15</i>	Solubilities of L-isoleucine in various alcohol-water mixtures: PC-SAFT (a) prediction, (b) correlation	146
<i>Figure 6.16</i>	The osmotic coefficients in aqueous DL-alanine solutions at 298.15 K.....	149
<i>Figure 6.17</i>	Symmetric activity coefficients in aqueous DL-alanine solutions at different temperatures (saturated conditions)	149
<i>Figure 6.18</i>	Solubilities of L-serine in pure ethanol (k_{ij} adjusted to the pure alcohol)	150
<i>Figure 6.19</i>	Solubilities of L-serine in water at different temperatures (saturated conditions).....	150
<i>Figure 6.20</i>	Solubilities of L-isoleucine in various 1-propanol-water mixtures (PC-SAFT EoS prediction, amino acid considered as an associating molecule).....	151

<i>Figure 6.21</i>	Densities of aqueous glycine solutions at different temperatures.....	154
<i>Figure 6.22</i>	Vapor pressures in aqueous glycine solutions	154
<i>Figure 6.23</i>	Unsymmetric activity coefficients in aqueous glycine solutions	154
<i>Figure 6.24</i>	Water activities in aqueous glycine solutions.....	154
<i>Figure 6.25</i>	Solubilities of glycine in water at different temperatures.....	154
<i>Figure 6.26</i>	Densities of aqueous DL-alanine solutions at different temperatures	155
<i>Figure 6.27</i>	Vapor pressures in aqueous L-alanine solutions.....	155
<i>Figure 6.28</i>	Osmotic coefficients in aqueous DL-alanine solutions	155
<i>Figure 6.29</i>	Water activities in aqueous DL-alanine solutions	155
<i>Figure 6.30</i>	Solubilities of DL-alanine in water at different temperatures	155
<i>Figure 6.31</i>	Densities of aqueous glycine solutions at different temperatures.....	158
<i>Figure 6.32</i>	Vapor pressures in aqueous glycine solutions.....	159
<i>Figure 6.33</i>	Unsymmetric activity coefficients in aqueous glycine solutions	159
<i>Figure 6.34</i>	Water activities in aqueous glycine solutions.....	159
<i>Figure 6.35</i>	Solubilities of glycine in water at different temperatures.....	159
<i>Figure 6.36</i>	Densities of aqueous DL-alanine solutions at different temperatures.....	159
<i>Figure 6.37</i>	Vapor pressures in aqueous L-alanine solutions.....	160
<i>Figure 6.38</i>	Osmotic coefficients in aqueous DL-alanine solutions	160
<i>Figure 6.39</i>	Water activities in aqueous DL-alanine solutions	160
<i>Figure 6.40</i>	Solubilities of DL-alanine in water at different temperatures	160
<i>Figure 6.41</i>	Solubilities of glycine in different pure alcohols: (a) k_{ij} adjusted to the pure solvent, (b) k_{ij} adjusted to the mixed solvent.....	160
<i>Figure 6.42</i>	Solubilities of DL-alanine in different pure alcohols: (a) k_{ij} adjusted to the pure solvent, (b) k_{ij} adjusted to the mixed solvent.....	161
<i>Figure 6.43</i>	Solubilities of glycine in various alcohol-water mixtures	161
<i>Figure 6.44</i>	Solubilities of DL-alanine in various alcohol-water mixtures.....	161
<i>Figure B.1</i>	Mechanism of the reaction of ninhydrin.....	200
<i>Figure C.1</i>	Calibration curve for L-serine	201
<i>Figure C.2</i>	Calibration curve for L-isoleucine	202
<i>Figure C.3</i>	Calibration curve for L-threonine	202

LIST OF TABLES

	<i>Page</i>
<i>Table 2.1</i>	Thermodynamic properties of amino acids and peptides in aqueous solutions..... 15
<i>Table 2.2</i>	Solubility of amino acids and peptides in aqueous alcohol solutions 17
<i>Table 2.3</i>	Solubility of amino acids and peptides in aqueous electrolyte solutions 19
<i>Table 2.4</i>	Thermodynamic properties of amino acids and peptides in aqueous electrolyte solutions 19
<i>Table 2.5</i>	Models to describe thermodynamic properties of amino acids and peptides in aqueous solutions..... 26
<i>Table 2.6</i>	Models to describe thermodynamic properties of amino acids and peptides in aqueous-alkanol solutions..... 29
<i>Table 2.7</i>	Models to describe thermodynamic properties of amino acids and peptides in aqueous electrolyte solutions..... 34
<i>Table 3.1</i>	Sources and purities of the used compounds..... 39
<i>Table 3.2</i>	Comparison between <i>initial</i> and measured amino acid solubilities, S (g of amino acid/1000 g of water)..... 46
<i>Table 3.3</i>	Comparison of the solubilities of amino acids (g of amino acid/1000 g of water) in pure water 51
<i>Table 3.4</i>	Solubilities of glycine in water/ethanol mixtures at different temperatures..... 52
<i>Table 3.5</i>	Solubilities of amino acids in water/ethanol mixtures at different temperatures..... 53
<i>Table 3.6</i>	Solubilities of glycine in water/1-propanol at 298.15 K..... 56
<i>Table 3.7</i>	Solubilities of amino acids in water/1-propanol mixtures at different temperatures..... 56
<i>Table 3.8</i>	Solubilities of glycine in water/2-propanol solvent mixtures at 298.15 K..... 61
<i>Table 3.9</i>	Solubilities of amino acids in solutions containing 2-propanol at different temperatures..... 61
<i>Table 3.10</i>	Solubilities of amino acids (g of amino acid/1000 g of water) in aqueous solutions of KCl at two temperatures 298.15 K and 323.15 K..... 66

<i>Table 3.11</i>	Solubilities of DL-alanine in aqueous solutions of KCl	67
<i>Table 3.12</i>	Solubilities of amino acids (g of amino acid/1000 g of water) in aqueous solutions of $(\text{NH}_4)_2\text{SO}_4$ at two temperatures, 298.15 and 323.15 K	69
<i>Table 3.13</i>	Solubilities of glycine and DL-alanine (g of amino acid/1000 g of water) in aqueous solutions of Na_2SO_4 at 298.15 K.....	77
<i>Table 4.1</i>	Parameters for water/KCl interactions at 298.15 K (Hu and Guo, 1999)	87
<i>Table 4.2</i>	Model parameters and RMSDs for KCl aqueous solutions with glycine and DL-alanine (without solubility).....	88
<i>Table 4.3</i>	Coefficients q_1 , q_2 and q_3 for water/KCl interaction parameters.....	90
<i>Table 4.4</i>	Model parameters and RMSDs for aqueous KCl solutions with amino acids.....	93
<i>Table 4.5</i>	Solubilities of DL-alanine (g amino acid/100 g of water) in aqueous KCl solutions at 333.15 K: experimental and predicted values.....	97
<i>Table 5.1</i>	Database on amino acid solubility data in aqueous alkanol solutions: experimental temperature range (first row), number of data points (second row), data sources (third row)	111
<i>Table 5.2</i>	NRTL parameters (cal.mol^{-1}) between water (1) and alcohols (2)	112
<i>Table 5.3</i>	NRTL $\tau_{aa,i}^o$ (cal.mol^{-1}) and $\tau_{aa,i}^t$ ($\text{cal.mol}^{-1}.\text{K}^{-1}$) parameters	113
<i>Table 5.4</i>	Modified NRTL parameters τ_{ij}^o (cal.mol^{-1}) and τ_{ij}^t ($\text{cal.mol}^{-1}.\text{K}^{-1}$) between water (1) and alcohols (2).....	114
<i>Table 5.5</i>	Standard partial molar volumes of amino acids ($\text{cm}^3.\text{mol}^{-1}$).....	114
<i>Table 5.6</i>	Modified NRTL $\tau_{aa,i}^o$ (cal.mol^{-1}) and $\tau_{aa,i}^t$ ($\text{cal.mol}^{-1}.\text{K}^{-1}$) parameters.....	115
<i>Table 5.7</i>	Modified UNIQUAC parameters a_{ij}^o (K) and a_{ij}^t between water (1) and alcohols (2).....	116
<i>Table 5.8</i>	Structural parameters (r_i and q_i).....	116
<i>Table 5.9</i>	Modified UNIQUAC parameters $a_{aa,i}^o$ (K) and $a_{aa,i}^t$	116
<i>Table 5.10</i>	The alcohol-water interaction parameters (A_{ji})	117
<i>Table 5.11</i>	Ternary interaction parameters ($C_{j,i,aa}$).....	117
<i>Table 5.12</i>	Average relative deviations (%).....	119

<i>Table 6.1</i>	Pure component PC-SAFT parameters for water (Fuchs et al., 2006).....	133
<i>Table 6.2</i>	Experimental data used to estimate the pure amino acid PC-SAFT parameters: average relative deviation (ARD)* for correlation (first row), number of data points (NDPs, second row), experimental temperature range (third row), data source (fourth row)	135
<i>Table 6.3</i>	Pure component PC-SAFT parameters for amino acids.....	136
<i>Table 6.4</i>	Binary interaction PC-SAFT parameters of amino acid/water systems	136
<i>Table 6.5</i>	Hypothetical fusion properties for amino acids.....	137
<i>Table 6.6</i>	Pure component PC-SAFT parameters for alcohols (Fuchs et al., 2006).....	139
<i>Table 6.7</i>	Binary interaction PC-SAFT parameters of amino acid/alcohol systems	141
<i>Table 6.8</i>	Binary interaction PC-SAFT parameters of water/alcohol systems (Fuchs et al., 2006)	142
<i>Table 6.9</i>	PC-SAFT parameters for DL-alanine	148
<i>Table 6.10</i>	Average relative deviation (%) obtained for the different thermodynamic property using different PC-SAFT parameters for DL-alanine	148
<i>Table 6.11</i>	Hypothetical properties for DL-alanine	148
<i>Table 6.12</i>	Pure component PC-SAFT parameters for L-isoleucine (associating substance)	151
<i>Table 6.13</i>	Pure component PC-SAFT parameters for amino acids given by Fuchs et al. (2006).....	152
<i>Table 6.14</i>	Binary interaction PC-SAFT parameters of amino acids/solvent systems given by Fuchs et al. (2006)	152
<i>Table 6.15</i>	Hypothetical melting properties given by Fuchs et al. (2006).....	153
<i>Table 6.16</i>	RMSD for each alcohol system with glycine and DL-alanine	162
<i>Table A.1</i>	α -Amino acids found in proteins.....	197

CHAPTER 1.

INTRODUCTION

1.1 IMPORTANCE AND MOTIVATION

The biological and industrial importance of amino acids is well known as well as the knowledge of their physical and chemical properties. Amino acids are the basic building blocks of proteins and peptides, and the development of more accurate and efficient processes for their separation, concentration and purification of those has been a subject of main interest, particularly for pharmaceutical and food industries. These methods are still of current interest because of their high cost in comparison to the total manufacturing cost.

The knowledge of solid-liquid equilibrium data is essential for the design, optimization and scale-up of the separation processes. One of the most effective methods used in purification is the re-crystallization since it does not require excessive heating. To reduce drastically the solubility of the solute in the mixture a second miscible solvent, called salting-out agent or anti-solvent, is added. The choice of the suitable separating agent and operating conditions is very limited since amino acids can denaturize. The industrial synthesis of L-serine (food and pharmaceutical industries) involves a fermentation process, and its recovery and purification utilizes cooling re-crystallization by addition of methanol as an anti-solvent (Charmolue and Rousseau, 1991).

Salt-induced precipitation of proteins provides one of the simplest precipitation techniques and it is used extensively in the biotechnology and pharmaceutical industries (Foster et al., 1973, 1976). Ammonium sulphate precipitation is still the preferable method for protein purification, especially in large-scale separation and it is perhaps, the most inexpensive technique available, the simplest one to operate, and does not damage most of the proteins and

enzymes (Scopes, 1994). On other hand, the reverse micellar extraction of proteins or amino acids with an electrolyte or an organic solvent has been recently considered of great interest (Lye et al., 1994; Su and Chiang, 2003). Thus, factors such as chemical structure, pH, surface charge distribution, ionic strength, and type of electrolyte or alcohol must be given special attention.

Solubility data and thermodynamic models are essential for any of the separation and purification methods mentioned. Although some studies have been published concerning the measurement and thermodynamic modelling of aqueous solutions of amino acids with electrolytes and alcohols, a great lack of information on solubility data still remains in terms of studied systems and/or condition (e.g. temperature range, pH, and ionic strength). So, it is evident the need to carry out further measurements.

In this work, a systematic programme to measure the solubilities of particular amino acids in aqueous solutions with alcohols and electrolytes, at different temperatures, was implemented. The establishment of a reliable and consistent data base, that includes the information already available from the open literature, and the new data obtained with the measurements carried out, were fundamental and essential to validate and develop thermodynamic models. In an attempt to overcome the drawbacks found in the representation of phase equilibria and achieve an acceptable quantitative performance for industrial applications, g^E models (NRTL, modified NRTL, modified UNIQUAC, and the model presented by Gude et al., 1996a,b) and an associative equation of state (Perturbed-Chain Statistical Associated Fluid Theory) for the correlation and prediction of the thermodynamic properties were applied.

1.2 OBJECTIVES

The main objective of this thesis is the study of solid-liquid equilibrium of amino acids, namely in pure water, and in aqueous alcohol or electrolyte solutions.

Chapter 2 is an introduction to the thermodynamics of amino acid solutions. The history, chemistry and use (market overview and prospects) of the amino acids are briefly discussed. A critical review of the available information from the open literature, concerning

experimental data, thermodynamic models and their modelling capabilities to correlate and/or predict thermodynamic properties and phase equilibria is also addressed.

Chapter 3 presents the techniques chosen to perform the experimental measurements, details of the procedure and the experimental values of amino acid solubilities. The analytical gravimetric method is used to measure the amino acids solubilities in electrolytes solutions. Concerning the mixed solvent solutions, the solid content is measured differently depending on the alcohol mass fraction in amino acid free basis in the mixed solvent ($w'_{alcohol}$): when $w'_{alcohol} < 0.8$, the gravimetric method is applied; at higher alcohol concentrations ($w'_{alcohol} \geq 0.8$), the spectrophotometric ninhydrin method is used for the analysis of low amino acid concentrations. The experimental results for the solubilities of the amino acids glycine, DL-alanine, L-serine, L-threonine and L-isoleucine in pure solvents, in aqueous alcohol solutions (ethanol, 1-propanol or 2-propanol) and in aqueous electrolyte solutions [KCl or $(\text{NH}_4)_2\text{SO}_4$] in the temperature range between 298.15 and 333.15 K, as well as a critical analysis are shown.

Modelling solid-liquid equilibrium of the amino acids in aqueous electrolyte solutions is presented in chapter 4. The solubility data obtained in this work and activity coefficient data collected from literature were used to study the ability of the Pitzer-Simonson-Clegg equations in the thermodynamic description of the ternary systems water-KCl with glycine, DL-alanine or L-serine at different temperatures. Due to the lack of experimental information it was impossible to extend this study to other systems.

Chapters 5 and 6 present thermodynamic modelling of the solid-liquid equilibrium of the amino acids aqueous alcohol solutions. Two different approaches were considered. In chapter 5 the potentialities of the excess solubility approach combined with conventional thermodynamic models of g^E such as the NRTL, the modified NRTL, the modified UNIQUAC equations, and also with the model presented by Gude et al. (1996a,b) were explored. In chapter 6, the solubility of the amino acids in pure and mixed solvents was calculated using the recently developed Perturbed-Chain Statistical Associated Fluid Theory equation of state (PC-SAFT EoS) (Gross and Sadowski, 2001, 2002).

The main conclusions that have been derived from the present work and suggestions that might be considered as future work are presented in chapter 7.

CHAPTER 2.

THERMODYNAMICS OF AMINO ACID SOLUTIONS

2.1 INTRODUCTION

Amino acids became a very important studied subject due to their biological and industrial importance. Historically, the first amino acid was isolated by Vauquelin and Robiquet in 1806 (Drauz et al., 2007) and, since then, the physical and chemical properties of the amino acids have been the issue of many studies.

In this chapter some aspects on the history of amino acids are given; it was necessary more than one hundred years to identify the 20 amino acids present in proteins. After, a market overview and outlook of the worldwide growth of the sector of amino acids is shown. Amino acids are valuable as basic elements in all forms of life and also of great importance in industrial processes, particularly for food, chemical, medical, pharmaceutical and cosmetics industries. Amino acids have also interesting properties due to the variety of their structural parts and so the chemistry of amino acids will also be considered in this chapter. Finally, experimental techniques applied for the determination of the solubility, experimental data (solubilities, vapor pressures, water activity, osmotic and activity coefficients), and modelling work for the correlation or/and prediction of those thermodynamic properties concerning aqueous amino acid solutions, with or without a salt, or an alcohol, are overviewed.

2.2 HISTORIC PREVIEW

In accordance to Drauz et al. (2007), the history of amino acid chemistry began in 1806 when two French researchers, Vauquelin and Robiquet, isolated asparagine from asparagus plant

juice. Their work was followed in 1812 when William Hyde Wollaston found a substance in urine (a new type of bladder stone), identified as a cystic oxide, and later named cystine. Henri Braconnot discovered glycine and leucine in 1820. Thirty years later, the first amino acid was synthesised; Adolph Strecker synthesised alanine from acetaldehyde via its condensation product with ammonia and hydrogen cyanide. In 1895, Sven Hedin isolated the compound arginine and, with the help of his colleague Albrecht Kossel, discovered histidine. Lysine was identified three years later by Edmund Dreschel. Several other amino acids were discovered towards the end of the 19th century; history of amino acids discovery is closely related to advances in analytical methods (Araki and Ozeki, 2003). Between 1899 and 1908, Fischer gave his great contributions to the knowledge of proteins. Until then, scientists were uncertain of the relationship between amino acids and protein molecules. Fischer discovered a new type of amino acids, the cyclic amino acids (e.g. proline) and showed how the various amino acids can be combined with each other forming a protein molecule. Fischer was able to establish the type of bond that would connect amino acids together in chains, the peptide bond, and obtained the dipeptides, and later the tripeptides and polypeptides. In 1901, in collaboration with Fourneau, the synthesis of glycylglycine was discovered. Simultaneously, tryptophan and its important role on the diet was showed by Frederick Gowland Hopkins. It was not until 1925 that all the 20 amino acids present in proteins were identified; the last to be known, threonine, was identified by William Cumming Rose. In 1942, Rose and collaborators turned their attention to the amino acid requirements for humans extending their studies to quantify the dietary requirements for each amino acid. This quantitative work distinguished the amino acids that are absolutely essential from those that are necessary only for optimal growth.

Degussa pioneered commercial production of synthetic DL-methionine during 1948 in Germany and two years later all L-amino acids were already manufactured by isolation from protein hydrolysis or by separation of L-amino acids from the synthesized racemic mixtures. Since the mid-1950s, methods of production of L-amino acids have changed extensively. The first significant change occurred when a new fermentation process using the so-called glutamic acid bacteria to produce L-glutamic acid was used. Afterwards, fermentation processes were developed to economically produce many other amino acids, and enzymatic processes were developed to produce L-aspartic acid, L-alanine, L-tryptophan, L-cysteine, L-serine, L-lysine, L-phenylalanine, from chemically synthesized substrates. Glycine,

DL-alanine, DL-methionine, and DL-cysteine, and some other amino acids, are still produced by chemical synthesis (Araki and Ozeki, 2003; Drauz et al., 2007).

2.3 THE USE OF AMINO ACIDS - MARKET OVERVIEW AND PROSPECTS

Since their isolation in the 19th century, the physical and chemical properties of amino acids became a very important studied subject, not only because of their value as basic elements in all forms of life, but also for their importance in industrial processes, particularly for food, chemical, medical, pharmaceutical and cosmetics industries.

Aspects of the use of amino acids in various branches of industry, with special attention for the use as food additives, surface-active agents, in the production of polymeric materials, in electrochemical manufacture, photography, pesticides, motor-fuel additives and cosmetics, were surveyed by Sadovnikova and Belikov (1978) on the basis of works published in the seventies. Recently, a mini-review describing the possibilities to generate (new) chemical products using amino acids from biomass instead of fossil resources, and showing that the production of those products can be more attractive to the current methods, was presented by Scott et al. (2007).

In addition to their nutritive value, amino acids are important flavour precursors and each amino acid has its characteristic taste of sweetness, sourness, saltiness and bitterness. In foods for humans, the flavour uses of amino acids represent the dominant factor in total market value. In animal nutrition, amino acids are used in the agricultural products (feedstuff for domestic animals) almost exclusively for their nutritive value. The 1999 world market for amino acids was estimated at more than 1.6 million tons, with approximately 95% of the volume for sodium L-glutamate (used as a taste enhancer), DL-methionine, and L-lysine·HCl (both used to improve the nutritive value of animal feeds) (Araki and Ozeki, 2003). In 2003, the total annual worldwide consumption of amino acids was estimated to be over 2 million tons. Around 1.5 million tons of L-glutamic acid was produced, and the glutamic acid market was growing by about 6% per year. Applications of amino acids in food and pharmaceuticals, or in animal feed nutrition, were expected to grow further in the following years (Hermann, 2003).

In terms of market value, L-lysine, DL-methionine, L-threonine and L-tryptophan (the so-called feed amino acids) constituted in 2004 the largest share (56%) corresponding approximately to 3.4 billion euros. The food sector dominates, essentially with three amino acids (L-glutamic acid in the form of the flavour enhancer mono sodium glutamate, L-aspartic acid and L-phenylalanine). The remaining amino acids were required mainly as raw materials for synthesis of chiral active ingredients, used in pharmaceutical, cosmetic, and agricultural industries (Leuchtenberger et al., 2005). According to a report from the Business Communication Company (2005) the sector of the amino acids with the greatest worldwide growth and highest return is the synthesis market, in particular, for applications in pharmaceuticals and biotechnology, since more drugs enter into the market that are protein based. These applications report for just over an half of the total, but the fast rising at an average annual growth rate (AAGR) of 9.8% is predicted. The worldwide market for amino acids for synthesis is projected to rise at an AAGR of 7% through 2009, from 550 to 750 million euros. The market for amino acids for beverages, health foods and supplements has also expanded and it is already at about 17000 ton.

During the last decades huge efforts were made to raise the productivity and to reduce the production expenses. Biotechnological production of amino acids today serves a market with strong prospects to grow.

The development over the last 20 years is due to major successes in cost effective production and isolation of amino acids products. Of four production methods for amino acids (extraction, synthesis, fermentation and enzymatic catalysis), the economic and ecological advantages of the last two biotechnological based processes are responsible for this impressive growth (Leuchtenberger et al., 2005). Furthermore, the recovery and separation processes of such compounds showed considerable technical advances.

2.4 THE CHEMISTRY OF AMINO ACIDS

2.4.1 CLASSIFICATION

The entire class of amino acids has a common backbone of an organic carboxylic acid group and an amino group attached to a saturated carbon atom. According to the location of the

amine group on the carbon chain that contains the carboxylic acid function, amino acids are classified as α , β , γ , and so on.

When the carboxyl group of one molecule reacts with the amino group of other molecules, releasing a water molecule, the resulting substance is an amide. These amine linkages between amino acids are known as peptide bonds. From the union of two amino acids by a peptide bond results a dipeptide, and it can be unitary increased to tripeptide, tetrapeptide... A peptide chain with more than two amino acids is also called a polypeptide. Proteins are relatively large compounds made of amino acids arranged in a linear chain joined by a peptide bond. Proteins are naturally occurring polypeptides with more than 50 amino acid units (Carey, 2003).

A great number of different amino acids are known to occur naturally, however, a group of twenty of them have a special feature; they are the building blocks of proteins and enzymes. All the amino acids from which proteins are derived are α -amino acids, and all but one of those contains a primary function conform to the general structure shown in Figure 2.1.

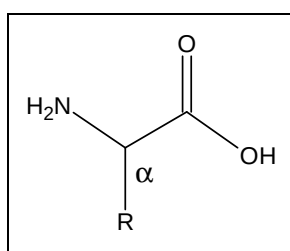


Figure 2.1 General structure of α -amino acids.

Proline (Figure 2.2), a secondary amine in which the amino nitrogen is incorporated into a five-membered ring, is the exception.

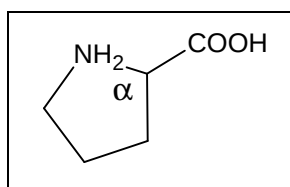


Figure 2.2 General structure of α -proline.

Glycine is the only amino acid that is achiral, the saturated carbon atom is unsubstituted, rendering it optically inactive. The α -carbon atom is a stereogenic center in all the other amino acids. The rest of the most common amino acids are optically active, existing as both

D- and L- stereoisomers. Naturally occurring amino acids that are incorporated into proteins have (L-) configuration.

The different physical and chemical properties result from variations in the structures of the R group, frequently referred as the amino acid "side chain". These twenty amino acids, along with the respective structures, common names, and the customary three- and one-letter codes that abbreviate their names, polarity, and acidity or basicity character can be consulted in Table A.1 (Appendix A). A brief description and comparison between amino acid's structures are also given in appendix A as well as the structure influence on their properties.

2.4.2 ACID-BASE BEHAVIOUR: ZWITTERIONS

Amino acids are crystalline solids with relatively high melting points, so they decompose rather than melt when heated. Most of them are fairly insoluble in non-polar solvents and quite soluble in water. An important characteristic of amino acids is their amphoteric character, they contain both acidic and basic functional groups, and therefore they can function as either acids or bases (Vollhardt and Schore, 2003). In aqueous solutions, the amino acids exist as neutral dipolar ions, also called zwitterions; equilibrium exists between the dipolar ions, and the anionic and cationic forms of an amino acid (Figure 2.3). The predominant form of the amino acid present in solution depends on the *pH* of the solution and on the nature of the amino acid. In strongly acidic solutions all amino acids are present primarily as cations; in strongly basic solutions they are present as anions. At intermediate *pH* values, and more specifically at the isoelectric point (*pI*), the concentration of the dipolar ion is at its maximum. Each amino acid has a particular isoelectric point.

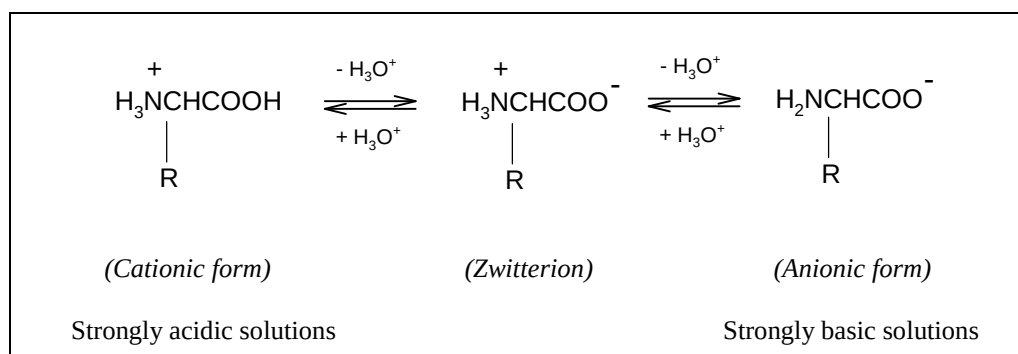


Figure 2.3 General acid-base equilibria for an amino acid.

2.5 STATE OF THE ART

As mentioned in chapter 1 it is intended to study thermodynamic properties of aqueous amino acid solutions with or without a salt or an alcohol.

In any scientific work a detailed and up-dated literature review is fundamental. In this particular case it will help deciding the systems and conditions to perform the experimental measurements, the kind of experimental methods and analytical techniques to implement, and it will also be possible to review the models already proposed and to develop new ones and new methodologies with an acceptable quantitative performance for industrial applications.

2.5.1 EXPERIMENTAL METHODS FOR SOLUBILITY MEASUREMENTS

The analytical method, which consists on the preparation of a saturated solution at constant temperature, has been widely applied for the determination of the solubility of amino acids and peptides. Traditionally, a jacketed glass container is charged with known amounts of all the components and the amino acid is added in an excess amount to that required for saturation. The temperature is maintained constant during the stirring time necessary to reach the solution equilibrium. Then, mixing is stopped to settle the undissolved amino acid particles and a sample of the supernatant phase is withdrawn. To measure the amino acid content several techniques have been applied. The solubility of the amino acids is commonly measured gravimetrically (Cohn et al., 1934; Gekko, 1981; Khoshkbarchi and Vera, 1997; Nozaki and Tanford, 1971; Pradhan and Vera, 2000; Soto et al., 1998a), but HPLC analysis (Givand et al., 2001; Gude et al., 1996a; Orella and Kirwan, 1989), titration (Islam and Wadi, 2001), spectroscopic method (Breil et al., 2004) and recently the attenuated total reflection-Fourier transform infrared (ATR-FTIR) spectroscopy (Fuchs et al., 2006), among others, have been used, as well as the combination of the gravimetric and one of the other methods (Fuchs et al., 2006; Gude et al., 1996a; Orella and Kirwan, 1989). However, the gravimetric analysis proved to be, with the exception of very low solubilities, the most accurate and reproducible method of analysis.

Another important and quite simple analytical technique for amino acid quantification is the spectrophotometric ninhydrin method. This method was introduced in the 1940s (Moore and

Stein, 1948) and since then considerable changes including different heating times, heating temperatures, buffer systems, pH values of buffer solutions and solvents for ninhydrin reagent have been introduced in order to improve it (Sun et al., 2006).

The reaction of ninhydrin with a primary amino group to form a coloured reaction product, diketohydrindylidene-diketohydrindamine (also called Ruhemann's purple – RP – since it was discovered by Siegfried Ruhemann in 1910), has been extensively used in quantitative and qualitative amino acid analysis (Fang and Liu, 2001; Friedman, 2004; Jones et al., 2002; Moore and Stein, 1948, 1954; Prochazkova et al., 1999; Sun et al., 2006). Compared to other methods, such as HPLC, the ninhydrin method still holds several advantages because no expensive equipment is required, and it is suitable for the routine analysis of large number of samples (Sun et al., 2006).

Initially, this method was developed for chromatographic elution from amino acid analyzer (Moore and Stein, 1948, 1954), but it has been extended for the determination of amino group in food samples (Hurst et al., 1995), pharmaceutical products (Amin et al., 2000; Frutos et al., 2000), evaluation of chitosan (Prochazkova et al., 1999), and quantification of collagen-like polymer (Yin et al., 2002) among others (Friedman, 2004) which points out the continued popularity of the method (Sun et al., 2006). This reaction has been widely studied (Joullié et al., 1991; McCaldin, 1960) and the mechanism of the reaction is presented in Appendix B.

Rather than the widely applied analytical methods, the synthetic methods were seldomly used to measure amino acid solubilities. In this method, the cell is charged with mixtures of known composition and then heated to the observed temperature of disappearance of the last crystal of amino acid (polythermal synthetic method) or a known amount of solvent is added (isothermal synthetic method). Messer et al. (1981) measured the water solubilities of single amino acids and pairs of amino acids over the temperature range between 293.15 and 413.15 K, using the polythermal synthetic method. When compared with other experimental data, the results showed certain discrepancies for the solubilities of individual amino acids in water, but gave consistent information on the phase equilibria of the pairs.

Recently, Yi et al. (2005) developed a small-scale automated apparatus for solubility measurements in small solution volumes (1 mL), for pharmaceutical applications. The device operates non-isothermally (polythermal synthetic method) and comparisons of solubilities of

test compounds obtained with this apparatus and data from the literature showed that the experimental error was within 5% (mass fraction), thus advocating for the accuracy of the technique.

2.5.2 EXPERIMENTAL DATA

2.5.2.1 *Water/Amino Acid*

In the 1930s Dalton and Schmidt (1933, 1935) pointed out a “striking factor”: the properties of the amino acids were, until then, studied from many perspectives such as nutritional, optical and physicochemical, but the most elementary properties of their aqueous solutions, solubility in water and the temperature effect, or the densities of their solutions, were fragmentary. Dunn et al. (1933) noticed that the number of solubility data for the amino acids given in the literature was very limited and many of those values were unreliable since the experimental conditions were not precise or not reported. Among others, they have made important pioneering research in the development of a precise experimental work to determine the solubility of amino acids in water over several temperature ranges. Equations have been devised for each compound to express the solubility as a function of temperature. A compilation of the early experimental and theoretical work on the solution behaviour of amino acids was given by Cohn and Edsall (1943).

Concerning the solubility of an amino acid in a solution of other amino acids the data it is still nowadays very scarce, and only few authors focused their work on this subject (Carta, 1999; Cohn et al., 1939; Jin and Chao, 1992; Kuramochi et al., 1996; Messer et al., 1981; Sexton and Dunn, 1947; Soto et al., 1999). Those studies relied on the addition of an excess amount of one amino acid to a solution containing the other amino acid until saturation. The solid phase was considered pure and only the liquid phase was studied. Inversely, Kurosawa et al. (2004) reported data on the compositions of the solid and liquid phases at equilibrium in the system of two amino acids in water.

The solubility of several amino acids in water was measured at pressures up to 400 MPa by Matsuo et al. (2002) and a variety of solubility phenomena were observed. The solubility of glycine decreased with increasing pressure while that of L-alanine increased. A solubility

maximum, around 100 MPa, was observed for L-valine and L-isoleucine and a solid-phase transition was shown for L-leucine. The high-pressure method used is advantageous since thermal decomposition of the amino acid is avoided.

The solubility of five cyclic dipeptides in water at 298.15 K were determined by Kleut and Sijpkens (1994) and the respective molar Gibbs free energies, molar enthalpies and molar entropies of dissolution and the corresponding heat-capacity changes calculated.

An extended study of the thermodynamic properties, namely, osmotic and activity coefficients of amino acids and related compounds, in aqueous solutions at 298.15 K, was developed by Smith and Smith (1937a,b; 1939; 1940a,b). The vapor pressures of the amino acid solutions were measured by the isopiestic technique using sucrose as the reference standard. Activity coefficients at 298.15 K of L-arginine.HCl and L-serine compared with KCl and sucrose, respectively, were measured by Hutchens et al. (1963) using a modified isopiestic method.

Richards (1938), Robinson (1952), Hutchens et al. (1963) and Ellerton et al. (1964) reported osmotic and activity coefficients for aqueous solutions of several amino acids, at 298.15 K, computed from isopiestic vapor pressure measurements. Relative viscosities, density and apparent and partial molar volume data, obtained from density were also reported. Later, Hutchens (1976) compiled some physical and thermodynamic properties of aqueous amino acid solutions at 298.15 K.

Kuramochi et al. (1997) measured vapor pressures for aqueous solutions of amino acids at 298.15 K by the differential pressure method. Activity and activity coefficients of water were determined and used to obtain the activity coefficients of the amino acids in water. Romero and González (2006) studied the effect of temperature (288.15 – 303.15 K) on the osmotic and activity coefficients of some α -amino acids in aqueous solutions using the isopiestic method.

Experimental data on water activity, pH and density of some aqueous amino acid solutions were presented by Ninni and Meirelles (2001) at 298.15 K in three different types of solvents (water, acid and basic buffers). More recently, Pinho (2008) developed a simple, fast and reliable experimental procedure, as alternative to the classical isopiestic method, to measure water activity in aqueous amino acid system with or without a salt (potassium chloride) at

298.15 K. The dependence of the solubilities on pH has also been studied (Brown and Rousseau, 1994; Carta, 1998; Pradhan and Vera, 1998; Zumstein and Rousseau, 1989).

Finally, the volumetric and other thermochemical properties of aqueous amino acids systems (e.g. densities, viscosity, apparent molar volumes, apparent molar heat capacities, partial molar volumes and compressibility) have also been measured by several authors at different temperatures (Banipal et al., 2007; Duke et al., 1994; Hakin et al., 1997; Kikuchi et al., 1995; Lark et al., 2004; Yan et al., 1999). A compilation of all these experimental works is presented in Table 2.1.

Table 2.1 Thermodynamic properties of amino acids and peptides in aqueous solutions.

Thermodynamic Properties	Temperature Range (K)	References
Solubility of amino acids	298.15-373.15	Cohn and Edsall (1943); Dalton and Schmidt (1933, 1935) Dunn et al. (1933); Messer et al. (1981)
Solubility of amino acids in water solutions	298.15	Carta (1999); Cohn et al. (1939); Jin and Chao (1992) Kuramochi et al. (1996); Kurosawa et al. (2004) Sexton and Dunn (1947); Soto et al. (1999)
	293.15-413.15	Messer et al. (1981)
Solubility of peptides	298.15	Kleut and Sijpkens (1994)
Solubility high pressures (up to 400 MPa)	298.15	Matsuo et al. (2002)
Osmotic and activity coefficients of amino acids	298.15	Ellerton et al. (1964); Hutchens et al. (1963) Kuramochi et al. (1997); Richards (1938) Robinson (1952) Smith and Smith (1937a,b, 1939, 1940a,b)
	288.15-303.15	Romero and González (2006)
Vapor pressure	298.15	Kuramochi et al. (1997)
Water activity	298.15	Ninni and Meirelles (2001); Pinho (2008)
pH influence on solubility	298.15	Brown and Rousseau (1994); Carta (1998) Pradhan and Vera (1998); Zumstein and Rousseau (1989)
Volumetric properties	288.15-328.15	Banipal et al. (2007); Duke et al. (1994); Hakin et al. (1997) Kikuchi et al. (1995); Lark et al. (2004); Yan et al. (1999)

2.5.2.2 Water/Alcohol/Amino Acid

As far as solid-liquid equilibrium data of amino acids in mixed-solvent solutions is concerned, a literature survey shows a considerable lack of information and the majority of the data available is at 298.15 K only.

Cohn et al. (1934), McMeekin et al. (1935), Nozaki and Tanford (1971) measured the solubility of several amino acids in water-ethanol mixtures at 298.15 K. Dunn and Ross (1938) studied the solubility of eight amino acids at five different ethanol concentrations at four temperatures (273.15, 298.15, 318.15 and 338.15 K). After, Conio et al. (1973) reported values of solubilities of glycine and some glycyl peptides in aqueous ethanol mixtures at 293.15 K, 298.15 K, 316.65 K and 333.15 K, and more recently the solubility equilibria of threonine, also in water-ethanol mixtures, was measured by Sapoundjiev et al. (2006) in the temperature range between 283.15 K and 319.15 K. It is worth to mention that these are some of the few works that consider the temperature influence on the solubility of amino acids in mixed-solvent solutions.

Amino acid solubilities in aqueous methanol solutions were measured by Gekko (1981) at 298.15 K. Charmolue and Rousseau (1991) measured the solubility of L-serine in water-methanol mixtures over the range 0 to 100 vol.% methanol at 283.15 K and 303.15 K. Later, the solubility of L-serine in water-methanol mixtures (0, 5, 10, 16 and 22% mol methanol) over a temperature range from 278 K to 333 K has been determined by Luk and Rousseau (2006).

Amino acid solubility in the mixed solvents water-1-propanol and water-2-propanol were reported by Orella and Kirwan (1989, 1991), and more recently the system water-1-butanol was studied by Gude et al. (1996a,b), all at 298.15 K only.

Two concentrations of *t*-butanol (8 and 15% mol) in water were used by Givand et al. (2001) to quantify the impact of the alcohol on the solubility of L-isoleucine and L-leucine. Very recently, solubility measurements (by gravimetry) were performed on glycine polymorphs in aqueous solutions containing methanol, ethanol, 2-propanol or acetone at 310 K (Bouchard et al., 2007). Table 2.2 compiles the information concerning systems and temperature ranges for solubility studies in aqueous alcohol solutions.

Concerning the measurements of the amino acids activity coefficients in water/alkanol mixed solvents, no literature data was found.

Table 2.2 Solubility of amino acids and peptides in aqueous alcohol solutions.

Solubility	Solvent	Temperature Range (K)	References
Amino acids	Ethanol	298.15	Cohn et al. (1934) McMeekin et al. (1935) Nozaki and Tanford (1971)
		273.15 – 338.15	Bouchard et al. (2007) Conio et al. (1973) Dunn and Ross (1938) Sapoundjiev et al. (2006)
	Methanol	298.15	Gekko (1981)
		278.15-333.15	Bouchard et al. (2007) Charmolue and Rousseau (1991) Luk and Rousseau (2006)
	1-Propanol	298.15	Orella and Kirwan (1989, 1991)
	2-Propanol	298.15	Orella and Kirwan (1989, 1991)
		310	Bouchard et al. (2007)
	1-Butanol	298.15	Gude et al. (1996a,b)
	t-Butanol	298.15	Givand et al. (2001)
Peptides	Ethanol	293.15 -333.15	Conio et al. (1973)

2.5.2.3 Water/Electrolyte/Amino Acid

Cohn and Edsall (1943) compiled the few and rather old experimental measurement of the solubility of amino acids in aqueous electrolyte solutions. The experimental work carried out so far has been most focused on the study of the effect of the electrolyte on the solubility of different amino acids (Islam and Wadi, 2001; Khoshkbarchi and Vera, 1997; Pradhan and Vera, 2000; Soto et al., 1998a) and peptides (Breil et al., 2004, Lampreia et al., 2006).

The measurement of the electrolyte activity coefficient in the presence of an amino acid (Bower and Robinson, 1965; Chung and Vera, 2002; Dehghani et al., 2005; Gao and Vera, 2001; Kamali-Ardakani et al., 2001; Khavaninzadeh et al., 2002, 2003; Khoshkbarchi and Vera, 1996a,b,c; Phang, 1978, Phang and Steel, 1974; Rodríguez-Raposo et al., 1994; Schrier and Robinson, 1971, 1974; Soto et al., 1997a,b, 1998b) or a peptide (Breil et al., 2001; Chung and Vera, 2001) have been also the subject of important experimental studies.

Although some studies have been published concerning those measurements (solubilities and activity coefficients), the use of electrolytes has been focused on a restrict number of salts: NaCl, KCl, NaBr, KBr, NaNO₃, KNO₃, Na₂SO₄, (NH₄)₂SO₄.

It is worthwhile to mention that the great majority of the measurements were carried out at 298.15 K, but the temperature influence on the solubilities was considered by Islam and Wadi (2001). Activity coefficients of amino acids in aqueous electrolyte solutions at different temperatures were presented by Kamali-Ardakani et al. (2001) and Khavaninzadeh et al. (2002).

The effect of the presence of another electrolyte in the mixture of electrolyte and amino acids was considered by Dehghani et al. (2005, 2006a). They presented data for the activity coefficients of glycine in mixed electrolyte solutions containing NaBr and K₃PO₄; first at constant molality of K₃PO₄ and different molalities of NaBr and glycine (Dehghani et al., 2005) considering two temperatures (298.15 and 308.15 K), and after, at constant molality of NaBr, using different molalities of K₃PO₄ and glycine (Dehghani et al., 2006b) at 298.15 K.

Various reports on volumetric and thermochemical properties (densities, apparent molar volumes, apparent molar adiabatic compressibilities, partial molar volumes, heat capacities, viscosities) of amino acids in aqueous electrolyte solutions, are also available and have been reviewed by several authors (Akhtar, 2007; Banipal et al., 2004, 2007; Lark et al., 2004, 2007; Ramasami and Kakkar, 2006; Singh et al., 2007; Soto et al., 1998c; Yuan et al., 2006).

The information concerning the solubility of amino acids and peptides in aqueous electrolyte solutions (electrolyte molality range, temperature range and sources) is presented in Table 2.3. Table 2.4 compiles information concerning other thermodynamic properties.

Table 2.3 Solubility of amino acids and peptides in aqueous electrolyte solutions.

Thermodynamic Properties	Electrolyte (molality range/m)	Temperature Range (K)	Reference
Solubility of Amino acids	NaCl, KCl (0 - 1.5m)	298.15	Khoshkbarchi and Vera (1997)
	NaNO ₃ , KNO ₃ / (0 – 1.5 m)		Pradhan and Vera (2000)
	NaCl, KCl, NaNO ₃ /(0 – 1.6 m)		Soto et al. (1998a)
Solubility of Peptides	Na ₂ SO ₄ (0 – 1.5 m)	288.15 – 308.15	Islam and Wadi (2001)
	NaCl (0 – 6 m)	298.15	Breil et al. (2004)
	Na ₂ SO ₄ , (NH ₄) ₂ SO ₄ (0 – 1 m)		
	NaCl (0.1 – 1 m)	288.15 – 313.15	Lampreia et al. (2006)

Table 2.4 Thermodynamic properties of amino acids and peptides in aqueous electrolyte solutions.

Thermodynamic Properties	Electrolyte	Temperature Range (K)	Reference
Activity coefficients of amino acids	NaCl NaBr KCl KBr NaNO ₃	298.15	Bower and Robinson (1965); Chung and Vera (2002)
			Cohn and Edsall (1943); Dehghani et al. (2005)
			Gao and Vera (2001); Kamali-Ardakani et al. (2001)
			Khavaninzadeh et al. (2002, 2003)
			Khoshkbarchi and Vera (1996a,b,c)
			Phang (1978); Phang and Steel (1974)
			Rodríguez-Raposo et al. (1994)
			Schrier and Robinson (1971, 1974)
			Soto et al. (1997a,b, 1998b)
	KCl NaCl	298.15, 308.15	Kamali-Ardakani et al. (2001) Khavaninzadeh et al. (2002)
	NaBr + K ₃ PO ₄	298.15–308.15	Dehghani et al. (2005, 2006a)
Activity coefficients of peptides	NaCl	298.15	Breil et al. (2001)
	NaCl, NaBr, KCl, KBr	298.15	Chung and Vera (2001)
Volumetric Properties	Cu(NO ₃) ₂ , NiCl ₂	288.15–310.15	Akhtar (2007); Banipal et al. (2004, 2007)
	CH ₃ COONa		Lark et al. (2004, 2007)
	(CH ₃ COO) ₂ Mg		Ramasami and Kakkar (2006)
	MgCl ₂ ; Na ₂ SO ₄		Singh et al. (2007); Soto et al. (1998c)
	KCl		Yuan et al. (2006)

2.5.3 MODELLING

2.5.3.1 Water/Amino Acid

Despite the values of the solubility of amino acids in water at different temperatures are known since the works by Dalton and Schmidt (1933, 1935) and Dunn et al. (1933), only during the last two decades the thermodynamic description of that property gained increased interest and several models have been proposed (Chen et al., 1989; Gupta and Heidemann, 1990; Kuramochi et al., 1996, 1997; Nass, 1988; Peres and Macedo, 1994; Pinho et al., 1994).

The successful representation of the solubilities is directly related to the ability to correlate and predict the activity coefficients of amino acids in solution. In this way, several studies have been performed for the correlation of the activity coefficients of amino acids in water (usually at 298.15K) and their aqueous solubilities as a function of the temperature and pH. In most cases g^E models (Wilson, NRTL, UNIFAC, UNIQUAC equations) as well as equations of state (simplified perturbed-hard-sphere model, or a hydrogen-bonding lattice-fluid equation of state) were used to correlate activity coefficients and solubility of amino acids in water. Nass (1988) assumed that there are two different terms, chemical and physical, so the g^E (and thus γ_i) was expressed as a sum of two separate contributions: the chemical reaction equilibria allows the introduction of pH, and the physical contribution is accounted by the Wilson equation; other contributions (e.g. long-range electrostatic interactions) were given by the addition of other physically based terms. Nass was able to successfully correlate the solubility data of phenylalanine, tyrosine and diiodotyrosine as a function of pH and temperature. The correlation of the activity coefficients for alanine, serine, and threonine in water was also in good agreement with the experimental data. Due to the lack of a comprehensive excess Gibbs energy expression to fully describe the physical interactions among the true species of the biomolecular systems, Nass's work was limited to aqueous single amino acid systems using a number of parameters between three to ten.

In the thermodynamic framework proposed by Chen et al. (1989) two different contributions for the calculation of the excess Gibbs energy of the system were proposed: the long-range interactions represented by a Pitzer-Debye-Hückel term and the local interactions formulated by a modified form of the NRTL equation. With two adjustable energy parameters, for each

amino acid/water pair, satisfactory results have been obtained in representing and predicting the solubilities of amino acids and small peptides in aqueous solutions. In addition to temperature and pH effects studied by Nass (1988), the influence of other dipolar or ionic species on aqueous amino acid solubility could be correlated with this model.

In the work of Gupta and Heidemann (1990), an attempt has been made to build a predictive model able to describe the activity coefficients of amino acids in water considering only short-range interactions, using the modified UNIFAC model. However, very large groups were defined (glycine and proline) which were not consistent with the original group contribution concept. In average, the model proved to be a flawed tool for correlation or prediction of the activity coefficients and solubilities of the amino acids. The results were not particularly successful for amino acids containing methyl and methylene groups as a side chain, but good for glycine and serine.

In 1994, a new model that combines chemical equilibria with a UNIFAC-Debye-Hückel approach has been proposed by Pinho et al. (1994) for the correlation and prediction of activity coefficients. New charged groups have been defined, taking into account the charges in the zwitterionic, the anionic, and the cationic forms of the amino acids. The results for correlation were satisfactory, while for predictions the model gave poor results. Difficulties arose in getting good correlations using amino acids with long hydrocarbon chain, confirming that the $\text{CH}_2/\text{H}_2\text{O}$ interaction parameters, from original UNIFAC, were not suitable to represent these kind of mixtures. The influence of pH and of temperature on the solubility was also studied and satisfactory qualitative and quantitative results were found.

In the same way, a molecular thermodynamic framework for the representation of the solubilities of several binary systems, amino acids-water (nine systems) and peptide-water (five systems), was developed and tested by Peres and Macedo (1994). Similarly, chemical and physical equilibrium were taken into account. A UNIQUAC model combined with a Debye-Hückel term was developed and new UNIQUAC parameters were estimated. The results obtained with this method were very satisfactory even when considering the influence of pH and temperature on the solubilities.

In order to overcome the difficulties found by Pinho et al. (1994), Kuramochi et al. (1996) proposed new CH_2 amino acid groups ($\alpha\text{-CH}$ and sc-CH_2), allowing a more accurate

representation of the activity coefficients of amino acids and peptides in aqueous solutions than with the previous UNIFAC models. The calculated solubilities of two amino acids in water showed also to be in good agreement with the experimental data.

In all the above mentioned studies, the activity coefficients of amino acids in aqueous solutions were represented by empirical local composition or by group-contribution models. On another way, Khoshkbarchi and Vera (1996d) proposed the use of the perturbation theory for modelling the activity coefficients of amino acids and peptides in aqueous solutions. To do so, they developed a two-parameter theoretical model based on a simplified perturbation theory of a hard-sphere reference system. Since amino acids have very large dipole moments, the interaction energies for the perturbation term were considered to be due to dispersion forces, represented by a Lennard-Jones expression, and to dipole-dipole interactions, represented by an angle-averaged dipole-dipole interaction in the form of the Keesom equation (calculated by a quantum mechanical approach). The activity coefficients of amino acids, calculated with the model, have been applied to correlate the solubilities of amino acids in aqueous solutions; results showed that the model can accurately correlate the activity coefficients and the solubilities of amino acids over a wide range of temperatures with two adjustable parameters. This model considered only the dispersion and dipole-dipole interactions between biomolecules and neglected those between solvents and between solvent and solute molecules.

After, Khoshkbarchi and Vera (1998) improved the theoretical structure of the perturbed hard sphere model considering for both reference system and the perturbation terms the radial distribution function obtained from the solution of the Percus-Yevick equation for hard sphere systems. The model can accurately correlate the activity coefficients of several amino acids and peptides in aqueous solutions over a wide range of concentrations. A comparison of the results obtained with those given by a simplified version presented before (Khoshkbarchi and Vera, 1996d) suggested that although the new model was more accurate, for engineering applications, the simplified version was more adequate.

A non-primitive perturbation model for chain-like molecules was used by Liu et al. (1998) to correlate the activity coefficients of amino acids and peptides in aqueous solutions. All the interactions between solvent and solute molecules were considered. In this model, the mixed hard-sphere segments were used as a reference system and the interactions of dispersion,

dipole-dipole and the hard-chain formation energy were calculated as perturbed terms. The parameters for water were obtained correlating liquid density and vapor pressure data, and the parameters of amino acids and peptides were evaluated by fitting their activity coefficient data in aqueous solution. With four parameters for each component (solvent and amino acid or peptide) the model can correlate accurately the activity coefficients of amino acids and peptides. The solubilities of several amino acids in pure water were predicted, with acceptable results over a wide range of temperatures, using experimental values of standard entropy and enthalpy changes in the dissolving process. No additional adjustable parameters were needed.

An equation of state applicable to associating systems was used by Park et al. (2003). They have chosen the hydrogen-bonding nonrandom lattice fluid equation of state to account for the specific interactions as well as physical interactions. Pure water parameters were fitted to vapor pressure and liquid density, and amino acids parameters were fitted to activity coefficients and partial molar volumes at infinite dilution. The calculated solution densities and activity coefficients as function of compositions, and solubilities as function of temperature, for some amino acids and simple peptides in aqueous solutions showed good agreement with experimental data. The results of amino acids activity coefficients in aqueous solutions were compared with those obtained by Chen et al. (1989), Khoshkbarchi and Vera (1996d), and Pinho et al. (1994), presenting smaller root mean square deviations (RMSD) than those of g^E models but higher than those of perturbation models (Khoshkbarchi and Vera, 1996d). The calculated solubility values when compared with the results of Liu et al. (1998) show also lower RMSD.

A new two-parameter model based on the perturbation of a hard-sphere reference was developed by Mortazavi-Manesh et al. (2003) to correlate the activity coefficients of several amino acids and simple peptides in aqueous solutions. The hard-sphere equation of state used by Khoshkbarchi and Vera (1996d), as the reference part of the model is replaced by the hard-sphere equation of state proposed by Ghotbi and Vera (2001). The Lennard-Jones and Keesom potential functions are used to represent the dispersion and dipole-dipole interactions, respectively. The proposed model was applied to correlate the activity coefficients of amino acids and simple peptides in aqueous solutions, and after, used to correlate the solubility of amino acids in aqueous solutions at various temperatures. The results for the correlation of activity coefficients were compared with those obtained with other models (Chen et al., 1989;

Gupta and Heidemann, 1990; Khoshkbarchi and Vera, 1996d, 1998; Pinho et al., 1994), and present a more accurate correlation. Using only two adjustable parameters per amino acid or peptide, the model is also able to accurately correlate the solubility data of several amino acids in aqueous solutions over a wide range of temperature.

The modified Wilson model originally proposed to represent the phase behaviour of polymer aqueous solutions was applied to calculate activity coefficients and the solubility of amino acids, and small peptides, in aqueous solutions (Xu et al., 2004). The interaction parameters estimated from the activity coefficients were used to evaluate the solubility of amino acids. The model could accurately represent the activity coefficients and solubilities of amino acids with only two adjustable parameters per system. After, a temperature dependence was introduced on the energy parameters and better predictions of the solubility at higher temperatures were obtained. It can also be used to calculate solubilities in systems containing two amino acids.

Pazuki and Nikookar (2006) used the local composition models; modified Wilson, NRTL and modified NRTL models to predict the activity coefficients and solubilities of amino acids in water with accurate results. The performance of a three parameter model based on the perturbation theory was also studied (Pazuki et al., 2006). The calculated activity coefficients of amino acids and simple peptides show that this equation of state can be more accurate for correlation than the other models, and it can also correlate accurately the solubility of amino acids in aqueous solutions over a wide range of temperatures.

The modified Wilson model was used by Pazuki et al. (2007); the parameters for amino acid-water pairs were obtained from the least squares fit of the model to the corresponding experimental data. A modification was performed using the new local mole fraction proposed by Zhao et al. (2000). The results obtained showed that this model can accurately correlate the activity coefficients and solubility of amino acids and simple peptides in aqueous solutions.

The modified polymer-electrolyte Wilson model (Sadeghi, 2005), where the combinatorial contribution term defined to account the entropy of mixing for molecules of different sizes is omitted and polymer molecules are replaced by small amino acid molecules, was extended to describe the activity coefficients and solubility of those in aqueous solutions (Sadeghi, 2007). The model was also used to represent the solubility of an amino acid in aqueous solutions of

another amino acid. The results were compared with those obtained from the NRTL model (Chen et al., 1989) and the conclusion is that both have similar behaviour. Good agreement with experimental data was obtained.

Recently, Cameretti and Sadowski (2008) applied an equation of state based on the Perturbed-Chain Statistical Associated Fluid Theory (PC-SAFT) to correlate the vapor pressures, liquid densities and solubilities of aqueous amino acid and oligopeptide solutions. Five pure-component model parameters for each amino acid as well as the melting enthalpies and temperatures were fitted to experimental data. The authors noticed that the obtained melting temperatures appear to have no physical meaning since those were much higher than the decomposition temperatures of the amino acids. One additional temperature independent binary interaction parameter k_{ij} was necessary to describe the solubilities, while for vapor pressures and densities this binary parameter was not necessary. Moreover, densities and vapor pressures of aqueous solutions of homopeptides and heteropeptides were modeled using the same parameters for the amino acids and only the segment number of the polypeptides was readjusted.

Even if a full comparison between all the approaches is not practicable it is possible to observe that despite the relative success obtained with the ongoing models, they exhibit limitations and for some of the fitted parameters there is no physical meaning. The work development in this field is still a growing challenge. The results produced using the g^E models are quite acceptable, however the equations of state became a very attractive alternative. Since experimental data are often scarce, from a practical and critical point of view, an equation of state is more robust for predictions beyond the region where model parameters were estimated. A list of the works mentioned above is presented in Table 2.5.

Table 2.5 Models to describe thermodynamic properties of amino acids and peptides in aqueous solutions.

Author	Applications	Description	Parameters
Nass (1988)	Correlation of the solubility data of as function of pH (phenylalanine, tyrosine and diiodotyrosine) Correlation of the pH and temperature dependence of solubility data in water (diiodotyrosine) Correlation of the activity coefficients in water (alanine, serine, and threonine)	g^E model Contributions: chemical equilibria; physical (Wilson equation); other contributions (e.g. long-range electrostatic interactions)	From three to ten per system
Chen et al. (1989)	Correlation and predict of the solubilities of amino acids and small peptides in aqueous solutions as function of temperature and pH	g^E model Contributions: long-range interactions (Pitzer-Debye-Hückel term); local interactions (modified form of the NRTL equation).	Two per system
Gupta and Heidemann (1990)	Modelling of the effects of temperature and pH on the solubility of amino acids in water (eight amino acids) Correlation and prediction of activity coefficients and solubility of amino acids in water	Modified UNIFAC model: new amino acid groups (glycine and proline)	Ten
Pinho et al. (1994)	Correlation and prediction of activity coefficients and solubility of 14 amino acids and 5 small peptides in water Influence of pH and of temperature on the solubility	UNIFAC-Debye-Hückel approach (new charged groups defined)	Seventeen
Peres and Macedo (1994)	Representation of the solubilities of several binary systems: amino acids-water (nine systems) and peptide-water (five systems) the influence of pH and temperature on the solubilities	UNIQUAC combined with the Debye-Hückel term (new UNIQUAC parameters)	Two per system
Kuramochi et al. (1996)	Correlation and prediction of activity coefficients of amino acids and peptides in aqueous solutions Solubility prediction of mixed amino acids in water	Larsen's UNIFAC model (three new UNIFAC groups)	Twenty eight
Khoshkbarchi and Vera (1996d)	Correlation of the activity coefficients of amino acids and peptides in aqueous solutions Correlation and prediction of the solubilities of amino acids at different temperatures	Simplified perturbation hard-sphere model	Two per component
Khoshkbarchi and Vera (1998)	Correlation of the activity coefficients of amino acids and peptides in aqueous solutions	Theoretically improved perturbation model	Two per component

Table 2.5 Models to describe thermodynamic properties of amino acids and peptides in aqueous solutions (continuation).

Author	Applications	Description	Parameters
Liu et al. (1998)	Correlation of the activity coefficients of amino acids and peptides in water Prediction of solubilities of amino acids in water over a wide range of temperatures	Perturbation theory	Four per component
Park et al. (2003)	Correlation of activity coefficients and solution densities as function of compositions, and of solubility as function of temperature for some amino acids and simple peptides in aqueous solutions	Hydrogen-bonding lattice fluid equation of state	Two per component One binary
Mortazavi-Manesh et al. (2003)	Correlate the activity coefficients of several amino acids and simple peptides in aqueous solution Correlate the solubility of amino acids in aqueous solutions over a wide range of temperature	Hard-sphere equation of state	Two per component
Xu et al. (2004)	Correlation of activity coefficients and solubilities of amino acids in water Effect of temperature on the solubility of amino acids Solubility of two amino acid mixtures	Modified Wilson model	Two per system
Pazuki and Nikookar (2006)	Prediction of the activity coefficients and solubilities of amino acids and peptides in water	Modified Wilson, NRTL and modified NRTL models	Two per system
Pazuki et al. (2006)	Correlation of the activity coefficients of amino acids and simple peptides in aqueous solutions Correlate the solubility of amino acids in aqueous solutions over a wide range of temperature	Perturbation theory	Three per component
Pazuki et al. (2007)	Correlate and prediction of the activity coefficients and solubility of amino acids and simple peptides in aqueous solutions	Modified Wilson model	Two per system
Sadeghi (2007)	Correlation of the activity coefficients and solubility of amino acids in aqueous solutions	Modified Wilson model	Two per system
Cameretti and Sadowski (2008)	Modeling of vapor pressures, liquid densities and solubilities of aqueous amino acid and oligopeptide solutions.	PC-SAFT EoS	Five per component One binary

2.5.3.2 Water/Alcohol/Amino Acid

Modelling solubilities of amino acids in mixed-solvent solutions has received much less attention. Chen et al. (1989) derived a correlation of the solubilities of amino acids in ethanol-water mixtures using the electrolyte-NRTL model.

Orella and Kirwan (1989, 1991) and Gude et al. (1996a,b) gave important contributions to this subject. Orella and Kirwan (1989, 1991) correlated the solubilities of amino acids in mixed-solvent systems containing water and aliphatic alkanols (from methanol to 2-propanol) using an excess solubility approach with various excess Gibbs energy models (Margules, Wilson, NRTL), and satisfactory fits of experimental data were obtained using the Wilson equation. In order to reduce the number of parameters to be estimated a constraint was established based on the fact that the ratio of the activity coefficients of amino acids in pure solvents should be inversely proportional to the ratio of pure solvent solubilities. Unfortunately, that constraint originates impossible values for the Wilson parameters, since in some cases, like for the 1-propanol/water or 2-propanol/water mixed solvent systems, with L-alanine, the parameters are negative.

Gude et al. (1996a,b) presented a simpler model which is a combination of a combinatorial term based on the Flory-Huggins theory with a Margules expression. Their methodology suggested the simultaneous representation of solid-liquid and liquid-liquid equilibria with one ternary Margules interaction parameter for each amino acid. The experimental data was successfully correlated using a unique specific interaction parameter for each amino acid in different aqueous-alkanol solutions. Similar results were achieved to those obtained by Orella and Kirwan (1991), but with a much lower number of parameters, which turned out their model very attractive. Recently, Fuchs et al. (2006) modelled the solubility of amino acids (DL-methionine, glycine and DL-alanine) in water and alcohols (from methanol to 1-butanol) using the PC-SAFT equation of state. Pure-component parameters of amino acids were fitted to the vapor pressures and to densities of their aqueous solutions. One constant (temperature-independent) binary parameter k_{ij} for each solute/solvent system was introduced to correlate the solubility data of the amino acids in pure solvents. Based on those binary systems, the solubility at different pH values, as well as in water-alcohol mixtures, was predicted without

the addition or refitting of model parameters, and the calculated results showed a fair agreement with the experimental data.

Models used to describe thermodynamic properties of amino acids and peptides in aqueous-alkanol systems are presented in Table 2.6.

Table 2.6 Models to describe thermodynamic properties of amino acids and peptides in aqueous-alkanol solutions.

Author	Applications	Description	Parameters
Chen et al. (1989)	Solubilities of glycine and β -alanine in ethanol-water mixtures	g^E model Contributions: long-range interactions (Born equation); local interactions (modified form of the NRTL equation).	Two per system One per component
Orella and Kirwan (1989, 1991)	The measured solubilities of glycine, L-alanine, L-isoleucine, L-phenylalanine, and L-asparagine monohydrate in aqueous solutions of methanol, ethanol, 1-propanol, and 2-propanol were correlated	Excess solubility approach and the Wilson activity coefficient formulation	Two per system
Gude et al. (1996a,b)	Correlation of the solubilities of seven amino acids in aqueous alkanol solutions	Excess solubility approach which makes use of a simple excess Gibbs energy expression (combinatorial term based on Flory-Huggins theory and a Margules residual expression)	One per amino acid
Fuchs et al. (2006)	Modelling of the solubility of amino acids in pure solvents. Prediction of the amino acids solubility in mixed solvents (ternary systems) without any additional fitting of the parameters	PC-SAFT EoS	Five per component One binary

2.5.3.3 Water/Electrolyte/Amino Acid

Kirkwood (1934, 1939) was a pioneer in the qualitative representation of the behaviour of water-electrolyte-amino acid systems at low electrolyte and amino acid concentrations, developing two models to represent the electrostatic interactions between amino acid and ions.

More than fifty years later, Chen et al. (1989) developed an electrolyte NRTL model, which combined a long-range interaction term given by a Pitzer-Debye-Hückel term, and a short-range term described by a modified version of the NRTL equation, and the salt-amino acid energy parameters were adjusted to represent the ion-molecule physical interactions in the liquid phase. The results obtained in the representation of the solubilities of amino acids in the presence of salts were satisfactory within this general framework.

Fernández-Mérida et al. (1994) and Rodríguez-Raposo et al. (1994) used a modified form of the Pitzer model for aqueous solutions of an electrolyte and a non-electrolyte to model the activity coefficients in the water-electrolyte-amino acid systems, which employs an electrostatic term and a virial series expansion to account for all other interactions. The original Pitzer model yields good fittings of the results for solutions containing two electrolytes as well as for a ternary electrolyte + non-electrolyte + water system, but it fails if the non-electrolyte is polar. This is the case for solutions of pure amino acids since they have a dipolar-ion structure (zwitterions). The modification proposed considers that the ion-non-electrolyte interactions should depend of the ionic strength of the medium and of the non-electrolyte molality.

An important contribution was given by J. H. Vera and collaborators who have developed and tested several approaches. The activity coefficients of amino acids in aqueous electrolyte solutions were modelled by Khoshkbarchi and Vera (1996a), using an expression for g^E based on the contribution of a long-range interaction term represented by the Bromley equation or the K-V equation (Khoshkbarchi and Vera, 1996e) and the short-range interaction term represented by the NRTL or the Wilson equations. The model requires two parameters that should be regressed from ternary data; all other parameters were evaluated from binary experimental data of water-amino acid and water-electrolyte systems.

Later on, Khoshkbarchi and Vera (1996f) proposed a model based on the perturbation of a hard-sphere system in which the contribution of the electrostatic interactions is represented by a mean spherical approximation model (MSA). After, a model has been developed to correlate the solubilities of amino acids in aqueous electrolyte solutions over a wide range of electrolyte concentration, and the perturbed hard-sphere model proposed by Khoshkbarchi and Vera (1996f) was employed to represent the activity coefficients of amino acids in electrolyte solutions (Khoshkbarchi and Vera, 1997).

Hu and Guo (1999) applied the Pitzer-Simonson-Clegg equations to calculate, with high accuracy, the solubility of electrolytes in electrolyte-non-electrolyte-water systems at 298.15 K, using parameters estimated only from activity coefficients data. In the mole-fraction-based model, the excess Gibbs energy is assumed to be the sum of short-range forces and long-range forces (Debye-Hückel term).

After, a model based on the simplified perturbation theory developed earlier to correlate the activity coefficients of amino acids and peptides in aqueous solutions (Khoshkbarchi and Vera, 1996d) was proposed by Gao and Vera (2001) to represent also the activity coefficients for ternary amino acid-electrolyte-water systems. Two parameters were regressed from the ternary system, while the other parameters were obtained from binary aqueous amino acid systems and from the data of pure ions. The amino acid molecules are characterized only by a size and an energy parameter (Gao and Vera, 2001).

A simple model based on the Wilson equation (Khoshkbarchi and Vera, 1996a) was used by Chung and Vera (2001) to correlate the activity coefficients of glycylglycine in four aqueous electrolyte solutions. The model fails at low electrolyte concentrations, but the results were satisfactory at molalities of the electrolyte equal or higher than 0.3.

The extended UNIQUAC model was used by Breil (2001) to correlate the activity coefficients of amino acids and peptides in binary and ternary aqueous solutions, and the results were satisfactory. Still, difficulties encountered in the calculation of the solubility of the biomolecules in aqueous electrolyte systems, were very evident; the model gives a poor prediction of the solubility but can predict its trend.

Later, Pazuki et al. (2005) calculated the activity coefficients of electrolyte in aqueous amino acid solutions using an expression for the Gibbs free energy based on the contribution of a long-range interaction term represented by Khoshkbarchi-Vera model (Khoshkbarchi and Vera, 1996a,e) and a short-range interaction term represented by local composition models such as Wilson, NRTL and the NRTL-NRF models. All these models require two parameters which should be regressed from ternary data. All other parameters were evaluated from binary experimental data of water-amino acid and water-electrolyte system. The results show that the local composition models can accurately correlated 30 water-amino acid (peptide)-electrolyte systems. However, the deviation obtained on activity coefficient from the Wilson model is smaller than those from the NRTL and the NRTL-NRF models.

In order to represent the experimental data, virial expansions with up to six parameters (Gao and Vera, 2001; Kamali-Ardakani et al., 2001; Khavaninzadeh et al., 2002, 2003; Khoshkbarchi and Vera, 1996a,b,c; Khoshkbarchi et al., 1997; Phang, 1978; Phang and Steel, 1974; Soto et al., 1997a,b, 1998b) were used to correlate the ratio of the mean ionic activity coefficients of the electrolytes in the presence and in the absence of the amino acids. However, the change of activity coefficients of different electrolytes in the presence of DL, α -aminobutyric acid showed distinctive patterns between dilute and concentrated region of the electrolyte and more complex fitting expressions were required (Chung and Vera, 2002). No simple equation was suitable to fit both concentration regions and attempts to fit the dilute electrolyte region with a single function were unsuccessful. To fit the ratio of the mean ionic activity coefficients of an electrolyte in the presence to those in the absence of a peptide a three-parameter expression was used (Chung and Vera, 2001). As observed for amino acids, the effect of individual ions can be quite distinctive for the dilute and concentrated regions. A different fitting equation, that may generate complex algebraic functions after differentiation and integration, was necessary for each different system since a single equation did not provide a satisfactory fit for all systems. Like the authors refer, as more experimental data becomes available, with special attention for the effect of salts with larger variation in charge density, the development of more sophisticated models will be possible (Chung and Vera, 2001).

More recently, systems containing amino acids or peptides + water + one electrolyte were modelled by different types of neural networks (Dehghani et al., 2006b). They designed an

artificial network that can predict the mean ionic activity coefficient ratio of electrolytes in presence and in absence of amino acid in different mixtures better than the common proposed polynomial equations for this kind of predictions. Table 2.7 summarizes the works mentioned in this section.

The different models were applied to several water-electrolyte-amino acid systems and showed to be able to correlate the experimental data accurately over a wide range of amino acid and electrolyte concentrations. However, a unified approach was not yet proposed, and the accuracy of the models depends both on the concentration and the type of electrolyte and amino acid.

Once again, models based either on g^E models or equations of state have been presented and their performance to describe the thermodynamic properties of the amino acid in aqueous electrolyte solutions discussed. The establishment of a reliable model able to represent the activity coefficients of amino acids in aqueous electrolyte solutions is essential for the development of accurate solubility models. Although some local composition or group-contribution methods are able to satisfactorily correlate the activity coefficient data, they provide limited information about the interactions among the components. In this way, the equations of state became very attractive. In addition, it is also possible to extend the prediction to a wide range of amino acid and electrolyte concentrations.

Table 2.7 Models to describe thermodynamic properties of amino acids and peptides in aqueous electrolyte solutions.

Author	Applications	Description	Parameters
Kirkwood (1934, 1939)	Influence of simple electrolytes and of the dielectric constant of the solvent on the solubilities of the aliphatic amino acids in alcohol water mixtures	Kirkwood's model describe the amino acids using various shapes and consider various positions for the charges on them	Three per component
Chen et al. (1989)	Correlation of the solubilities of amino acids in the presence of salts	Long-range interactions (Pitzer-Debye-Hückel term) Short-range interactions (modified form of the NRTL equation).	Two per system
Fernández-Mérida et al. (1994)*	Model the activity coefficients in water-electrolyte-amino acid systems	Modified form of the Pitzer model	Five per system
Khoshkbarchi and Vera (1996a)	Correlation of the activity coefficients of amino acids in aqueous electrolyte solutions	Long-range interactions (Bromley model/K-V model) Short-range interactions (NRTL/Wilson model)	Two per system
Khoshkbarchi and Vera (1996f)	Correlation and prediction of the activity coefficients of amino acids in aqueous electrolyte solutions	A perturbed hard-sphere model with mean spherical approximation (MSA)	Two per system
Khoshkbarchi and Vera (1997)	Correlation and prediction of the activity coefficients of amino acids in aqueous electrolyte solutions Correlation of the solubility of amino acids in aqueous electrolyte solutions.	A perturbed hard-sphere model as proposed by and Vera (1996f)	One per system
Hu and Guo (1999)	Correlation of the solubility of electrolytes in electrolyte-non-electrolyte-water systems	Pitzer-Simomson-Clegg equations Sum of short-range and long-range (DH term) forces	Five per system
Gao and Vera (2001)	Correlation of the activity coefficients for seven binary aqueous amino acid systems obtained from literature and the mean ionic activity coefficients measured for the four ternary systems	Simplified perturbation hard-sphere model	Two per system
Chung and Vera (2001)	Correlation of the activity coefficients of glycylglycine in four aqueous electrolyte solutions	Wilson model, Khoshkbarchi and Vera (1996a)	Four per system
Breil (2001)	Correlation of binary and ternary aqueous solutions containing amino acids or peptides.	Extended UNIQUAC model	Two per binary
Pazuki et al. (2005)	Correlation of the activity coefficients of 30 amino (peptide)-water-electrolyte systems	g^E model, long-range interactions (K-V model) Short-range interactions (Wilson/NRTL/NRTL-NRF models)	Two per system

* Rodríguez-Raposo et al. (1994).

2.6 CONCLUSIONS

An extensive literature search on the available solubility data and experimental methods was essential to establish the systematic experimental program and to choose the most appropriated techniques to measure the solubility of the amino acids in aqueous solutions containing alcohols and electrolytes. The available solubility data is very scarce in terms of the number of studied systems and rather old leaving some doubts about their quality, so it is fundamental to extend the experimental database already available.

The main goal is to use the already available experimental information from the open literature together with the new data measured to validate and develop thermodynamic models. Thus, the review of the proposed models was also of extreme relevance. Thermodynamic models have not been widely used in biotechnological industry as is the case for the chemical industry, but it is becoming more important because of the increasing demand for computer aided design and optimisation of processes (Breil et al., 2004). The available thermodynamic models and their capabilities to correlate and/or predict the thermodynamic properties of the amino acids in aqueous systems containing alcohol or electrolytes were briefly presented and discussed. g^E models (Wilson, modified Wilson, NRTL, electrolyte NRTL, UNIQUAC, modified UNIQUAC and UNIFAC models) as well as equations of state (simplified perturbation theory, simplified perturbed-hard-sphere model; a hydrogen-bonding lattice-fluid equation of state, PC-SAFT) have been applied to model and predict the thermodynamic properties for several binary and ternary aqueous amino acid systems at various temperatures. As could be understood over the years, models, or their modifications, have been developed in an attempt to overcome the drawbacks found in the representation of phase equilibria. It is foreseen and still needed the development of new frameworks that can accurately represent the solubility behaviour of amino acids and small peptides as affected by temperature, ionic strength, dipolar species concentrations, solvent compositions and pH.

CHAPTER 3.

SOLID–LIQUID EQUILIBRIUM: EXPERIMENTAL STUDIES

3.1 INTRODUCTION

As mentioned before, the development of more sophisticated and efficient processes for separation, concentration and purification of valuable biomolecules such as peptides and proteins have been a subject of main interest for the biochemical industry. These processes stand to benefit from understanding the solution and solubility behavior of the amino acids, which are among the simplest biochemicals. So, extending and establishing a reliable and consistent database in order to review proposed models, such as conventional thermodynamic models, or develop new ones in an attempt to overcome the drawbacks found in the representation of phase equilibria, is fundamental, and one of the aims of the present work.

The extensive literature search on the available solubility data and experimental methods, cited in Chapter 2, was fundamental to establish a systematic experimental program and to implement the most appropriated techniques to determine the solubilities of the amino acids in aqueous solutions containing alcohols or electrolytes. In fact, the existing information is very scarce, most of it old, limited to very few solvents/salts and/or conditions, and the temperature influence is almost ignored; the vast majority of the published data is at 298.15 K.

In this chapter, details of the experimental techniques, the analytical methods, and the measured experimental solubilities are given, as well as a critical analysis of the obtained data. Solubility data was measured in a temperature range between 298.15 K and 333.15 K at ambient pressure and without pH adjustment. Amino acids with different characteristics, namely size and functional groups were preferred; glycine, DL-alanine, L-serine, L-isoleucine

and L-threonine. Concerning the use of electrolytes the influence of the size, the anion or the cation, as well as the ionic strength were considered; the KCl and $(\text{NH}_4)_2\text{SO}_4$ were selected and the analytical gravimetric method was chosen to perform these measurements. Besides water, the solvents chosen for the experimental study were ethanol, 1-propanol and 2-propanol. The solid content of these solutions was measured differently depending on the alcohol mass fraction in amino acid free basis in the mixed solvent (w'_{alcohol}): when $w'_{\text{alcohol}} < 0.8$, the gravimetric method was applied; at higher alcohol concentrations ($w'_{\text{alcohol}} \geq 0.8$), the spectrophotometric ninhydrin method was used for quantitative determination of the extremely low solubility of the amino acids.

The gravimetric analysis is one of the most accurate methods of analysis (Cohn et al., 1934; Gekko, 1981; Khoshkbarchi and Vera, 1997; Nozaki and Tanford, 1971; Pradhan and Vera, 2000; Soto et al., 1998a) and the reaction of ninhydrin with a primary amino group to form a coloured reaction product, diketohydrindylidene-diketohydrindamine, also called Ruhemann's purple (RP), has been known and studied for years and extensively used for amino acid analysis (Fang and Liu, 2001; Friedman, 2004; Jones et al., 2002; Moore and Stein, 1948; Sun et al., 2006). Even though the success and high reproducibility of the experimental technique used, the quality of the experimental data was checked and accuracy tests were made.

The experimental results are presented divided into three parts: solubility of the different amino acids in pure water, in aqueous alcohol solutions and in aqueous electrolyte solutions.

3.2 ANALYTICAL METHOD

The analytical method, which consists in the preparation of a saturated solution at constant temperature, was the technique chosen to perform the measurements. This has been the experimental technique generally presented in the literature to determine the solubility of amino acid since it is simple, accurate and presents high reproducibility.

3.2.1 CHEMICALS

In all experiments double-ionized water, supplied by Fresenius Kabi Pharma, was used. The supplier and purities of compounds used are listed in Table 3.1. All the chemicals were used as received with no further purification. In order to avoid water contamination the amino acids were kept in a dehydrator with silica gel. The salts were oven-dried ($T = 343.15\text{ K}$) and used after cooling in a dehydrator with silica gel.

Table 3.1 Sources and purities of the used compounds.

Substance	Supplier	Purity (mass %)
Glycine	Merck	99.7
DL-alanine	Merck	99.0
L-serine	Sigma	99.0
L-isoleucine	Fluka	99.0
L-threonine	Fluka	99.0
Ethanol	Merck	99.8
1-Propanol	Merck	99.8
2-Propanol	Merck	99.9
KCl	Merck	99.5
$(\text{NH}_4)_2\text{SO}_4$	Merck	99.5
Na_2SO_4	Merck	99.0
$\text{NaOAc} \cdot 3\text{H}_2\text{O}$	Merck	99.5
Hydrindantin	Sigma	98.0
CH_3COOH	Merck	99.8
DMSO	Merck	99.9
Ninhydrin	Merck	—

3.2.2 APPARATUS DESCRIPTION

The apparatus used in the present work to measure the solubility of the amino acid in aqueous solutions containing salt or alcohol is shown in Figure 3.1. This apparatus was initially designed and build to measure the solid-liquid equilibrium of binary and multi-component sugar/solvent and sugar/mixed solvents systems. It can operate from 293.15 K to 353.15 K , at ambient pressure.

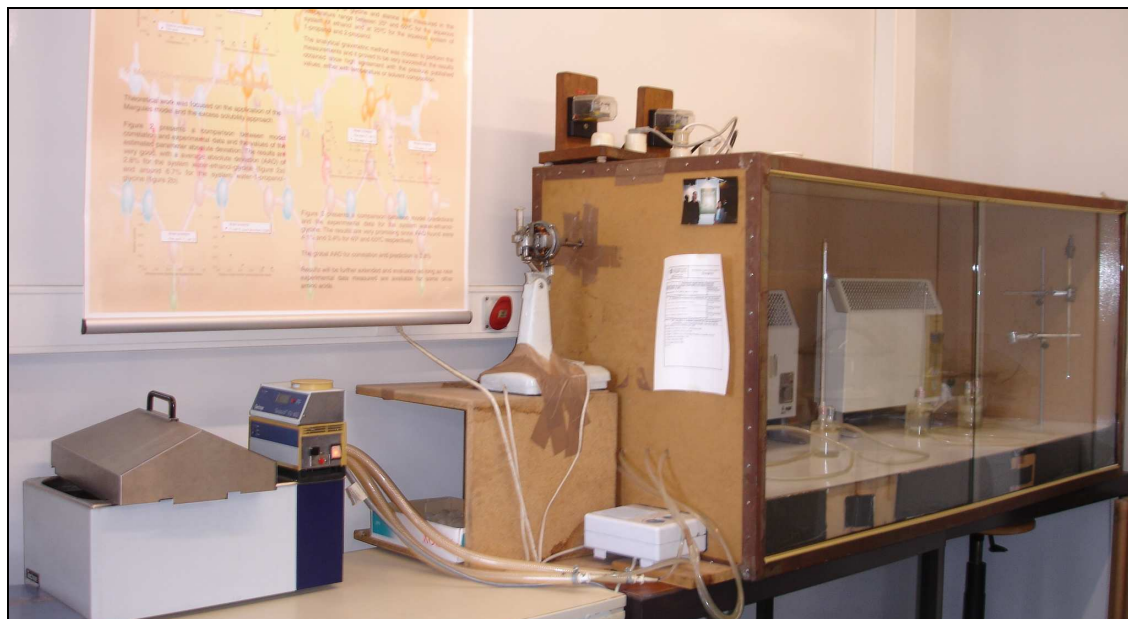


Figure 3.1 Experimental apparatus.

Four equilibrium jacketed glass cells, based on the ones used by Pinho (2000) were designed with a capacity of about 120 mL and used simultaneously (Figure 3.2). The cells have two vertical orifices on the top, one for the mercury thermometer and the other to collect the samples and two lateral orifices placed in opposite sites, one on the bottom (entry) and the other on the top (exit) to circulate the heating water in the jacket. The temperature of the water in the jacket cell is controlled by a thermostated water bath, equipped with a digital controlled immersion circulator (Tempunit TU16D, Techne). Due to the long period of time required to reach the equilibrium, the thermostatic bath was not enough to guarantee the maintenance of the constant temperature inside of the equilibrium cell. Therefore, the cells were placed inside an insulated box specially designed for the effect. Inside the box there are two heaters responsible for keeping the temperature constant, and a fan to ensure its uniformity.

Magnetic stirrers (Agimatic-N, Selecta) and magnetic bars (3.5 cm length) are used in the mixing process. The magnetic stirrers are placed at the bottom of the box and separated from the glass cells by an isolating plaque to prevent their heating during the experiments. When necessary they are also cooled using a fan. To promote an efficient contact between the solid

and the liquid phase, without breaking the crystals, the stirrer speed was usually set at 500 rpm.

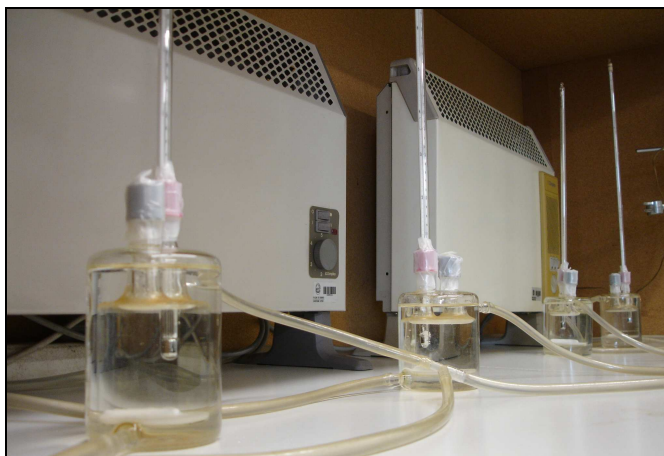


Figure 3.2 Equilibrium jacketed glass cells.

A mercury thermometer (Ammarell Precision with ± 0.1 °C resolution) is used to measure directly the solution temperature.

During the drying process, a heating plate (Stuart, SB 300) and a drying stove (Scientific, series 9000) are used. An electronic balance (Adam, model AAA 250 L, with 0.1 mg precision) is used to carry out all the weights.

For the spectrophotometric ninhydrin method, a Thermo electron corporation UV1, using 1 cm quartz cells was used to measure the absorbance. The pH measurements were made using a digital SympHony, VWR, pH meter.

3.2.3 PROCEDURE

The following section reports the preparation of the different solutions and the procedure for the amino acid solubility measurements in an aqueous solution containing either an alcohol or a salt.

3.2.3.1 Preparation of the Different Solutions

Preparation of the water/alcohol mixed solutions - Desired amounts of each solvent, starting with the less volatile, are weighted into a balloon-flash, in the precision electronic balance to prepare approximately 100 g of solvent mixture. After, to ensure the complete mixing, the resulting solution is vigorously shaken for a few minutes.

Preparation of the water/electrolyte mixed solutions - The solutions (100 g) were prepared at different molalities of electrolyte. The electrolyte is weighted into a balloon and the water is also weighed. The solution is stirred to promote complete mixing.

Preparation of sodium acetate buffer (4N) - The sodium acetate buffer (4N) is prepared dissolving 108.8 g of NaOAc.3H₂O in 80 mL of double-ionized water. Then, 20 mL of glacial acetic acid is added to bring the pH to 5.2 and the solution made up to 200 mL with double-ionized water. The buffer is stored at 277.15 K (Jones et al., 2002).

Preparation of ninhydrin solution - The ninhydrin solution is prepared as follows: 2 g ninhydrin and 0.3 g hydrindantin are dissolved in 75 mL dimethylsulfoxide (DMSO) under a stream of nitrogen gas, 25 mL sodium acetate buffer (pH 5.2) is added, and the resulting dark red solution is further bubbled with nitrogen for at least 2 min. The solution is stored refrigerated (277.15 K) in a dark bottle with dispenser. Fresh ninhydrin solution was prepared every working day.

3.2.3.2 Experimental Procedure

Each jacketed glass cell is charged with the amino acid, previously weighted in a small excess over the estimated solubility, and with the prepared mixed solvent solution. The magnetic bar is introduced and the thermometer placed inside the cell. During the heating process, if necessary, more amino acid is added until slightly excess remains. The cell is conveniently closed and the stoppers surrounded with parafilm, preventing the change of the solvent composition. The thermometer should be sufficiently immersed in the solution in order to measure the real temperature of the solution. To reach the solution equilibrium conditions, stirring is promoted during 48 hours at constant temperature, controlled by circulating

thermostated water in the jacket. After, the magnetic stirrer is stopped and the solution is allowed to settle before sampling. For each determination 3 samples of the saturated solution, of approximately 5 mL (20 mL for pure alcohol saturated solutions), are withdrawn with preheated pipettes, inserted into the glass vessels (25 mL, previously weighted) and immediately weighted. To avoid precipitation of the solid during sampling, the pipettes were kept in the drying stove at a temperature higher than equilibrium. The glass vessels are then left to cool to room temperature (approximately during one hour) and weighted again. This last measurement is the one used for the calculations. When there is a considerable difference between the temperature of the sample and the room temperature, the reading value increases slightly. After, the samples are placed in a heating plate to blandly evaporate most of the solvent of the saturated solution and to enhance the formation of salt and/or amino acids crystals, which are then completely dried in the drying stove at 343.15 K. After cooling, in a dehydrator with silica gel, they are weighted and the process is repeated until a constant value is achieved. This process can, in some cases, take several weeks. The mass of dissolved amino acid is calculated from the knowledge of the initial concentration of the solution and the weight of the glass vessels empty, with solution and with the dry sample. Each experimental data point is an average of at least three different measurements obeying one of the following criteria: if the solubility is higher than 10 per cent (mass percentage), the quotient $2s/(\text{solubility} * 100)$, should be lower than 0.2, where s is the standard deviation within a set of different experimental results. The standard deviation s is defined by the following expression:

$$s = \sqrt{1/(n-1) \sum_{i=1}^n (x_i - \bar{x})^2} \quad (3.1)$$

where x_i is the experimental solubility of the sample i and $\bar{x}$ the arithmetical mean of n experimental results.

If the experimental solubility is less than 10 per cent this criterion is difficult to attain and in this case an equivalent criterion is that the standard deviation should be lower than 0.005 (Pinho and Macedo, 2002).

As stated before, the solubility of the amino acids in high alcohol concentrations ($w'_{alcohol} \geq 0.8$) it is extremely low, and since the analytical balance is not sufficiently accurate, the spectrophotometric ninhydrin method was used for the quantitative determination of this solutions. After a constant mass value of amino acid is achieved, for those samples the amino acid solution is prepared dissolving the crystals, with double-ionized water (100 g). Amino acid solution (1 mL), and ninhydrin solution (1 mL), added from the dispenser bottle, are placed in test tubes, immediately capped, briefly shaken by hand, and heated in a covered boiling water bath for 30 min. The tubes are then cooled below 303.15 K in a cold water bath. The content is diluted with 5 mL of 50% (v/v) ethanol/water and methodically stirred on a vortex mixer (15 s) to oxidize the excess of hydrindantin (Prochazkova et al., 1999). After standing at room temperature for 10 min, the absorbance of the mixture is measured using quartz cuvettes ($\lambda=570$ nm), zero-set against a similarly treated blank of water. Three independent experiments were conducted and the average used for the calculations. Calibration curves were prepared by assaying standard amino acid solutions at 8 different concentrations, ranging from 0.00005 to 0.0005 g amino acid/100g of water, obtained by dilution of an initial solution of known concentration (0.01 g amino acid/100g of water) (Appendix C). The linearity of the calibration curves ($R^2 > 0.99$) is determined by plotting the measured absorbance versus concentration standard solutions of amino acids. Calibration curves are made for each amino acid studied on each analysis day, using freshly prepared calibration standards. Using the spectrophotometric ninhydrin method, each experimental solubility data verifies the following criteria: the quotient $2s/\text{solubility} \times 100$ is lower than 10%, for L-threonine or L-serine solubility values inferiores to 1×10^{-5} (mass fraction), and lower than 6% for the other solubility values. For L-isoleucine, that quotient is lower than 8% for solubility values inferiores to 1×10^{-4} , and lower than 4% for the remaining solubilities.

3.2.3.3 *Stirring Time*

The stirring time is a fundamental parameter to achieve equilibrium. Several authors (Gekko, 1981; Khoshkbarchi and Vera, 1997; Pradhan and Vera, 2000; Soto et al., 1998a) agreed that the optimum time required is 48 hours; however, in this study preliminary tests were performed. The experiments were carried out following the procedure described in the

previous section but the stirring was stopped after different mixing time periods. The system chosen to present the results of the preliminary tests was the system water/L-serine; L-serine it is the amino acid which presents higher solubility values on water and since this is the solvent present in all the systems studied.

The results obtained for that system, at two different temperatures, are presented in Figure 3.4. It is shown that there are no detectable differences in the mass of amino acid dissolved after 24 hours of mixing; preliminary tests, performed with the other amino acids studied, showed similar equilibration curves. To insure that the equilibrium was attained, the mixing time established is 48 hours.

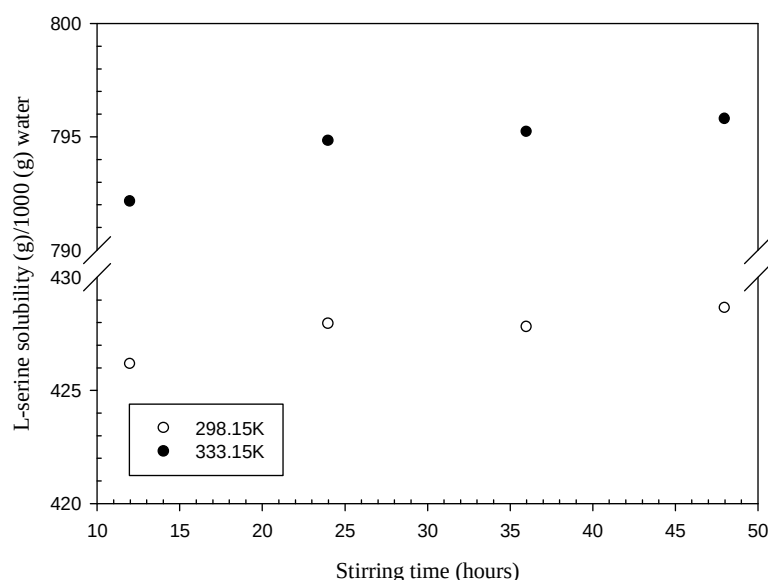


Figure 3.3 Solubility of L-serine in water versus stirring time at two different temperatures.

The influence of the starting temperature to reach equilibrium conditions was also checked. Two cells, prepared following the same procedure, were initially equilibrated at different temperatures, slightly above and below the desired temperature. Then, the two were brought to the equilibrium temperature and left to equilibrate during 48 hours. The final results revealed no significant differences since the solubility values were very close.

3.2.4 METHOD RELIABILITY

The analytical gravimetric method has already proved to be very successful with high reproducibility and accuracy, but even though some preliminary tests were carried out. The procedure followed was the one described in section 3.2.3.2 with the difference that now cells were charged with known amounts of amino acid and water, forming unsaturated solutions. The initial amount and the result found for the mass of L-serine and glycine dissolved in pure water, at different temperatures, and the respective deviations are presented in the Table 3.2. The results were in good agreement with the original concentration values, with deviations quite small indicating that the method is accurate.

Table 3.2 Comparison between *initial* and measured amino acid solubilities,
S (g of amino acid/1000 g of water).

Amino Acid	Temp. (K)	S _{Initial Concentration}	S _{Measured Concentration}	Deviation (%) [*]
L-serine	298.15	250.00	249.53	0.19
	333.15		250.42	0.17
Glycine	323.15	62.60	73.23	0.17

$$* \text{ Deviation (\%)} = \left| \frac{S_{\text{Measured Concentration}} - S_{\text{Initial Concentration}}}{S_{\text{Initial Concentration}}} \right| * 100$$

The accuracy and reproducibility of the spectrophotometric ninhydrin method was also verified. As described in section 3.2.3.2, newly prepared calibration standards are used to make the calibration curves and, the reliability of the regression curve, expressed as regression coefficient (R^2) was generally higher than 0.99. A simple test was preformed; a known amino acid concentration solution was prepared and the procedure of the spectrophotometric ninhydrin method followed. The initial amount (0.0100 ± 0.0001 g amino acid/100g of water) and the result given by the method (0.0096 ± 0.0008 g amino acid/100g of water) showed a very good agreement. The reaction of amino acids with ninhydrin is rapid, sensitive and with high reproducibility.

3.3 EXPERIMENTAL MEASURED DATA

In the following sections, the measured solubility data are reported. Depending on the systems, the maximum temperature studied changes; however the lower temperature studied was the same, 298.15 K.

3.3.1 BINARY SYSTEMS: WATER/AMINO ACID

The measured values of the solubility of the amino acids, glycine, DL-alanine, L-serine, L-isoleucine and L-threonine in water, between 298.15 and 333.15 K, expressed in grams of amino acid per 1000 grams of water ($S_{\text{this work}}$), are reported in Table 3.3 and presented in the Figure 3.4. It is possible to observe that, for all the amino acids the solubility increases with temperature and, at the same temperature, the solubility of the amino acids in water follows the sequence L-serine > glycine > DL-alanine > L-threonine > L-isoleucine. However, the increase rate of the amino acid solubility in water, with the temperature, follows a different sequence: L-serine > glycine > L-threonine > DL-alanine > L-isoleucine.

The amino acids studied have in common an amino and a carboxyl group but their side chain is different. The chemical structure of the amino acids is depicted in Table A.1. Glycine is the simplest amino acid with only two carbon atoms. DL-alanine and L-serine have the same number of $-\text{CH}_2$ groups; one $-\text{CH}_2$ group more than glycine. L-threonine and L-isoleucine have, respectively, two and four $-\text{CH}_2$ groups more than glycine. However, L-serine and L-threonine have an $-\text{OH}$ group in their hydrocarbon backbone. A comparison of the solubilities of glycine, DL-alanine and L-isoleucine shows, as expected, that the solubility in water decreases as the size of the hydrocarbon backbone increases. Examining the solubility of the pair DL-alanine/L-serine makes possible to verify that the solubility of DL-alanine in water is considerably lower than that of L-serine, conforming that the exchange of a hydrogen atom with a hydroxyl group makes the molecule more hydrophilic. The behaviour of the solubility L-threonine in water is more complex mainly because of the competitive effect between the aliphatic chain, considerably longer (with four carbon atoms), and $-\text{OH}$ group.

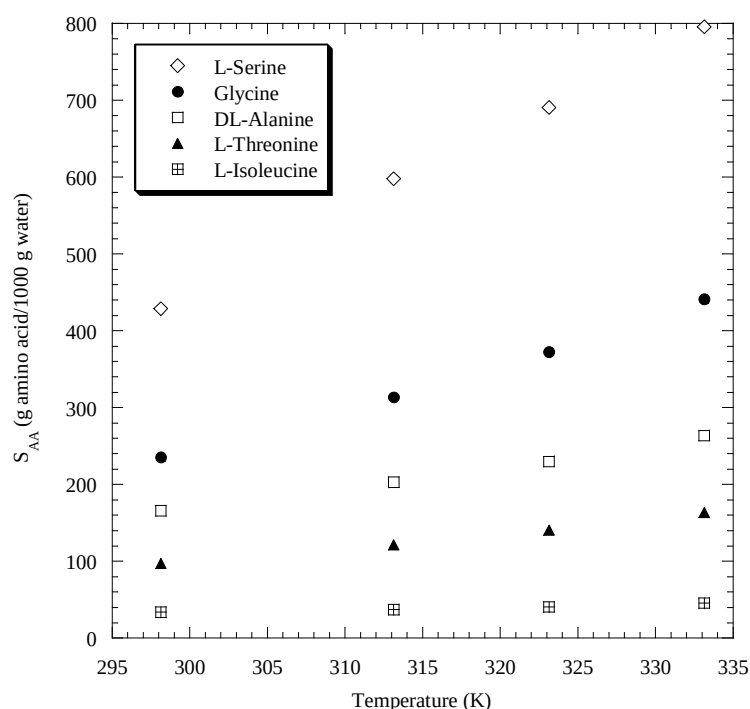


Figure 3.4 Solubilities of amino acids (S_{AA}) in water at different temperatures.

The solubility of the amino acids in pure water reported in the literature was reviewed and compared with the new solubility data. Table 3.3 lists the information compiled, the corresponding measured value from this work as well as the deviation to the average. For glycine, the deviation to the average, at 298.15 K (-12.68 g per 1000 g of water), 313.15 K (-11.64 g per 1000 g of water) and 323.15 K (-15.69 g per 1000 g of water) is considerable. At 298.15 K, the most frequent published value is around 250.00 g of glycine in 1000 g of water (Cohn et al., 1934; Dalton and Schmidt, 1933; Gekko et al., 1998; Gude et al., 1996b) which presents a deviation of 5.72% from the corresponding value from this work. This deviation increases considerably when compared with the value reported by Orella and Kirwan (1991), being 7.93% larger. However, the solubility of glycine presented by Jelińska-Kazimierczuk and Szydłowski (1996), and Carta and Tola (1996) is much similar to the one obtained in this work, with deviations of 1.57% and -0.64%, respectively. Matsuo et al. (2002) did not include the last two references (Carta and Tola, 1996; Jelińska-Kazimierczuk and Szydłowski, 1996) in their estimation of the solubility of glycine in water at 298.15 K. According to these authors, the addition of hydrogen chloride, or sodium hydroxide, to adjust the solution pH (Carta and Tola, 1996), may result in a deviation from the standard solubility and, as Jelińska-Kazimierczuk and Szydłowski (1996) measured the temperature dependency of the

solubility by rising the temperature of the sample mixtures at $0.067 \text{ K}\cdot\text{min}^{-1}$, the low solubility found may be due to insufficient equilibration time. In this work, those arguments are not suitable; there was no pH adjustment and the preliminary tests proved that 48 hours were enough to reach the equilibrium. It is also important to refer that, the value of the solubility of the glycine in water at 298.15 K is the result of an average of eight independent measurements with a standard deviation of 0.38 g of glycine per kg of water. At higher temperatures, the deviation to the average is considerable smaller, especially at 333.15 K; however the data available in literature is more limited. For the higher temperatures, the average is given by measurements made in the thirties (Cohn et al., 1934; Dalton and Schmidt, 1933) and the ones presented more recently by Jelińska-Kazimierczuk and Szydłowski (1996).

It can be easily verified that the solubilities measured in the thirties are larger than those observed in this work and by Jelińska-Kazimierczuk and Szydłowski (1996). The measured values for the solubility of glycine at 313.15 and 323.15 K are in agreement with the ones published by Jelińska-Kazimierczuk and Szydłowski (1996) but at 333.15 K, the value measured here is closer to the one by Dalton and Schmidt (1933).

The solubility of L-isoleucine in water, at 298.15 K, has also been measured by many authors. The deviations found, comparing this result with the higher and lowest results published before, is considerable, 3.75% and -4.97% respectively; however, the absolute deviation to the average of seven sources, is very reasonable (0.45 g of L-isoleucine per 1000 g of water). As mentioned before, at higher temperatures, the number of data becomes limited, but at 313.15 and 323.15 K the corresponding measured values from this work are slightly smaller than the average, while at 333.15 K a very good agreement is found (absolute deviation of 0.09 g per 1000 g water).

The results found for the solubility of L-threonine justifies special attention. The solubility measured is always superior to the average; the deviation found at 298.15 K is particularly high when compared to the ones obtained at the other studied temperatures. Nevertheless, the value given by Nozaki and Tanford (1970), at 298.15 K, is in good agreement with the corresponding measured value. From the correlation given by Profir and Matsuoka (2000), the solubility of L-threonine was calculated at the studied temperatures and the results compared. For 298.15 K the difference is of 5.96 g per 1000 g of water, while for the other temperatures

the solubility is inferior but of the same order of magnitude (difference around 3 g per 1000g of water).

Like happened for glycine, the average of the solubility of DL-alanine in water, is given by values available from the thirties (Cohn et al., 1934; Dalton and Schmidt, 1933) and solubility values presented more recently (Jin and Chao, 1992). The values measured in this work are lower than the average except at 333.15 K and surprisingly, a better agreement is found with the old data (Cohn et al., 1934; Dalton and Schmidt, 1933).

The data for solubility of L-serine in water has one common reference for all temperatures (Jin and Chao, 1992); the deviation is quite acceptable with a maximum relative deviation of 1.4% for the solubility at 298.15 K and a minimum of -0.03% at 333.15 K.

In conclusion, generally, a very satisfactory agreement is found; with the exceptions previously mentioned. For all the amino acids the number of data available in the literature decreases at higher temperatures; the common data available is at 298.15 K and some are rather old. In those cases, the chemical purity and accuracy of the experimental technique must have special awareness. Generally, a better agreement is found at high temperatures.

Table 3.3 Comparison of the solubilities of amino acids (g of amino acid/1000 g of water) in pure water.

Amino Acid	Temp. (K)	Solubility (S_{AA})			$S_{average}$	$S_{this\ work}$	$\Delta S/S_{average}$ (%)
L-serine	298.15	421.7 (a)	427.95 (b)	418.5 (c)	422.72	428.63	1.40
	313.15		592.0 (a, d)		592.0	598.03	1.02
	323.15		692.2 (a)*		692.2	690.84	-0.20
	333.15		796.0 (a)		796.0	795.78	-0.03
Glycine	298.15	250.4 (b) 251.48 (f) 249.9 (i)	250.0 (c, r) 234.21(g) 253.1 (j)	256.0 (e) 239.46 (h)* 250.9 (k)	248.38	235.70	-5.10
	313.15		318.8 (h)*	331.6 (i)	325.2	313.56	-3.58
	323.15		371.4 (h)*	391.0 (i)	387.97	372.28	-4.04
	333.15		423.9 (h)*	452.60 (i)	438.25	440.88	0.60
DL-alanine	298.15	169.30 (a)	167.20 (i)	165.8 (j)	167.43	165.77	-0.99
	313.15		205.50 (a)	202.90 (i)	204.2	202.95	-0.61
	323.15	230.8 (a)	230.9 (i)	234.8 (j)	232.17	229.86	-0.99
	333.15		263.2 (a)	262.70 (i)	262.95	263.61	0.25
L-threonine	298.15	95.9 (c) 93.0 (m)	97.7 (k) 99.1 (n)*	91.5 (l) [§]	95.44	97.46	2.12
	313.15		120.8 (n)	118.13(l) [§]	119.47	121.48	1.68
	323.15		138.3 (l) [§]		138.3	140.58	1.65
	333.15		160.36 (l) [§]		160.36	163.53	1.98
L-isoleucine	298.15	33.20 (b) 33.06 (f)	32.3 (c) 32.4 (o) 34.9 (q) [§]	32.0 (e) 34.1 (p) [§]	33.14	33.59	1.36
	313.15	37.50 (d)	38.24 (p) [§]	38.6 (q) [§]	38.11	37.18	-2.44
	323.15		42.2 (q) [§]	41.2 (p) [§]	41.7	40.57	-2.71
	333.15		46.56 (q) [§]	44.11 (p) [§]	45.34	45.43	0.20

$\Delta S = S_{this\ work} - S_{average}$; * interpolated; [§] calculated using equation given in the reference.

(a) Jin and Chao (1992); (b) Gude et al. (1996b); (c) Gekko et al (1998); (d) Hutchens (1976); (e) Orella and Kirwan (1991); (f) Matsuo et al. (2002); (g) Carta and Tola (1996); (h) Jelińska-Kazimierczuk and Szydlowski (1996); (i) Dalton and Schmidt (1933); (j) Dunn et al. (1933); (k) Nozaki and Tanford (1970); (l) Profir and Matsuoka (2000); (m) Chen et al. (2004); (n) Sapoundjiev et al. (2006); (o) Brown and Rousseau (1994); (p) Teja et al. (2002); (q) Zumstein and Rousseau (1989); (r) Cohn et al. (1934).

3.3.2 TERNARY SYSTEMS: WATER/ALCOHOL/AMINO ACID

The measured solubility data for mixed two-solvent systems are presented in sections 3.3.2.1 to 3.3.2.3, divided according to the mixed solvent studied. The amino acid solubility is expressed in grams of amino acid per 1000 grams of solvent (S_{AA}), and the solvent composition is expressed in mass fraction in amino acid free basis (w'). For a better understanding and description of the influence of the temperature, solvent composition and nature of the chemicals, the relative solubility, defined as the ratio of the mole fraction solubility of the amino acid in the mixed solvent to that in pure water is used in all figures.

3.3.2.1 Water/Ethanol/Amino Acid Systems

Table 3.4 presents the solubility of glycine in aqueous systems of ethanol at 298.15 K, 313.15 K, 323.15 K and 333.15 K. The experimental results for the solubility of DL-alanine, L-serine, L-isoleucine and L-threonine in aqueous ethanol mixed solvent systems (298.15 K, 313.15 K and 333.15 K) are compiled in Table 3.5.

Table 3.4 Solubilities of glycine in water/ethanol mixtures at different temperatures.

Amino acid	298.15 K		313.15 K		323.15 K		333.15 K	
	w'_{ethanol}	S_{AA}	w'_{ethanol}	S_{AA}	w'_{ethanol}	S_{AA}	w'_{ethanol}	S_{AA}
Glycine	0.0500	187.86	0.0500	258.15	0.0509	313.82	0.0500	377.09
	0.1500	110.90	0.1500	163.44	0.1501	209.10	0.1500	261.00
	0.2035	83.07	0.2000	128.48	0.2000	169.33	0.2000	214.51
	0.4003	27.33	0.4000	43.16	0.4002	58.58	0.4000	76.95
	0.6000	7.47	0.6000	11.42	0.6000	15.16	0.6000	20.12
	0.8003	1.02	0.8000	1.63	0.8000	2.00	0.8000	2.59
	1.0000	0.09	1.0000	0.09	1.0000	0.11	1.0000	0.13

The relative solubility of the five amino acids in water/ethanol mixed solvent system, with the relative solubility of the simplest amino acids, glycine, shown as a smooth line, are presented in Figures 3.5 to 3.7, at 298.15 K, 313.15 K and 333.15 K, respectively. As illustrated in those

figures, the change of the solvent from water to pure ethanol has a drastic effect on the solubility of amino acids. For glycine, with no side chain, L-serine and L-threonine, both with a polar side chain, the relative solubility is reduced by three orders of magnitude. For the amino acids with hydrophobic side chains, DL-alanine and L-isoleucine, the magnitude of the change is smaller.

Table 3.5 Solubilities of amino acids in water/ethanol mixtures at different temperatures.

Amino acid	298.15 K		313.15 K		333.15 K	
	w'_{ethanol}	S _{AA}	w'_{ethanol}	S _{AA}	w'_{ethanol}	S _{AA}
DL-alanine	0.1999	71.28	0.2000	98.59	0.1999	145.23
	0.4000	27.90	0.4000	42.21	0.4000	66.69
	0.6000	9.35	0.6000	13.78	0.6000	22.06
	0.8000	1.74	0.8000	2.58	0.8000	3.99
	1.0000	0.18	1.0000	0.23	1.0000	0.25
L-serine	0.2002	153.59	0.2000	250.91	0.2002	401.88
	0.3998	46.46	0.4001	76.41	0.4000	140.19
	0.6000	11.58	0.6002	17.76	0.6000	29.44
	0.8000*	0.99	0.8000*	2.25	0.8000*	2.69
	1.0000*	0.03	1.0000*	0.06	1.0000*	0.11
L-isoleucine	0.0501	29.15				
	0.2000	17.31	0.2001	23.94	0.2005	35.41
	0.4001	10.87	0.4002	16.49	0.4000	27.36
	0.6000	6.76	0.6000	10.12	0.6000	16.54
	0.8002*	2.63	0.8003*	4.17	0.8000*	4.71
	1.0000*	0.19	1.0000*	0.44	1.0000*	0.59
L-threonine	0.2002	35.41	0.2000	51.18	0.2000	79.70
	0.3998	12.82	0.4001	19.70	0.4001	33.30
	0.6000	4.52	0.6000	6.32	0.6000	10.77
	0.8000*	0.57	0.8000*	1.05	0.8000*	1.75
	1.0000*	0.0410	1.0000*	0.0456	1.0000*	0.0873

* Spectrophotometric ninhydrin method

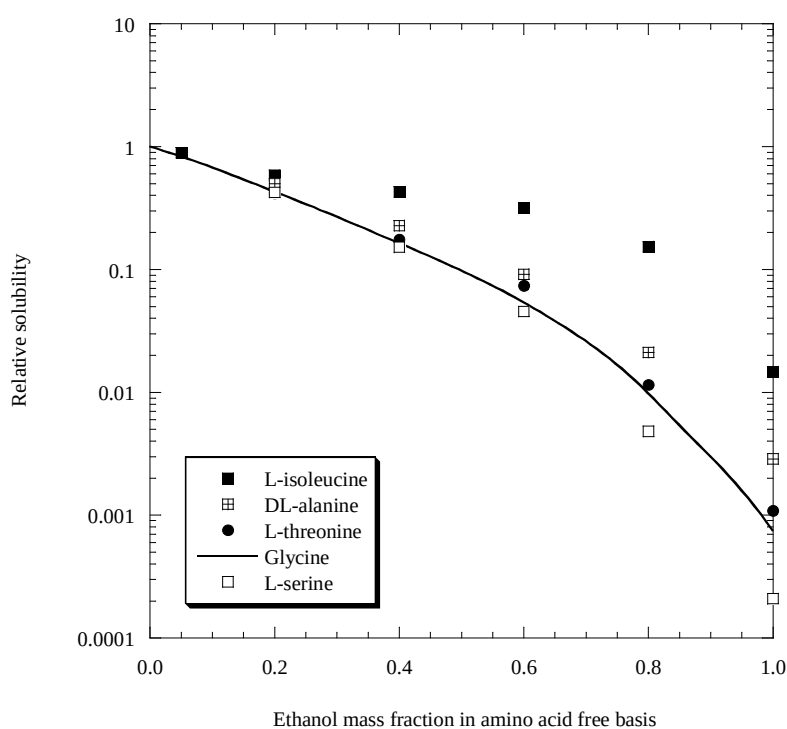


Figure 3.5 Relative solubilities of amino acids in water/ethanol solutions at 298.15 K.

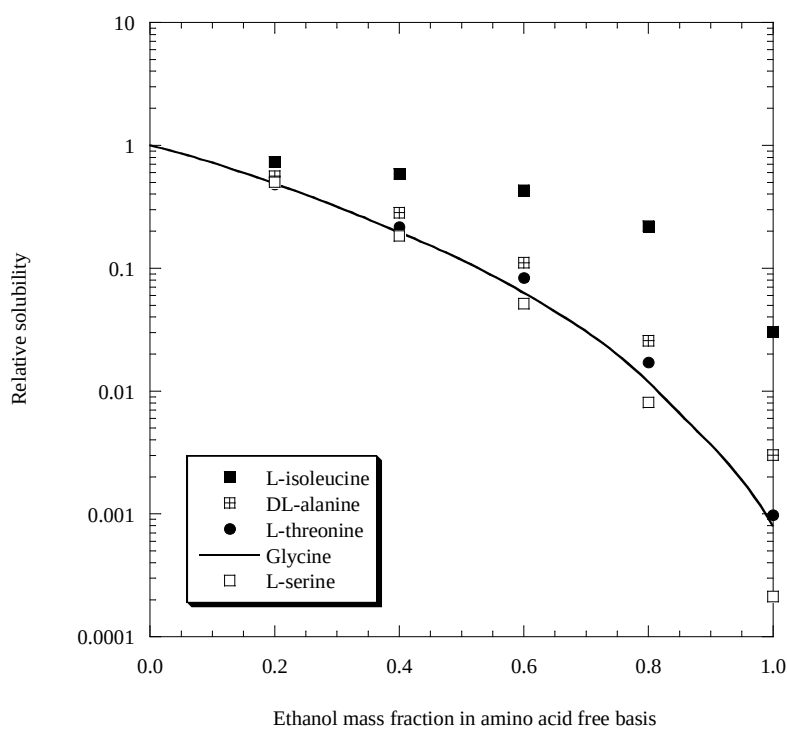


Figure 3.6 Relative solubilities of amino acids in water/ethanol solutions at 313.15 K.

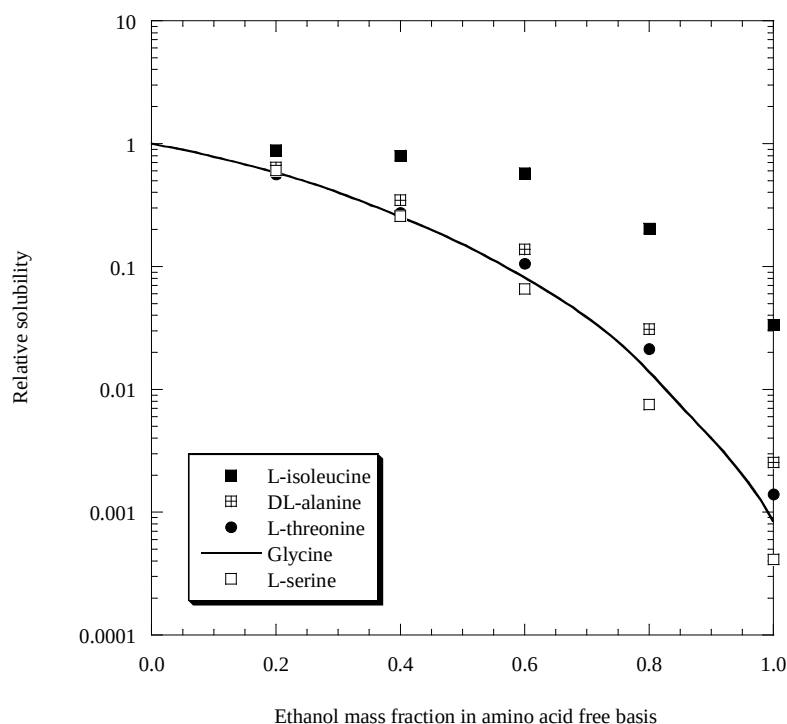


Figure 3.7 Relative solubilities of amino acids in water/ethanol solutions at 333.15 K.

3.3.2.2 Water/1-Propanol/Amino Acid Systems

Table 3.6 presents the measured solubilities of glycine in aqueous 1-propanol mixed solvent systems at 298.15 K. The measured solubilities for DL-alanine, L-isoleucine, L-threonine and L-serine in aqueous systems of 1-propanol, in the temperature range studied, are given in Table 3.7. Figures 3.8 to 3.10 show the relative solubilities of amino acids in water/1-propanol solutions at different temperatures.

Like for the previous system, the chemical nature of the amino acids side chain plays an important role in the influence of the amino acid on the solvent system. Glycine, L-serine and L-threonine, can see their solubility reduced by three orders of magnitude when the solvent changes from water to pure alcohol, however for the DL-alanine and L-isoleucine the effect is less pronounced.

Table 3.6 Solubilities of glycine in water/1-propanol at 298.15 K.

$w'_{1\text{-propanol}}$	S_{Glycine}
0.0501	188.11
0.1500	120.25
0.2001	101.52
0.4011	47.37
0.6000	15.08
0.8000	1.66
1.0000	0.14

Table 3.7 Solubilities of amino acids in water/1-propanol mixtures at different temperatures.

Amino acid	298.15 K		313.15 K		333.15 K	
	$w'_{1\text{-propanol}}$	S_{AA}	$w'_{1\text{-propanol}}$	S_{AA}	$w'_{1\text{-propanol}}$	S_{AA}
DL-alanine	0.2001	87.86	0.2000	111.25	0.2000	159.29
	0.4003	46.17	0.4000	60.18	0.4000	88.46
	0.6000	16.79	0.6000	22.25	0.6000	33.22
	0.8000	2.51	0.8000	3.33	0.8000	4.93
	1.0000	0.23	1.0000	0.28	1.0000	0.36
L-serine					0.8000*	4.94
	0.6500	14.52	0.7000	13.65	0.8500*	1.81
	0.7000	8.76	0.8000*	3.26	0.9000*	0.61
	0.8001*	2.39	0.9000*	0.32	0.9500*	0.27
	1.0000*	0.01	1.0000*	0.02	1.0000*	0.05
L-isoleucine	0.0501	29.64				
	0.2015	21.14	0.2021	27.96	0.1996	42.36
	0.3999	17.27	0.4008	24.50	0.4000	37.36
	0.6000	11.07	0.6000	15.54	0.6001	23.36
	0.8001*	4.31	0.8000*	5.04	0.8000*	5.80
	1.0000*	0.01	1.0000*	0.16	1.0000*	0.23
L-threonine	0.1996	42.43	0.2003	59.46	0.2000	90.41
	0.3998	20.77	0.4000	30.41	0.4000	46.87
	0.6000	7.99	0.6000	10.84	0.6000	16.57
	0.8000*	1.30	0.8000*	1.45	0.8000*	2.18
	1.0000*	0.02	1.0000*	0.02	1.0000*	0.04

* Spectrophotometric ninhydrin method

In order to better evidence an uncommon effect, a horizontal line has been drawn in the figures at the value of the relative solubility in pure water. With especial attention to Figure 3.10 it is possible to observe that the relative solubility of L-isoleucine (333.15 K), at low 1-propanol mass fractions, is superior to 1 indicating that, at this temperature and alcohol composition, the solubility is higher than the solubility in water at the same temperature. While the temperature dependence of the relative solubility of DL-alanine is weak, an unpredicted phenomenon was observed for the amino acids, L-serine, and glycine: the formation of two liquid phases. For glycine this was only observed at 313.15 K, while for L-serine it was observed in the entire temperature range. The solubility of L-serine in the miscible composition range of the solvent system was studied and, surprisingly, it was observed that the miscible composition range becomes shorter by increasing of the temperature.

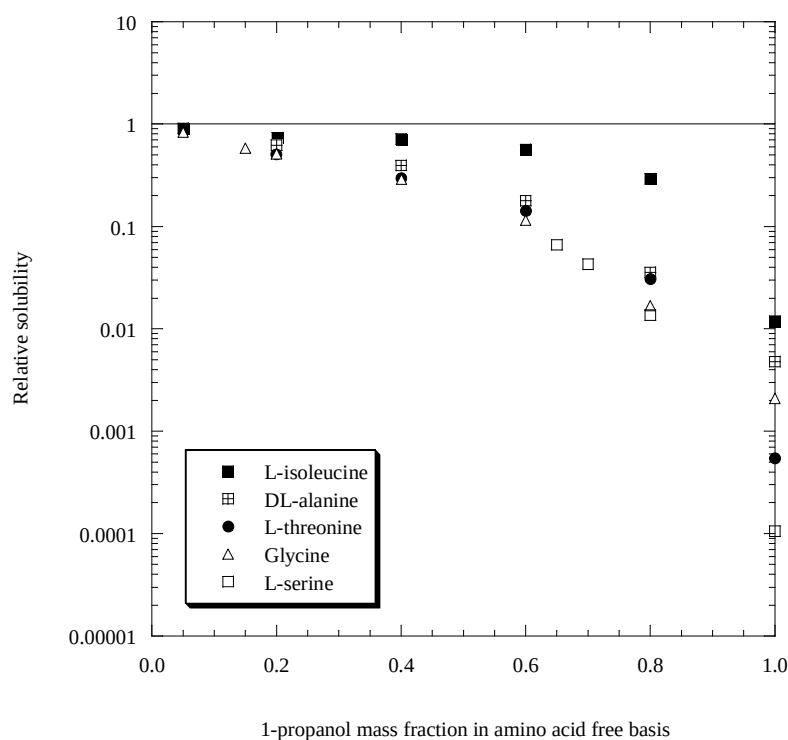


Figure 3.8 Relative solubilities of amino acids in water/1-propanol solutions at 298.15 K.

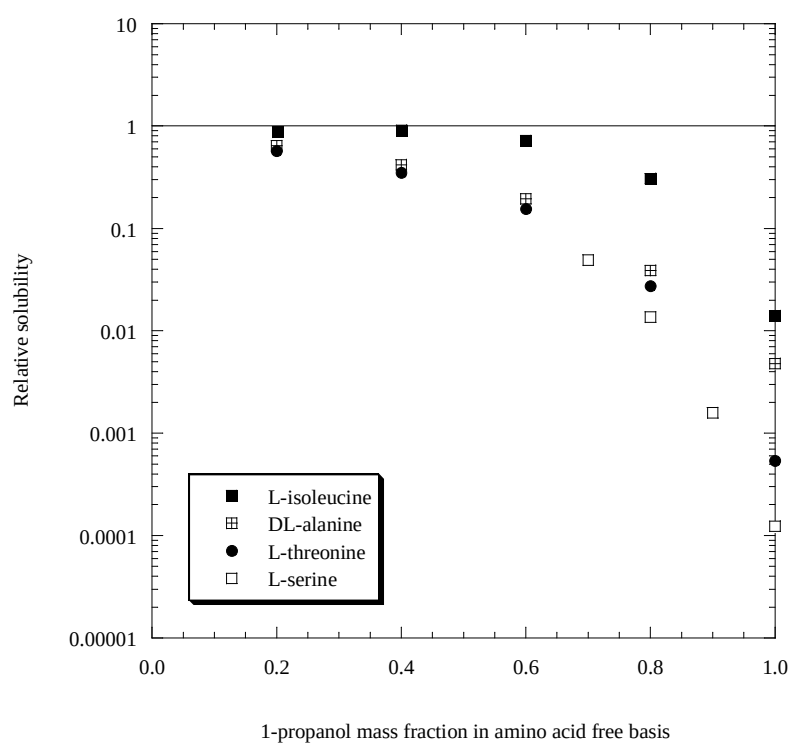


Figure 3.9 Relative solubilities of amino acids in water/1-propanol solutions at 313.15 K.

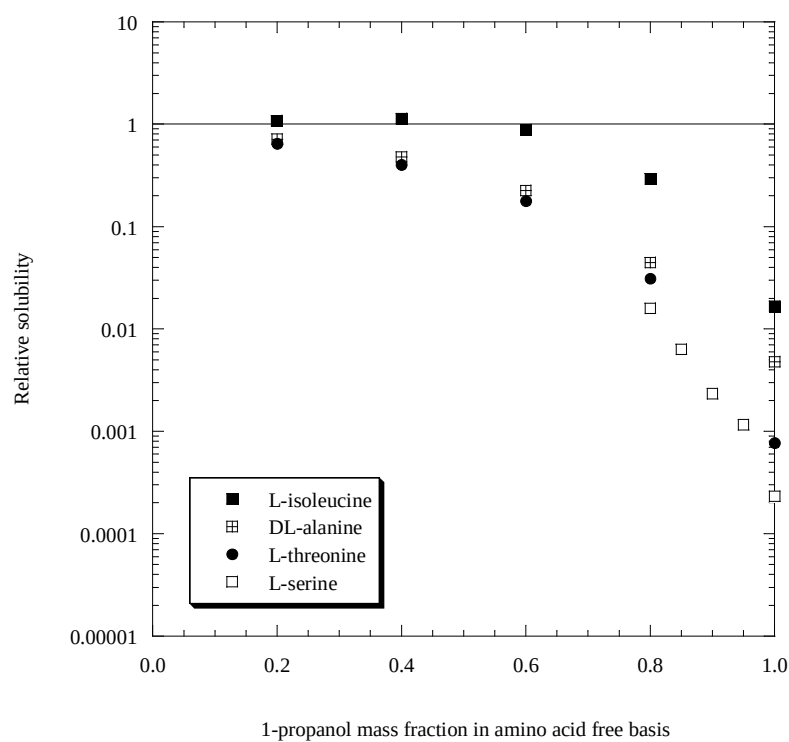


Figure 3.10 Relative solubilities of amino acids in water/1-propanol solutions at 333.15 K.

3.3.2.3 Water/2-Propanol/Amino Acid Systems

Figures 3.11 to 3.13 show the relative solubility of the different amino acids in water/2-propanol solutions in the temperature range 298.15 K – 333.15 K. The measured values of the solubilities are also presented in Tables 3.8 and 3.9. As shown in the figures the relative solubility of L-serine, glycine, L-threonine and DL-alanine comes closest to being monotonically decreasing. The data for L-isoleucine shows a region up to an alcohol mass fraction around 0.4 where the change is small, followed by a moderate decrease with the increase of the alcohol concentration on the solvent mixtures. The formation of two liquid phases was also observed for the amino acids glycine and L-serine in this mixed solvent system. For glycine this behavior was observed at 313.15 K and for the L-serine at 333.15 K. Only the solubilities of L-serine in the miscible composition range of the solvent system were observed.

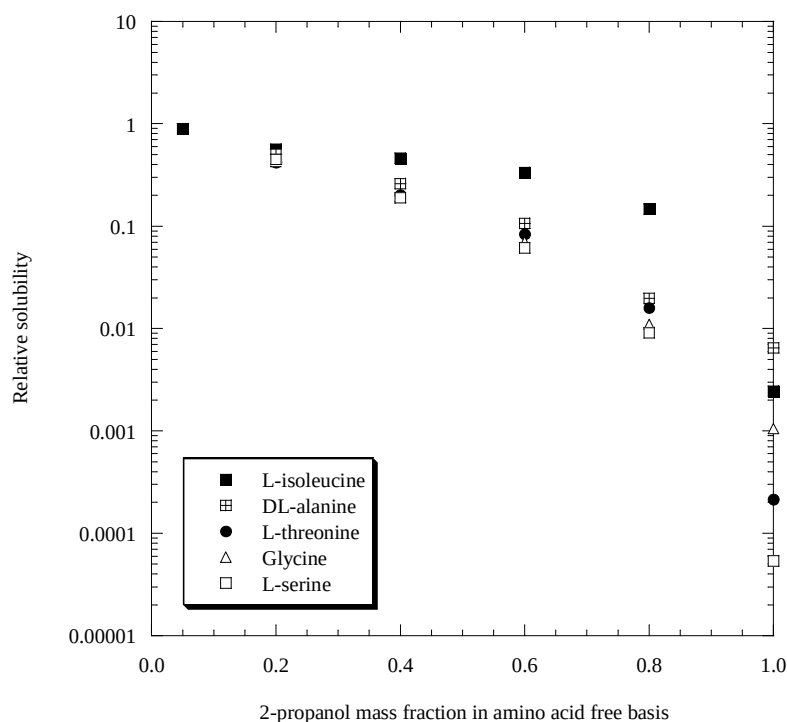


Figure 3.11 Relative solubilities of amino acids in water/2-propanol solutions at 298.15 K.

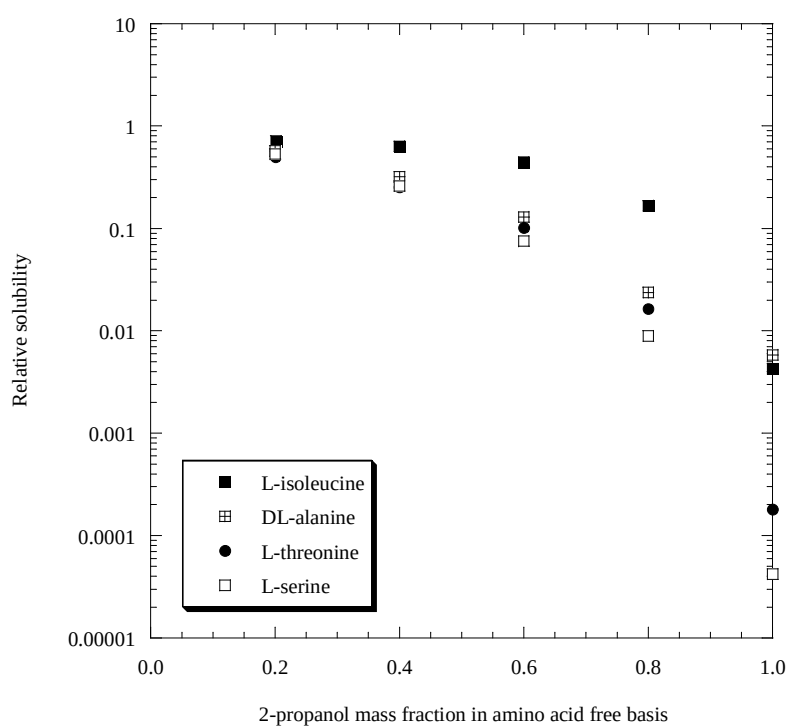


Figure 3.12 Relative solubilities of amino acids in water/2-propanol solutions at 313.15 K.

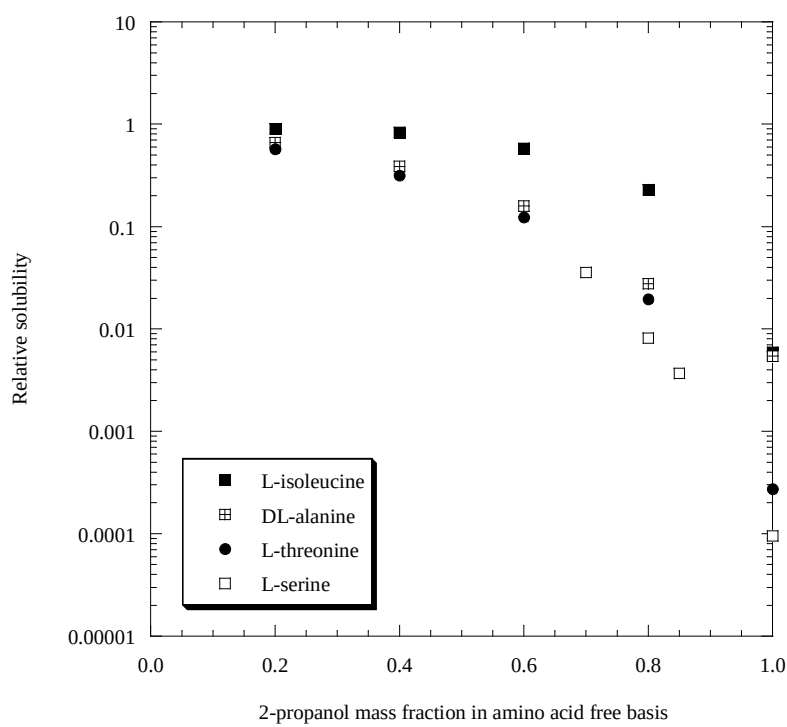


Figure 3.13 Relative solubilities of amino acids in water/2-propanol solutions at 333.15 K.

Table 3.8 Solubilities of glycine in water/2-propanol solvent mixtures at 298.15 K.

$w'_{2\text{-propanol}}$	S_{Glycine}
0.2000	84.07
0.4000	31.94
0.6000	9.15
0.8000	1.08
1.0000	0.07

Table 3.9 Solubilities of amino acids in solutions containing 2-propanol at different temperatures.

Amino acid	298.15 K		313.15 K		323.15 K	
	$w'_{2\text{-propanol}}$	S_{AA}	$w'_{2\text{-propanol}}$	S_{AA}	$w'_{2\text{-propanol}}$	S_{AA}
DL-alanine	0.2000	70.58	0.2000	98.35	0.2000	147.20
	0.4000	30.30	0.3999	45.45	0.4000	71.15
	0.6000	9.97	0.6000	14.80	0.6000	23.33
	0.8000	1.39	0.8000	2.04	0.8000	3.05
	1.0000	0.31	1.0000	0.34	1.0000	0.41
L-serine	0.2000	159.25	0.2001	261.61		
	0.4002	55.23	0.4000	104.57	0.7005	12.74
	0.6000	14.34	0.6000	23.81	0.8000*	2.5161
	0.8000*	1.6024	0.8000*	2.1230	0.8500*	1.0491
	1.0000*	0.0064	1.0000*	0.0069	1.0000*	0.0201
L-isoleucine	0.0500	28.84				
	0.2000	16.39	0.2013	22.82	0.2001	35.21
	0.4000	11.09	0.4001	16.93	0.4000	27.35
	0.6000	6.5121	0.6000	9.57	0.6000	15.35
	0.8000*	2.1702	0.8000*	2.7170	0.8000*	4.5724
	1.0000*	0.0246	1.0000*	0.0474	1.0000*	0.0804
L-threonine	0.2000	34.97	0.2006	51.56	0.2000	79.49
	0.4001	14.11	0.4000	21.81	0.4001	36.70
	0.6000	4.67	0.6000	7.02	0.6000	11.52
	0.8000*	0.6698	0.8000*	0.8615	0.8000*	1.3706
	1.0000*	0.0062	1.0000*	0.0065	1.0000*	0.0131

* Spectrophotometric ninhydrin method

3.3.2.4 Critical Analysis

Qualitative resemblances can be found in the different alcohol systems studied. Like expected the absolute values of the solubilities (S_{AA} /grams of amino acid per 1000 grams of solvent) increase with the temperature and decrease, at constant temperature, with the increasing alcohol concentration. For small concentrations of alcohol the solubility diminishes severely while for larger amounts of alcohol this effect is much less pronounced.

The balance between hydrophobicity and self-association has a major importance in the behaviour of the solubility of the amino acids in the solvent system (Pinho, 2008). Numerous “hydrophobicity scales” have been proposed (Karplus, 1997; Wilce et al., 1995) and fundamental differences can be found between them. However, among the amino acids studied, glycine and serine are ranked as less and isoleucine as the strongly hydrophobic, while threonine and alanine have similar hydrophobicity and are positioned in the middle. Amino acids also may present a tendency for self-association (Lin et al., 2000), and consequently, the interactions between side-chains are very important. As expected, glycine, L-serine and L-threonine can see their relative solubility reduced severely while the amino acids DL-alanine and L-isoleucine present smaller reductions.

When possible, our experimental data were compared with the solubility data available in the literature. The data for the relative solubility of glycine in ethanol solutions at 298.15 K and for L-isoleucine in solutions containing 1-propanol and 2-propanol are also presented in Figures 3.14 and 3.15, respectively. Generally, a very good agreement was found.

For the majority of the systems for which no literature data is available, the qualitative approach can confirm the quality of the measurements since it was in agreement with the expected: for amino acids with nonpolar side chain the relative solubility is reduced by a magnitude smaller than for amino acids with a polar side chain. Consequently, these alcohols will tend to be poorer crystallizing agents for the amino acids with nonpolar side chain (Orella and Kirwan, 1989).

The increase of the number of carbon atoms in the alcohol increases the attraction between the solute and solvent and the effect of the different alcohols on the amino acids solubility can be striking, as illustrated for L-isoleucine and L-serine in Figure 3.15. The reduction in the

relative solubility of the L-isoleucine is clearly affected by the increase of the number of carbon atoms in the alcohol; until moderate alcohol mass fraction values the relative solubility in water/ethanol and water/2-propanol are very similar and for water/1-propanol is always higher. For larger alcohol mass fractions a difference is observed, water/2-propanol presents the lowest relative solubility and water/ethanol the largest. As observed in section 3.3.2.2, the relative solubility of L-isoleucine in systems with a small mass fraction of alcohol can be superior to 1 indicating that the solubility in the mixed solvent is higher than in water at the same temperature. This kind of behavior was already observed for the phenylalanine in solutions of either 1-propanol or 2-propanol at 298.15 K (Orella and Kirwan, 1989, 1991). The solubility of the phenylalanine in aqueous 1-propanol can be 25% larger than the solubility in water at the same temperature due to the strong interactions between the highly nonpolar aromatic side chain of the phenylalanine and the solvents (Orella and Kirwan, 1989, 1991). For glycine and L-serine the formation of two liquid phases with the 1-propanol and 2-propanol was observed clearly influenced by the temperature and alcohol mass fraction. These results confirm the extreme interactions taking place between solute and solvent.

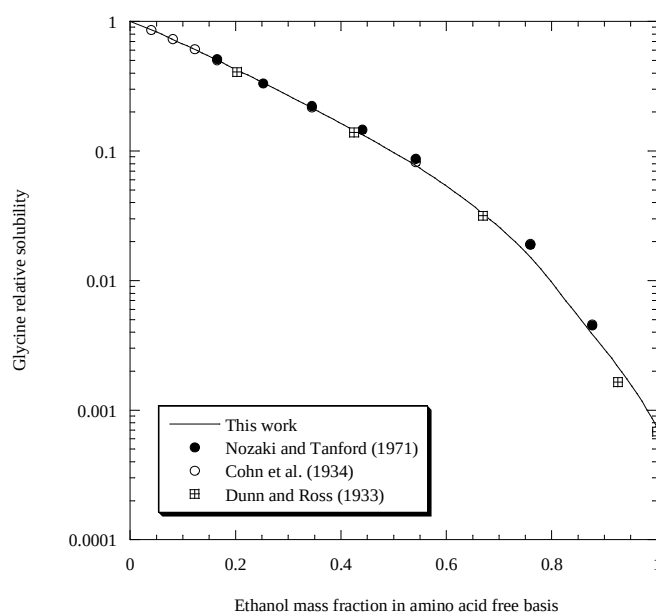


Figure 3.14 Glycine relative solubilities in water/ethanol solutions at 298.15 K: comparison with solubility data available in the literature.

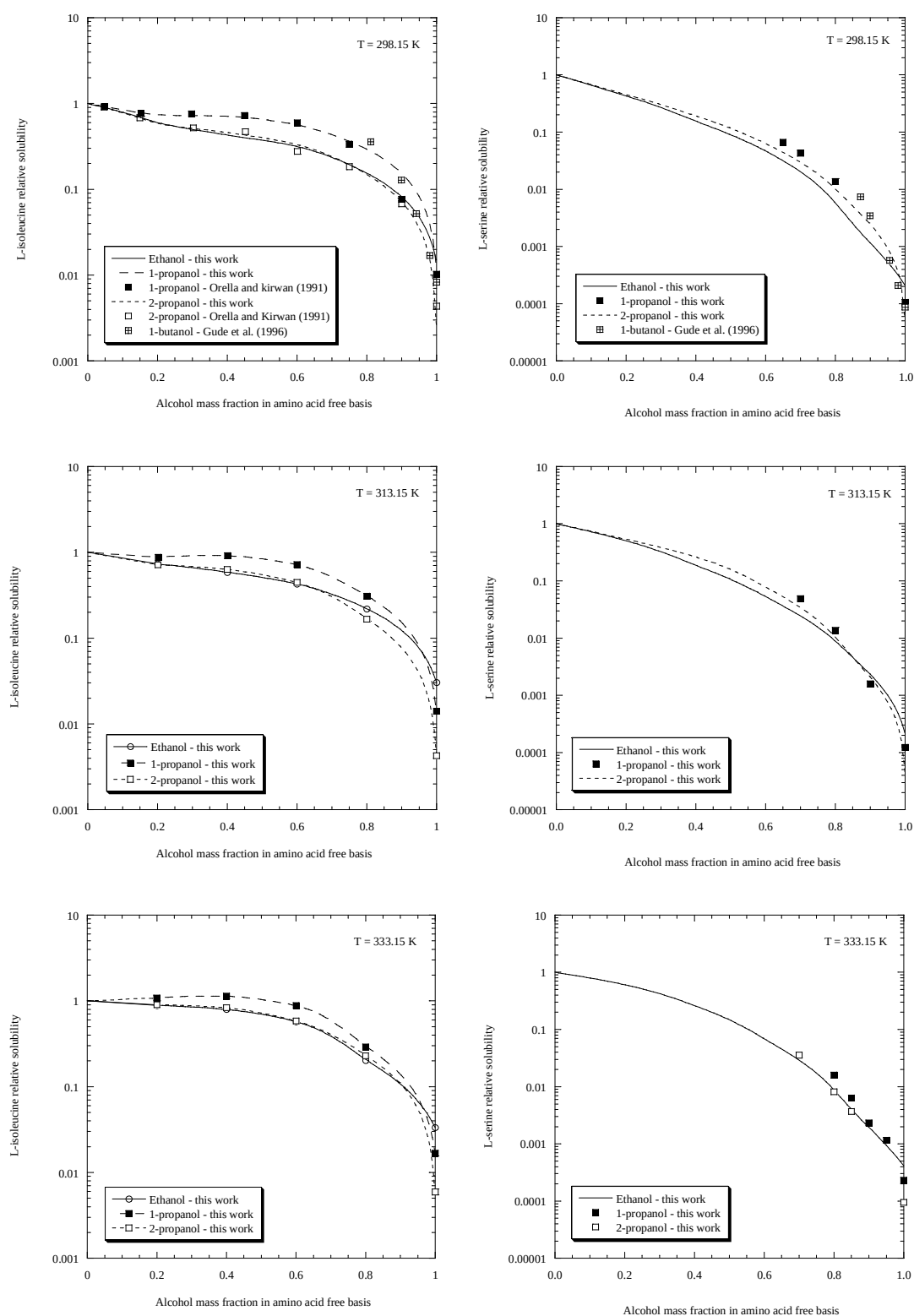


Figure 3.15 Effect of the different alcohols on the solubilities of L-isoleucine and L-serine at different temperatures.

3.3.3 TERNARY SYSTEMS: WATER/ELECTROLYTE/AMINO ACID

In the following two sections (3.3.3.1 and 3.3.3.2), the measured solubility data of the amino acids in aqueous systems of electrolytes is presented and expressed in grams of amino acid per 1000 grams of water (S_{AA}); the electrolyte concentration is expressed in molality.

3.3.3.1 Water/KCl/Amino Acid Systems

The experimental data for the solubility of the amino acids, glycine, L-serine, L-isoleucine and L-threonine in aqueous KCl solutions (molality ranging from 0.0 to 2.0) at two temperatures (298.15 and 323.15 K) is presented in Table 3.10. The measured values for the solubility of DL-alanine in aqueous KCl solutions at 298.15, 323.15 and 333.15 K are reported in Table 3.11. Figures 3.16 and 3.17 show the change of the relative solubility at 298.15 and 323.15 K, respectively, calculated as the ratio between the solubilities of the amino acid in the electrolyte solution to that in pure water, on the electrolyte molality. For a better comparison, the relative solubility for glycine, the simplest amino acid, is presented as a smooth line. As observed in the figures, the presence of the potassium chloride may lead to either a salting-in and/or a salting-out effect. At very low electrolyte molality, all the amino acids show a slightly salting-in behavior. However, the increase of the KCl concentration introduces differences between the amino acids, which are consistent with their chemical structure.

Examining Figure 3.16 it is possible to observe the slight increase of the glycine solubility with the increasing electrolyte concentration. DL-alanine, with one more hydrophobic $-\text{CH}_3$ group than glycine, shows an inverse dependence with the salt concentration, which leads to a moderate salting-out effect. The same effect is verified for L-isoleucine, even though, the salting-out effect is, as expected, more drastic since a bigger hydrocarbon chain is present introduced. The salt KCl has a very pronounced salting-in effect on the solubility of L-serine at 298.15 K. L-serine has the same number of $-\text{CH}_2$ groups as DL-alanine, but the polar $-\text{OH}$ group increases its tendency to dissolve in ionic solutions. On the other hand, L-threonine with one more aliphatic group than L-serine exhibits a moderate salting-in effect up to 1 KCl molal with the increase of the KCl molality, the salting-in behavior starts to decrease. Like mentioned before, the peculiar behavior showed by L-threonine is, somehow, a mixed result

that outcomes from the competitive effect of aliphatic and -OH groups present in the same molecule.

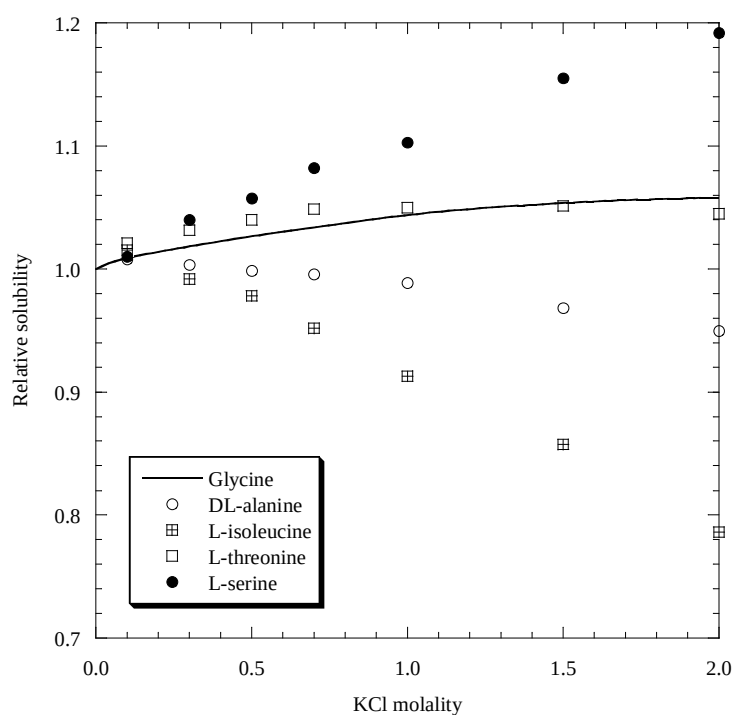
Comparing the results obtained at two different temperatures (Figures 3.16 and 3.17), a similar effect of the KCl concentration on the relative solubility of DL-alanine and L-isoleucine is found. For glycine, L-serine and L-threonine, the variation of the relative solubility with the potassium chloride concentration, at 323.15 K, is much less pronounced than at 298.15 K; and more similar between them. In almost all the electrolyte molality range L-threonine presents, now, the higher relative solubility.

Table 3.10 Solubilities of amino acids (g of amino acid/1000 g of water) in aqueous solutions of KCl at two temperatures 298.15 K and 323.15 K.

Temp. (K)	Electrolyte (molality)	S _{AA} (g of amino acid/1000 g of water)			
		Glycine	L-serine	L-isoleucine	L-threonine
298.15	0.000	235.70	428.63	33.59	97.46
	0.100	238.03	432.98	34.11	99.50
	0.300	240.00	445.82	33.32	100.55
	0.500	242.01	453.33	32.86	101.37
	0.700	243.63	463.80	31.97	102.23
	1.000	246.09	472.61	30.66	102.32
	1.500	248.35	495.16	28.80	102.45
	2.000	249.40	510.87	26.40	101.84
323.15	0.000	372.28	690.84	40.57	140.58
	0.100	375.49	693.53	41.09	142.13
	0.300	376.98	696.88	40.45	143.88
	0.500	377.89	700.92	40.04	144.56
	0.700	378.12	704.74	39.26	144.96
	1.000	379.83	706.78	37.64	145.48
	1.500	381.62	713.17	35.34	144.60
	2.000	382.08	719.69	33.33	143.27

Table 3.11 Solubilities of DL-alanine in aqueous solutions of KCl.

Amino Acid	Electrolyte (molality)	S_{AA} (g of amino acid/1000 g of water)		
		298.15 K	323.15 K	333.15 K
DL-alanine	0.000	165.77	229.86	263.61
	0.100	167.13	231.49	264.33
	0.300	166.33	230.63	263.03
	0.500	165.54	229.81	262.70
	0.700	165.06	229.17	261.20
	1.000	163.88	226.88	259.21
	1.500	160.52	223.57	255.34
	2.000	157.43	218.58	250.97

**Figure 3.16** Relative solubilities of different amino acids in water/KCl solutions at 298.15 K.

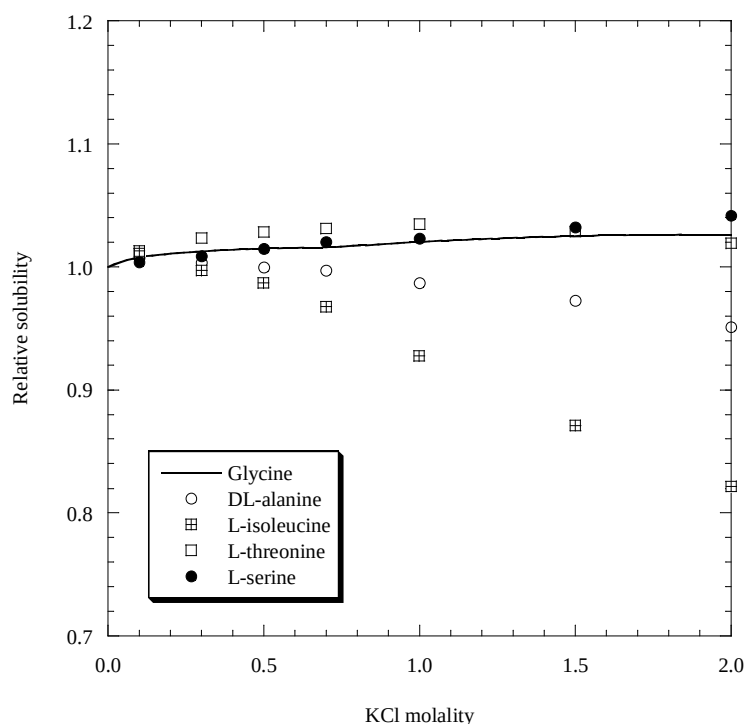


Figure 3.17 Relative solubilities of different amino acids in water/KCl solutions at 323.15 K.

3.3.3.2 Water/(NH₄)₂SO₄/Amino Acid Systems

Figures 3.18 and 3.19 show the relative solubility of glycine, DL-alanine, L-serine, L-isoleucine and L-threonine, in aqueous solutions of (NH₄)₂SO₄ at various electrolyte concentrations (molality ranging from 0.0 to 2.0) at the temperatures 298.15 K and 323.15 K. The measured values for the solubilities are presented in Table 3.12.

As observed in the previous section with KCl, depending on the amino acid it is possible to observe a salting-in or/and salting-out effect with the increase of the electrolyte concentration. Once again, a qualitative approach, based on the functional groups present in the amino acids molecules, was carried out. The relative solubility of glycine, shown as a smooth line, increases with the increase of the electrolyte concentration, however this increase is more flat at higher electrolyte concentrations. At very low ammonium sulfate molality, all amino acids show a slightly salting-in effect. L-isoleucine, DL-alanine and L-threonine show an initial increase in solubility until it reaches a maximum (L-isoleucine – around 0.2 KCl molal;

DL-alanine - around 0.5 KCl molal and; L-threonine – around 1 KCl molal;) and then the solubility decreases: moderately for DL-alanine and L-threonine, but strongly for L-isoleucine. The salting-out effect is more evident for the amino acid with the largest hydrocarbon backbone (L-isoleucine). L-threonine has a characteristic behavior due to its chemical structure. For L-serine a salting-in effect is observed in all the electrolyte concentration range studied but this effect is more pronounced at 298.15 K. The increase of the temperature has less influence in the change of the relative solubility with the electrolyte concentration, except for L-serine, that at 323.15 K shows a minor salting-in, comparable to the one obtained for glycine. This changing with the temperature is similar to the one observed for KCl electrolyte aqueous systems.

Table 3.12 Solubilities of amino acids (g of amino acid/1000 g of water) in aqueous solutions of $(\text{NH}_4)_2\text{SO}_4$ at two temperatures, 298.15 and 323.15 K.

Temp. (K)	Electrolyte (molality)	S_{AA} (g of amino acid/1000 g of water)				
		Glycine	DL-alanine	L-serine	L-isoleucine	L-threonine
298.15	0.000	235.70	165.77	428.63	33.59	97.46
	0.167	246.52	170.50	441.33(0.100)*	34.15	102.16(0.100)*
	0.233	249.40	172.28	461.90	33.76	105.85
	0.333	254.26	173.57	463.52(0.300)*	33.15	106.54(0.300)*
	0.500	259.71	173.99	481.75	32.04	108.88
	0.700	264.95	173.71	497.45	30.28(0.667)*	110.50
	1.000	271.24	172.95	515.12	27.03	110.39
	1.500	274.77	167.56	537.50	22.51	108.33
	2.000	277.17	160.74	557.78	18.67	104.39
323.15	0.000	372.28	229.86	690.84	40.57	140.58
	0.167	383.85	237.63	704.33	41.52	148.78
	0.233	388.90	239.16	710.52	41.77	150.03
	0.333	390.75(0.334)*	240.10	717.64	41.03	153.09
	0.500	398.46	241.42(0.501)*	727.12	39.84	155.26
	0.700	402.78	242.90	736.34	37.99(0.667)*	157.97
	1.000	411.55	242.00	747.89	35.53	158.70
	1.500	418.31	236.38	759.67	30.13	157.21
	2.000	420.53	229.99	767.02	25.53	152.87

* Molality of the electrolyte.

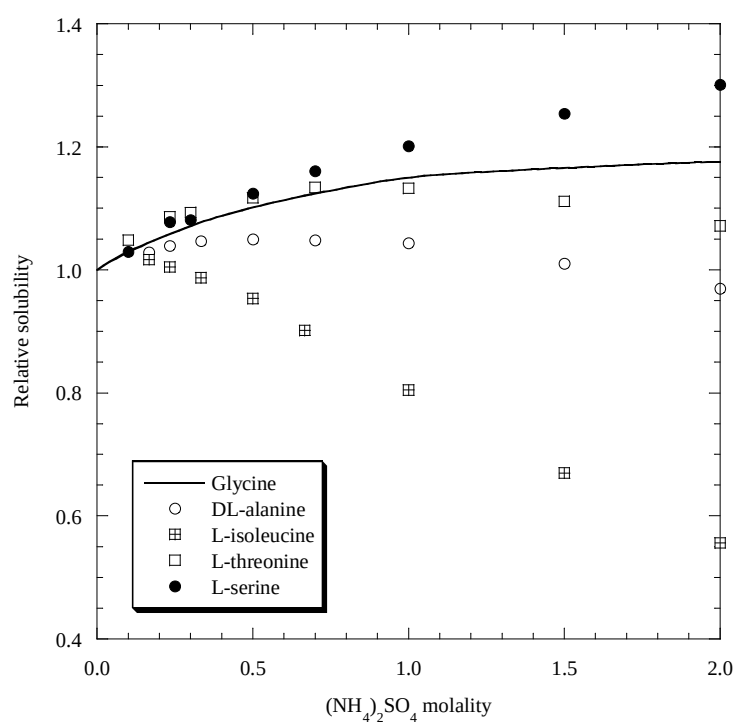


Figure 3.18 Relative solubilities of different amino acids in water/(NH₄)₂SO₄ solutions at 298.15 K.

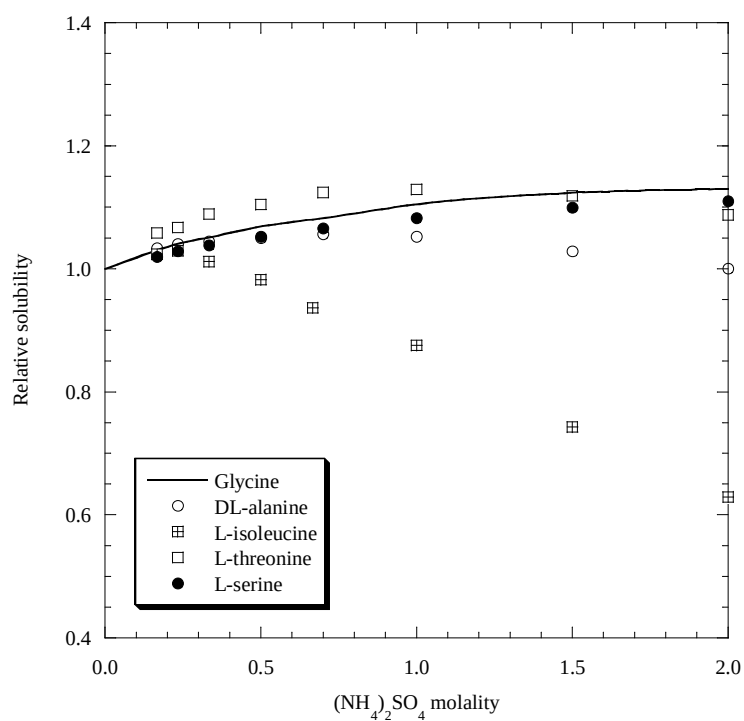


Figure 3.19 Relative solubilities of different amino acids in water/(NH₄)₂SO₄ solutions at 323.15 K.

3.3.3.3 Critical Analysis

For both electrolytes studied, a qualitative analysis, based on the functional groups present in the amino acids molecules, was performed and the consistency found confirmed the quality of the measured data. The study of the amino acids solubility change with the electrolyte concentration must take into account all the interactions present in these highly complex systems but, from a qualitative point of view, the indications that a polar –OH group present in the structure of the amino acid increases its tendency to dissolve, while an aliphatic group has the opposite effect, are consistent with all the experimental results shown for the two system studied, with either the KCl or the $(\text{NH}_4)_2\text{SO}_4$.

The influence of the size of the electrolytes in the solubility was also subject to a critical analysis. Figures 3.20 to 3.23 show the change of the relative solubility, for the electrolyte [KCl or $(\text{NH}_4)_2\text{SO}_4$] ionic strength, at 298.15 K and 323.15 K. As can be seen from Figures 3.20 and 3.21, glycine, DL-alanine and L-isoleucine show the same behavior with both salts at 298.15 K and 323.15 K but, at the same ionic strength, the relative solubility of the amino acids in aqueous ammonium sulfate solution are higher. Figures 3.22 and 3.23 compare the experimental data for aqueous KCl and $(\text{NH}_4)_2\text{SO}_4$ system with L-serine and L-threonine. The salting effect observed for each amino acid is the same with both salts at the two temperatures. In the case of L-threonine the size of the salt influences the maximum of solubility. For both electrolytes the solubility of L-threonine shows an initial salting-in until it reaches a maximum, and then the relative solubility starts decreasing the relative solubility. Depending on the electrolyte this maximum is reached at different ionic strength, around 1 molal for KCl and 2 molal for $(\text{NH}_4)_2\text{SO}_4$. The size of the electrolyte and the temperature have a major effect on the solubility of L-serine. At 298.15 K the relative solubility of the amino acid in aqueous KCl solutions is exceptionally superior to the one observed for the $(\text{NH}_4)_2\text{SO}_4$ at the same ionic strength.

All the amino acids, with the exception of L-serine at 298.15 K, present similar solubility trends with both electrolytes. At the same ionic strength, the solubility in the presence of $(\text{NH}_4)_2\text{SO}_4$ is higher than in the presence of KCl. This behavior is observed over the whole range of ionic strength studied and this difference is more significant with the increase of the ionic strength.

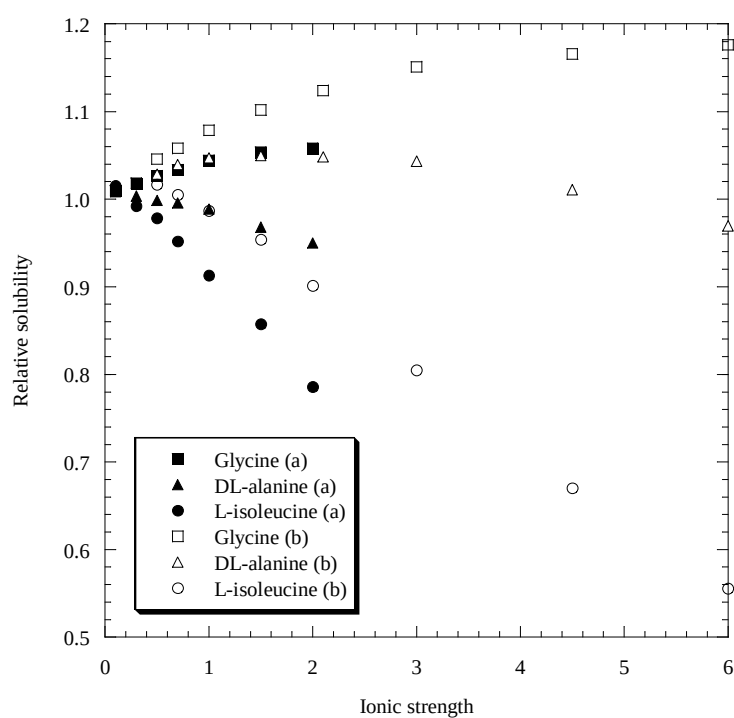


Figure 3.20 Relative solubilities of different amino acids in water/electrolyte solutions at 298.15 K: (a) KCl; (b) $(\text{NH}_4)_2\text{SO}_4$.

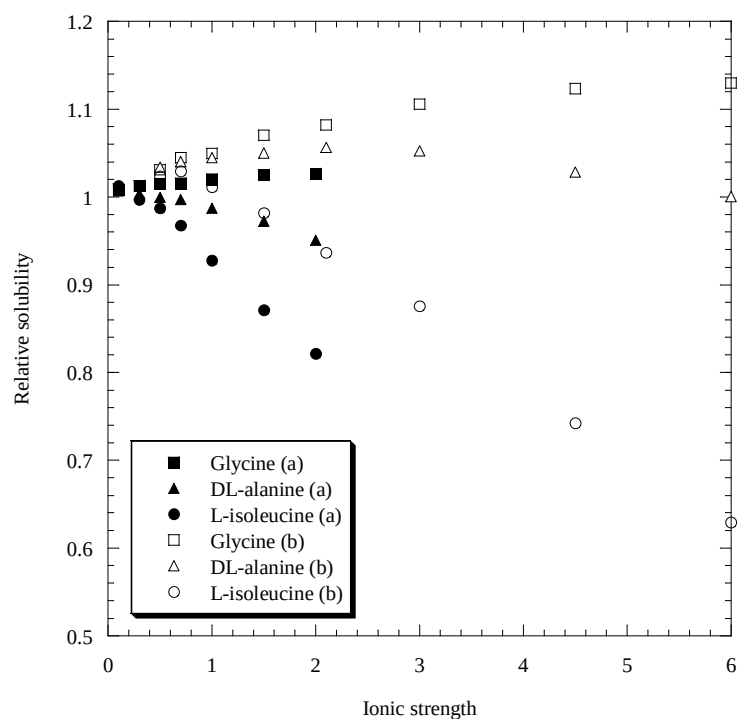


Figure 3.21 Relative solubilities of different amino acids in water/electrolyte solutions at 323.15 K: (a) KCl; (b) $(\text{NH}_4)_2\text{SO}_4$.

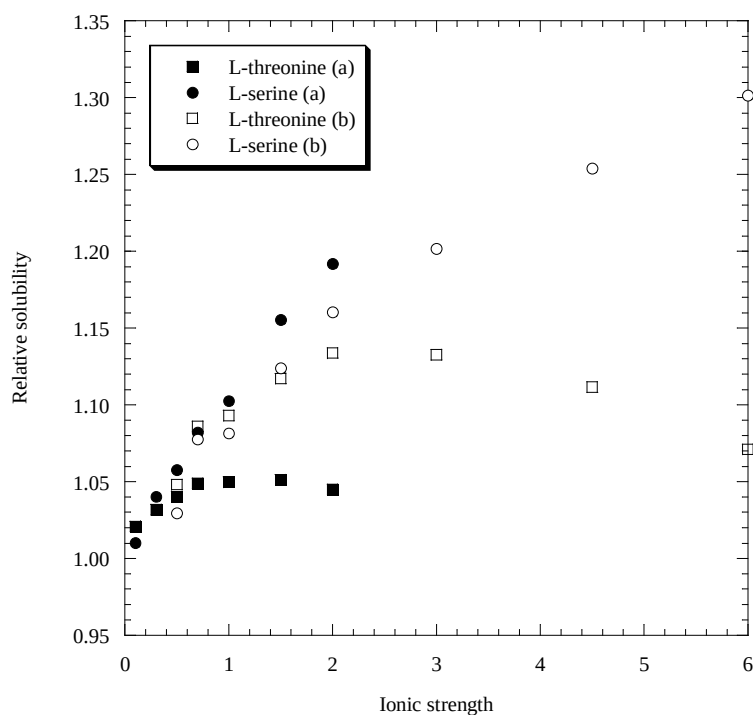


Figure 3.22 Relative solubilities of L-threonine and L-serine in water/electrolyte solution at 298.15 K: (a) KCl; (b) $(\text{NH}_4)_2\text{SO}_4$.

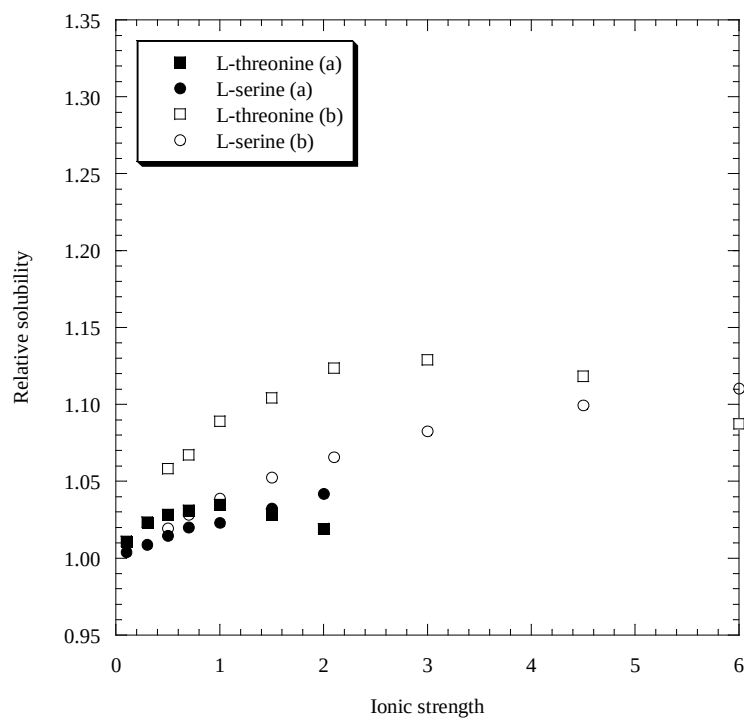


Figure 3.23 Relative solubilities of L-threonine and L-serine in water/electrolyte solution at 323.15 K: (a) KCl; (b) $(\text{NH}_4)_2\text{SO}_4$.

Figure 3.24 shows the solubility behavior of glycine and DL-alanine in aqueous solutions with the sulfate anion and two cations (sodium and ammonium) at 298.15 K. In this case, the influence of the cation, on the solubility of glycine is almost irrelevant, while for DL-alanine it is quite significant. For the same ionic strength the solubility of DL-alanine is higher in presence of the bigger cation and the difference increases with the increase of the ionic strength.

When possible, experimental results were compared with the values published in the literature. Figure 3.25 compares the data of this work for the aqueous KCl/glycine system with literature values (Khoshkbarchi and Vera, 1997) at 298.15 K. According to the data published by the other authors, at low electrolyte concentrations, the solubility of glycine decreases until it reaches a minimum, and after, the solubility increases significantly with the increase of the electrolyte concentration. Inversely, for the experimental data measured in this work, no minimum is observed nor a drastic effect on the solubility at high salt concentration.

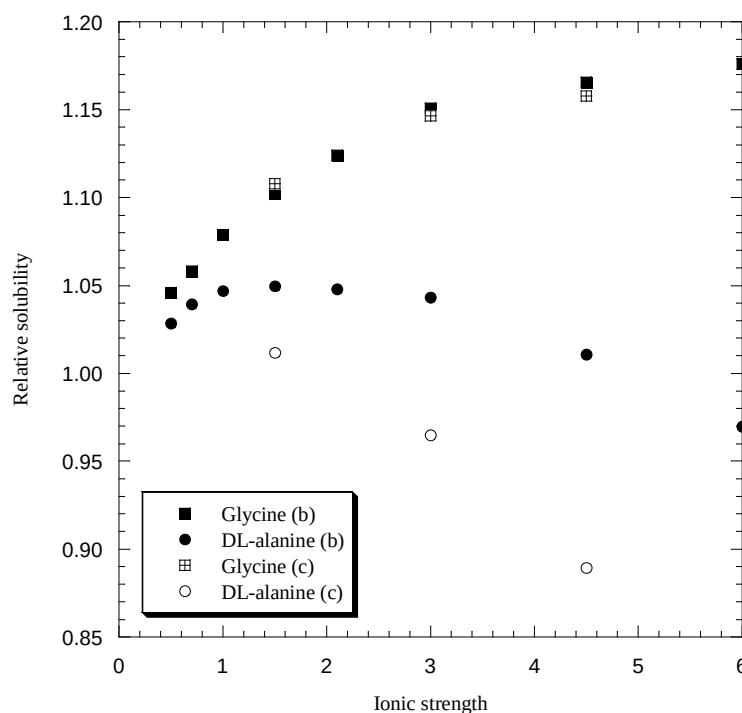


Figure 3.24 Relative solubilities of glycine and DL-alanine in water/electrolyte solution at 298.15 K:

(a) $(\text{NH}_4)_2\text{SO}_4$; (b) Na_2SO_4 .

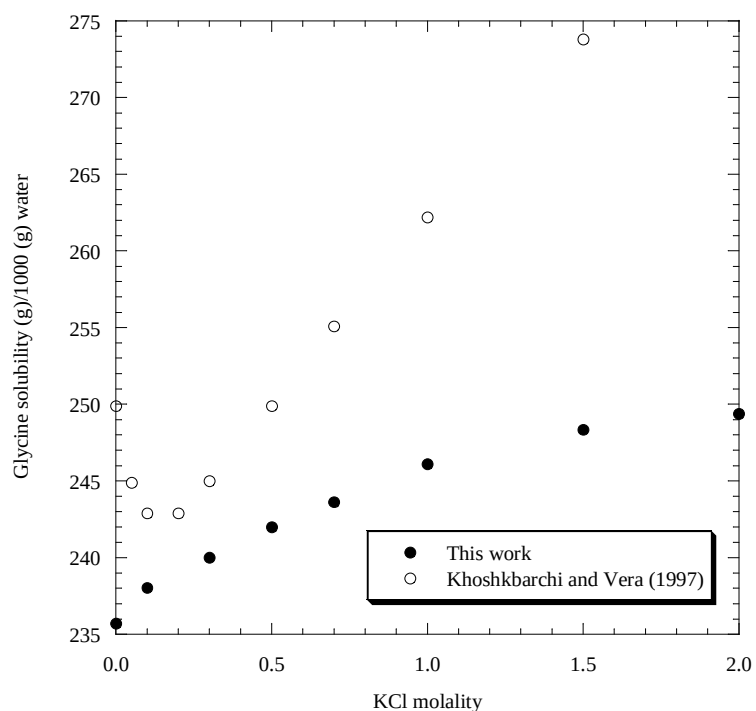


Figure 3.25 Comparison of solubility data of glycine in water/KCl solutions at 298.15 K.

The experimental data for the aqueous KCl/DL-alanine system at 298.15 K are compared in Figure 3.26. Looking to the literature data, the presence of potassium chloride results in a salting-in effect over the whole range of electrolyte concentration studied, whereas in this work, that situation only occurs at very low electrolyte concentrations and, from that range the solubility of the amino acid decreases, leading to a salting-out effect.

It should be mentioned that the experimental technique chosen, in both works, was the same, the analytical gravimetric method. Potassium chloride of 99% purity and glycine and DL-alanine of 99.0% purity obtained from A&C American Chemicals Ltd. (Montreal, Quebec, Canada) were used by Khoshkbarchi and Vera (1997). In this work, the purities are higher according to the manufacturers.

Like Khoshkbarchi and Vera (1997), we also tested the accuracy of the experimental technique and tried to find possible interfering parameters. A KCl solution (1 molal) was prepared and the cell charged in order to obtain a mixture of 170 g of DL-alanine per 1000 g of water (Test A). Examining Figure 3.26, this mixture has a global composition between the solubility values obtained in this work and those obtained by Khoshkbarchi and Vera (1997).

In fact, after carrying out all the procedure, it was possible to observe that some amino acid did not dissolve, confirming that the solubility value measured by Khoshkbarchi and Vera (1997) is too high indeed. Moreover, the final result was in very good agreement with your first measurement.

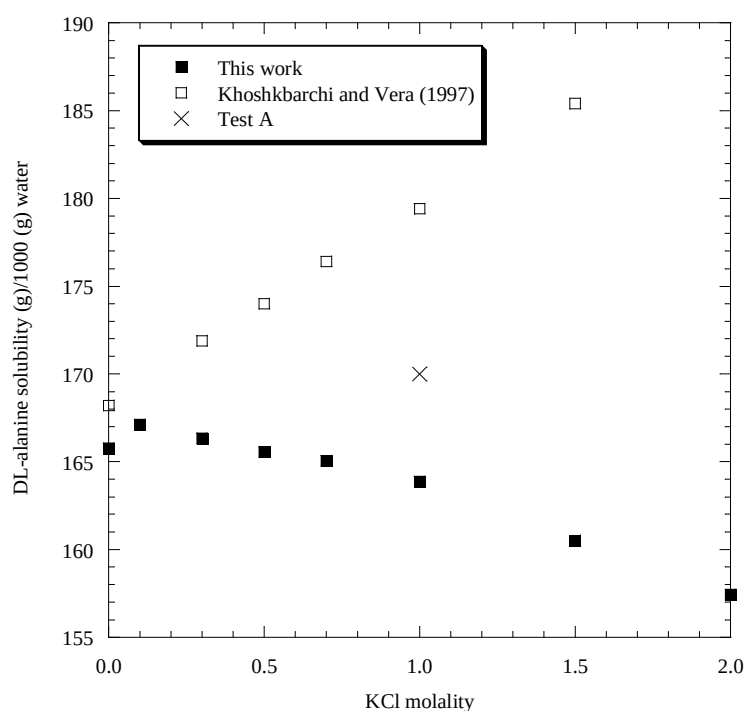


Figure 3.26 Comparison of solubility data of DL-alanine in water/KCl solutions at 298.15 K.

Possible interferences due to the purity and source of the amino acids were also checked out measuring their solubility in aqueous sodium sulfate solutions and comparing them with the values reported by Islam and Wadi (2001). The amino acids used by those authors were supplied by Sigma but there is no indication about the purity in the publication. The experimental results are presented in Table 3.13 and Figure 3.27. From that figure, it is possible to observe the very good agreement found; the trend for the change of the solubility of amino acid with the electrolyte concentration is the same, and the maximum deviation is 3% (g of amino acid)/(1000 g of water).

Table 3.13 Solubilities of glycine and DL-alanine (g of amino acid/1000 g of water) in aqueous solutions of Na_2SO_4 at 298.15 K.

Electrolyte (molality)	S_{AA} (g of amino acid/1000 g of water)	
	Glycine	DL-alanine
0.000	235.70	165.77
0.500	261.16	167.72
1.000	270.24	159.94
1.500	272.87	147.45

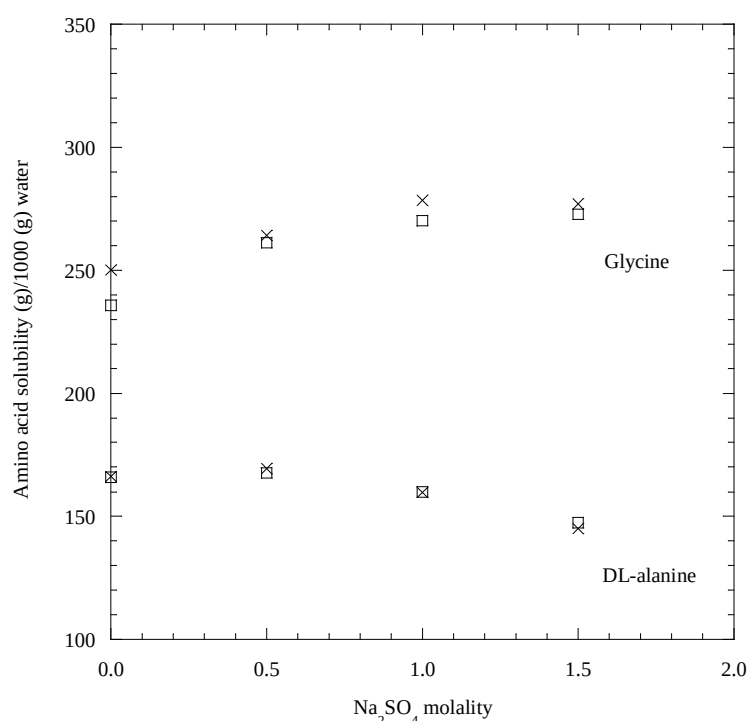


Figure 3.27 Comparison of solubility data of glycine or DL-alanine in water/ Na_2SO_4 solutions at 298.15 K:

×, Islam and Wadi (2001); □, this work.

Givand et al. (2001) determined the solubility of L-isoleucine at three different concentrations of ammonium sulfate ($m = 0.5, 1.08, 2.67$), in the temperature range 292.15 K – 331.55 K, without giving any indication about the purity and/or source of the amino acid and/or salt used in the solubility experiments. Figure 3.28 shows the change of the relative solubility with the temperature, that also includes the solubility of L-isoleucine in aqueous $(\text{NH}_4)_2\text{SO}_4$

solutions ($m = 0.5, 1.0, 2.0$). As reported by Givand et al. (2001), and shown in figure, the solubility of the amino acid is salted-out dramatically at the intermediate and highest $(\text{NH}_4)_2\text{SO}_4$ concentrations, and it is essentially unchanged at the lowest salt concentration. Comparing the two sources, a fair agreement is found for the lowest electrolyte molality, but with the increase of the ammonium sulfate concentration the deviations found are considerable. In fact, for the highest concentration ($m = 2.67$), the salting-out observed is lower than the one observed in this work at a lower electrolyte molality ($m = 2.0$). However, it is possible to verify, in both cases, a slight decrease of the salting-out effect increasing the temperature.

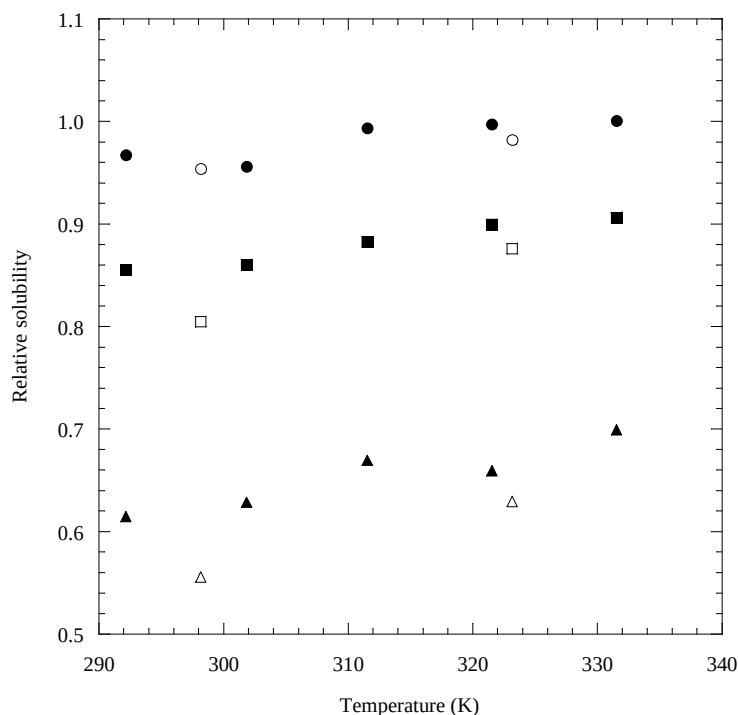


Figure 3.28 Relative solubilities of L-isoleucine in water/ $(\text{NH}_4)_2\text{SO}_4$ solutions versus temperature:

- Givand et al. (2001) ($m = 0.5$), ■ Givand et al. (2001) ($m = 1.08$), ▲ Givand et al. (2001) ($m = 2.67$),
- This work ($m = 0.5$), □ This work ($m = 1.0$), △ This work ($m = 2.0$).

3.4 CONCLUSIONS

The solubilities of the amino acids (glycine, DL-alanine, L-serine, L-isoleucine, L-threonine) have been measured in the systems water/alcohol (ethanol, 1-propanol or 2-propanol) and water/electrolyte [KCl or $(\text{NH}_4)_2\text{SO}_4$] in the temperature range 298.15 K - 333.15 K.

Concerning the measurement of the amino acids solubilities in electrolytes solutions the analytical gravimetric method was used. Regarding the mixed solvent solutions, the solid content was measured differently depending on the alcohol mass fraction in amino acid free basis in the mixed solvent (w'_{alcohol}): when $w'_{\text{alcohol}} < 0.8$, the gravimetric method was applied; at higher alcohol concentrations ($w'_{\text{alcohol}} \geq 0.8$), the spectrophotometric ninhydrin method was used. Both experimental techniques proved to be very accurate. The spectrophotometric ninhydrin method also showed to be particularly suitable for the analysis of low amino acid concentrations.

The experimental results were compared with literature data, when possible. Generally, the experimental results are in good agreement with the published data. However, for a few systems, some discrepancies were found and discussed.

The new experimental data and all the experimental information concerning different thermodynamic properties, compiled from the open literature, allowed the establishment of a more extensive and reliable database, that is very important and crucial for the improvement and development of the thermodynamic models, which will be presented in the following two chapters.

CHAPTER 4.

MODELLING AMINO ACID SOLUBILITY IN ELECTROLYTE SOLUTIONS

4.1 INTRODUCTION

As pointed out before, both solubility and activity coefficients are physical properties essential to the design and scale-up of chemical processes for separation, concentration, and purification of priceless biochemicals. Many factors, such as chemical structure, pH, and type and concentration of the electrolyte present, can affect their behavior in solution. For aqueous electrolyte solutions containing amino acids, the experimental work carried out so far has been most focused on the study of the effect of the electrolyte on the solubility of different amino acids and peptides, as well as on the measurement of the electrolyte activity coefficients in the presence of an amino acid or a peptide like described in section 2.5.2.3, which are both of high importance for the improvement of thermodynamic models capable of representing the solubility of amino acids in aqueous electrolyte solutions. Over the years, models, or their modifications, have been developed in an attempt to overcome the drawbacks found in the representation of this type of phase equilibria. Some important studies regarding activity coefficients of amino acids in aqueous systems without the presence of electrolytes have been conducted and reviewed with detail in section 2.5.3.1. However, concerning amino acid aqueous electrolyte solutions, the lack of reliable models for the calculation of activity coefficients has been, probably, the main barrier for the development of solubility approaches (section 2.5.3.3).

In Chapter 3, new experimental data for the solubility of glycine and DL-alanine in aqueous KCl solutions (molality ranging from 0.0 to 2.0) at 298.15 K are presented, which clearly contradict the data published by Khoshkbarchi and Vera (1997) (section 3.3.3.3). The quality

of the experimental data obtained was checked testing the experimental technique and measuring the solubility of the same amino acids in Na_2SO_4 aqueous solutions at 298.15 K in order to compare with the data published by Islam and Wadi (2001). In the present chapter the Pitzer-Simonson-Clegg (PSC) equations (Hu and Guo, 1999) are used to correlate the activity coefficients of KCl in aqueous solutions with glycine or DL-alanine, and to predict the amino acid solubility in those solutions. This model was chosen since Hu and Guo (1999) used it to calculate the solubility of NaCl in four electrolyte-non-electrolyte-water ternary systems at 298.15 K, with parameters estimated from activity coefficient data only, with high accuracy.

After, both types of data, the solubility measured in this work and the activity coefficients collected from literature are correlated simultaneously in order to study the ability of the PSC equations in the thermodynamic description of the ternary systems water-KCl with glycine, DL-alanine or L-serine at two different temperatures (298.15 and 323.15 K). The ability of the model to predict solubility data for DL-alanine at one temperature outside the range used in the correlation is also investigated. Finally, water activity in aqueous solutions of glycine, DL-alanine, or L-serine, with potassium chloride, at 298.15 K, is predicted.

Modelling was not carried out for the $(\text{NH}_4)_2\text{SO}_4$ containing systems as the only available thermodynamic properties for sulphate systems consists on the data measured in this work, which are presented on the previous chapter. Therefore the number of experimental data points is not enough to perform a parameter estimation which can be considered reliable.

4.2 THEORETICAL FUNDAMENTALS

The solubility of an amino acid, m_n , in an aqueous electrolyte solution, at a given temperature, can be expressed in terms of its saturation molality in the absence of an electrolyte, m_n^o , the ratio of its unsymmetrically normalized molal activity coefficients in the absence and in the presence of an electrolyte, $\gamma_n^{*o} / \gamma_n^*$, and the ratio of its solid state fugacities in the presence and in the absence of an electrolyte $\hat{f}_n^{So} / \hat{f}_n^S$ by (Khoshkbarchi and Vera, 1997; Soto et al., 1998a):

$$m_n = \frac{m_n^o \gamma_n^{*o} \hat{f}_n^{So}}{\gamma_n^* \hat{f}_n^S} \quad (4.1)$$

If both solid phases are pure and have the same crystalline, the $\hat{f}_n^{So} / \hat{f}_n^S$ becomes equal to unity, and equation 4.1 can be written as:

$$m_n = \frac{m_n^o \gamma_n^{*o}}{\gamma_n^*} \quad (4.2)$$

Therefore, choosing an appropriate method to calculate the activity coefficients, the solubility of an amino acid in aqueous electrolyte solutions can be obtained. In this work, the PSC equations are going to be implemented. This approach follows a mole fraction based model giving the rational activity coefficient of species i (f_i). So, by conventional thermodynamics (Prausnitz et al., 1999):

$$\ln f_i = \left(\frac{\partial g^E}{\partial n_i} \right)_{T,P,n_{j \neq i}} \quad (4.3)$$

where g^E is the excess Gibbs energy, T is the temperature, P is the pressure, and n the number of moles of the system.

The rational activity coefficient on the infinitely diluted reference state (f_i^*) is defined as $f_i^* = f_i / f_i^\infty$, where f_i^∞ is the rational activity coefficient of species i when infinitely diluted. The f_i^* calculated by the thermodynamic model is related to the corresponding activity coefficient on the molal scale (γ_i^*) by (Hu and Guo, 1999):

$$f_i^* = \gamma_i^* \left(1 + M_1 \sum_i^{N_{solu}} m_i \right) \quad (4.4)$$

where M_1 is the molar mass of water and N_{solu} is the number of solutes.

The PSC model gives the rational activity coefficient of each ion. Thus, in order to obtain the activity coefficient of a salt, mean ionic properties are needed. The mean ionic rational activity coefficient ($f_{\pm}^*$) of a salt is defined as:

$$f_{\pm}^* = \left[(f_M^*)^{\nu_M} (f_X^*)^{\nu_X} \right]^{1/\nu} \quad (4.5)$$

where ν_M and ν_X are the stoichiometric coefficients of the cation and anion, respectively, and ν is their sum. Naturally, all the different conversions between activity coefficients in different concentration scales or conventions of normalization are now easy to perform.

The potentialities of the PSC equations for the thermodynamic description of the ternary systems water-KCl with amino acids are going to be explored. This model was chosen since Hu and Guo (1999) used it to calculate the solubility of NaCl in four electrolyte-non-electrolyte-water ternary systems at 298.15 K, with parameters estimated from activity coefficient data only, with high accuracy.

In the mole-fraction-based model, the excess Gibbs energy of PSC model is assumed to be the sum of short-range forces (g^S) and long-range forces, expressed using a Debye-Hückel term (g^{DH}) (Hu and Guo, 1999):

$$g^E = g^S + g^{DH} \quad (4.6)$$

For the present ternary systems (water/electrolyte/amino acid) the short-range term is:

$$\begin{aligned} \frac{g^S}{RT} = & x_I (x_1 W_{1,MX} + x_n W_{n,MX}) + x_I^2 (x_1 U_{1,MX} + x_n U_{n,MX}) \\ & + x_I^2 (x_1^2 V_{1,MX} + x_n^2 V_{n,MX}) + x_1 x_n \left[w_{1,n} + u_{1,n} (x_1 - x_n) + x_I \left(Y_{1,n,MX}^{(0)} + Y_{1,n,MX}^{(1)} \frac{x_I^2}{4} \right) \right] \end{aligned} \quad (4.7)$$

and the long-range Debye-Hückel term is:

$$\frac{g^{DH}}{RT} = - \frac{4 A_x I_x}{\rho \ln(1 + \rho I_x^{1/2})} + \frac{x_I^2}{4 B_{MX} g(\alpha I_x^{1/2})} \quad (4.8)$$

In equations 4.7 and 4.8 R is the ideal gas constant, B_{MX} , $W_{1,MX}$, $U_{1,MX}$ and $V_{1,MX}$ are the model parameters to describe the interactions between water and the electrolyte, $w_{1,n}$ and $u_{1,n}$ the coefficients for the description of water-amino acid interactions, $W_{n,MX}$, $U_{n,MX}$, $V_{n,MX}$, $Y_{1,n,MX}^{(0)}$ and $Y_{1,n,MX}^{(1)}$ the model parameters which represent the interactions arising in mixtures including both non-ionic and ionic solutes, and α is a standard value equal to 13.0 (Clegg and Pitzer, 1992).

This approach uses species mole fractions (x_i), and the mole fractions of a 1:1 electrolyte MX and a non-electrolyte (n) in water (1) are related to the stoichiometric molalities as (Hu and Guo, 1999):

$$x_n = \frac{m_n M_1}{1 + M_1(2m_{MX} + m_n)} \quad (4.9)$$

$$x_M = x_X = \frac{m_{MX} M_1}{1 + M_1(2m_{MX} + m_n)} \quad (4.10)$$

The mole fraction of water, x_1 , is given by $x_1 = 1 - x_M - x_X - x_n$, while the total mole fraction of ions, x_I , is obtained by $x_I = 1 - x_1 - x_n = 2x_M = 2x_X$.

The mole fraction ionic strength (I_x) is defined as:

$$I_x = \frac{1}{2} \sum_i x_i z_i^2 \quad (4.11)$$

where z_i is the charge number for ion i .

The function $g(x)$, with $x = \alpha I_x^{1/2}$, is given by (Hu and Guo, 1999):

$$g(x) = 2 \left[\frac{1}{x^2} - \left(\frac{1}{x^2} + \frac{1}{x} \right) \exp(-x) \right] \quad (4.12)$$

and A_x is the Debye-Hückel parameter on a mole fraction basis (Hu and Guo, 1999):

$$A_x = \left(\frac{1}{3} \right) \left(\frac{2\pi N_a d}{M} \right)^{1/2} \left(\frac{e^2}{4\pi \epsilon_0 D k T} \right)^{3/2} \quad (4.13)$$

where N_a is the Avogadro's number, e is the electronic charge, ϵ_0 is the vacuum permittivity, k is Boltzmann constant, and d , M , and D are the density, molar mass, and relative permittivity of the solvent, respectively.

The “closest approach” distance for the ions (ρ) is defined as (Pitzer and Li, 1983):

$$\rho = 2150 \left(\frac{d_1}{D_1 T} \right)^{1/2} \quad (4.14)$$

where D_1 and d_1 denote the relative permittivity and density of water respectively.

From this model, and applying conventional thermodynamics, the expressions for the rational activity coefficient (f_1) of water and the mean ionic activity coefficient ($f_{\pm}^*$) of a 1:1 electrolyte-in-aqueous-nonelectrolyte solutions are given by:

$$\begin{aligned} \ln f_1 = & \frac{2A_x I_x^{3/2}}{1 + \rho I_x^{1/2}} - I_x^2 B_{MX} \exp(-\alpha I_x^{1/2}) + x_I [(1 - x_1)W_{1,MX} - x_n W_{n,MX}] \\ & + x_I^2 [(1 - 2x_1)U_{1,MX} - 2x_n U_{n,MX}] + x_I^2 [x_1(2 - 3x_1)V_{1,MX} - 3x_n^2 V_{n,MX}] \\ & + \left[2x_n I_x (1 - 2x_1)Y_{1,n,MX}^0 + \left(\frac{x_I^3}{4} \right) x_n (1 - 4x_1)Y_{1,n,MX}^1 \right] \\ & + x_n [(1 - x_1)w_{1,n} + (2(x_1 - x_n)(1 - x_1) + x_n)u_{1,n}] \end{aligned} \quad (4.15)$$

and

$$\begin{aligned} \ln f_{\pm}^* = & -A_x \left[\left(\frac{2}{\rho} \right) \ln(1 + \rho I_x^{1/2}) + \frac{I_x^{1/2}(1 - 2I_x)}{1 + \rho I_x^{1/2}} \right] \\ & + \left(\frac{I_x}{2} \right) B_{MX} [g(\alpha I_x^{1/2}) + \exp(-\alpha I_x^{1/2})(1 - x_I)] \\ & + (1 - x_I)[x_1 W_{1,MX} + x_n W_{n,MX}] + 2x_I(1 - x_I)[x_1 U_{1,MX} + x_n U_{n,MX}] \\ & + x_I(2 - 3x_I)[x_1^2 V_{1,MX} + x_n^2 V_{n,MX}] + x_1 x_n [(1 - 4I_x)Y_{1,n,MX}^0 + (3I_x^2 - x_I^3)Y_{1,n,MX}^1] \\ & - x_1 x_n [w_{1,n} + 2(x_1 - x_n)u_{1,n}] - W_{1,MX} \end{aligned} \quad (4.16)$$

The corresponding expression for the rational activity coefficient (f_n) of the amino acid is given by equation 4.17:

$$\begin{aligned} \ln f_n = & \frac{2A_x I_x^{3/2}}{1 + \rho I_x^{1/2}} - I_x^2 B_{MX} \exp(-\alpha I_x^{1/2}) + [-x_1 W_{1,MX} + (1-x_n) W_{n,MX}] x_I \\ & + x_I^2 [-2x_1 U_{1,MX} + (1-2x_n) U_{n,MX}] + x_I^2 [-3x_1^2 V_{1,MX} + x_n(2-3x_n) V_{n,MX}] \\ & + x_1 [(1-x_n) w_{1,n} + u_{1,n} [2(1-x_n)(x_1-x_n) - x_1]] + 2x_1 I_x (1-2x_n) Y_{1,n,MX}^0 \\ & + x_1 x_I^3 \left(\frac{1}{4} - x_n \right) Y_{1,n,MX}^1 \end{aligned} \quad (4.17)$$

4.3 SOLUBILITY PREDICTION

As mentioned before, two different approaches were considered, the first one consists in the application of the PSC equations to predict the solubility of the amino acids glycine and DL-alanine in aqueous KCl solutions, at 298.15 K, using activity coefficient data only. The purpose was to study the consistency of the experimental data since large discrepancies with the data available in literature were found.

For each type of experimental data the model parameters were estimated minimizing the following objective function (*FOBJ*):

$$FOBJ = \sum_k (Q_k^{\text{exp}} - Q_k^{\text{calc}})^2 \quad (4.18)$$

where Q_k^{exp} and Q_k^{calc} are the experimental and the calculated quantities, respectively.

4.3.1 PARAMETER ESTIMATION

The parameters used for water/KCl interactions at 298.15 K were already known in the work by Hu and Guo (1999) and are listed in Table 4.1.

Table 4.1 Parameters for water/KCl interactions at 298.15 K (Hu and Guo, 1999).

Solute	B_{MX}	$W_{1,MX}$	$U_{1,MX}$	$V_{1,MX}$
KCl ^a	4.4264	-2.8348	-1.4979	-0.3643

^a ρ is set equal to 14.0292; $A_x = 2.9094$.

For the binary systems glycine-H₂O and DL-alanine-H₂O, the model parameters $w_{1,n}$ and $u_{1,n}$ were determined by fitting equation 4.19 to published osmotic coefficient or to water activity data at 298.15 K.

$$\ln f_1 = x_n \left[(1 - x_1) w_{1,n} + (2(x_1 - x_n)(1 - x_1) + x_n) u_{1,n} \right] \quad (4.19)$$

The five parameters left to be estimated, which describe the interactions between electrolyte and amino acid, were calculated, fitting the parameters to experimental data on the ratio of the mean ionic molal activity coefficients of electrolytes in the presence of the amino acid to those in the absence of the amino acid at different electrolyte and amino acid molalities at 298.15 K. Table 4.2 lists the parameters estimated together with the root mean square deviation (RMSD) for each type of data, the number of data points (n), and the source of data. In this table, ϕ and a_w mean the osmotic coefficient and water activity in water/amino acid systems, respectively, and γ^r represents the ratio of the mean ionic activity coefficients of electrolytes in the presence of the amino acid to those in the absence of the amino acid.

Table 4.2 Model parameters and RMSDs for KCl aqueous solutions with glycine and DL-alanine (without solubility).

	$w_{1,n}$	$u_{1,n}$	$W_{n,MX}$	$U_{n,MX}$	$V_{n,MX}$	$Y_{1,n,MX}^{(0)}$	$Y_{1,n,MX}^{(1)}$
Glycine	-37.924	14.192	-5.8921	98.139	-625.03	-13.195	-4507.8
RMSD		$a_w = 0.0018$ ($n = 20$) (d, e)		$\phi = 0.0061$ ($n = 61$) (b, h)		$\gamma^r = 0.0030$ ($n = 157$) (c, a, f)	
DL-alanine	-17.826	5.836	47.307	69.450	-1296.2	-59.856	-2809.7
RMSD		$a_w = 0.0017$ ($n = 7$) (e)		$\phi = 0.0023$ ($n = 56$) (i, g)		$\gamma^r = 0.0028$ ($n = 45$) (j)	

(a) Bower and Robinson (1965); (b) Ellerton et al. (1964); (c) Kamali-Ardakani et al. (2001); (d) Kuramochi et al. (1997); (e) Ninni and Meirelles (2001); (f) Roberts and Kirkwood (1941); (g) Robinson (1952); (h) Smith and Smith (1937a); (i) Smith and Smith (1937b); (j) Soto et al. (1998b).

4.3.2 RESULTS AND DISCUSSION

With the set of estimated parameters, the solubility of the amino acid was calculated according to equation 4.2. The predictions for the glycine and DL-alanine solubility in aqueous systems with KCl at 298.15 K are shown in Figures 4.1 and 4.2, respectively. Two prediction curves are displayed in each figure, since the calculations are dependent on the amino acid solubility in pure water, which presents different values in this work and in the work by Khoshkbarchi and Vera (1997). As can be observed, for both systems, the model predictions are in better agreement with the solubility data measured in this work. For these data, the RMSD found were 4.4 and 3.3 g of amino acid per kg of water for the systems containing glycine and DL-alanine, respectively. Naturally, these predictions are useful from the qualitative point of view; the data published by Khoshkbarchi and Vera (1997) show higher discrepancies to the predicted values, especially for the system with DL-alanine (RMSD of 16.2 g of DL-alanine per kg of water), and the solubility trend is inversely predicted. Quantitatively, the predictions are of little use, which is in disagreement to the results found by Hu and Guo (1999) for the solubility of NaCl in sucrose or urea aqueous systems. The main reason for that is surely the fact that, here, electrolyte activity coefficient data is used only in order to predict the amino acid solubility, while Hu and Guo (1999) used the same kind of data to calculate electrolyte solubility. Thus, to extrapolate the calculation of the amino acid activity coefficients to the limits of the solubility concentration, using electrolyte activity coefficient data only, is much more difficult.

4.4 THERMODYNAMIC MODELLING

To study the ability of the model to represent the KCl activity coefficient ratio and the solubility of the amino acids, a simultaneous correlation was performed with both types of data. The capability to describe solubility changes with temperature was also considered. To do so, it was necessary to study the introduction of a temperature dependency on some of the model parameters trying to find the right balance between accuracy and simplicity. In the following, details about the calculation of the temperature dependency of some model parameters, as well as its estimation are given. For each type of experimental data the parameters were estimated minimizing the objective function expressed by equation 4.18.

4.4.1 PARAMETER ESTIMATION

To take into account the temperature influence on the Debye-Hückel parameter (A_x) and the “closest approach” distance for the ions (ρ), density (Perry and Green, 1984) and dielectric constant (Archer and Wang, 1990) data of pure water were found in the literature.

The model parameters for water/KCl pair; B_{MX} , $W_{1,MX}$, $U_{1,MX}$ and $V_{1,MX}$, were estimated based on osmotic coefficients and mean ionic activity coefficients for aqueous KCl solutions in the temperature range 298.15-333.15 K and molalities up to 4.8, calculated using the fundamental equations given in the extensive review by Archer (1999). The temperature dependency of each of those parameters is usually described in the literature by a five constant empirical equation (Farelo et al., 2002; Lopes et al., 2001). In order to reduce the number of fitted constants several sensitivity tests were made when correlating 290 osmotic and activity coefficient data points and, in the short temperature range studied in this work, the conclusion was that a three constant empirical equation allows a very good representation of the thermodynamic data, without loss of accuracy, when comparing to more complex forms. Therefore, the final expression used was:

$$P(T) = q_1 + q_2(T - T_r) + q_3 \ln(T/T_r) \quad (4.20)$$

where P is the model parameter and T_r is a reference temperature taken as 298.15 K. The fitted coefficients (q_1 , q_2 and q_3) are listed in Table 4.3, being the RMSD equal to 0.00028 for the activity coefficient data and 0.00038 for osmotic coefficients.

Table 4.3 Coefficients q_1 , q_2 and q_3 for water/KCl interaction parameters.

	B_{MX}	$W_{1,MX}$	$U_{1,MX}$	$V_{1,MX}$
q_1	1.5016	-4.6269	-6.0232	2.6628
q_2	-0.25055	-0.35551	-0.85655	0.42504
q_3	96.270	123.03	297.60	-147.71

Concerning the binary amino acid/water systems, experimental data on water activity and osmotic coefficients are available at 298.15 K but, at higher temperatures, that is uncommon; only a few data points could be found in the work published recently by Romero and González (2006). Therefore, no temperature dependency was considered either in $w_{1,n}$ or $u_{1,n}$ parameters. For systems containing DL-alanine or glycine amino acids the parameters used were the ones given in Table 4.2, while for L-serine they were also estimated using experimental osmotic coefficient (Hutchens, 1963) and water activity data (Kuramochi et al., 1997) and their values are presented in Table 4.4 together with the corresponding RMSDs values. Unfortunately, the lack of experimental data for systems containing L-isoleucine or L-threonine inhibited the application of the theoretical approach presented here.

The five parameters left to be estimated; $W_{n,MX}$, $U_{n,MX}$, $V_{n,MX}$, $Y_{1,n,MX}^{(0)}$ and $Y_{1,n,MX}^{(1)}$, that describe the interactions between electrolyte and amino acid, were obtained by fitting the parameters to experimental data on the ratio of the mean ionic activity coefficients of electrolytes in the presence of the amino acid to those in the absence of the amino acids, at different electrolyte and amino acid molalities at 298.15 K, simultaneously with the solubility values measured in this work at two different temperatures.

Considering the reduced number of experimental data points available at temperatures different than 298.15 K one concludes the need to implement an approach, other than equation 4.20, to consider the temperature influence on those parameters. Using the experimental data available at 298.15 K, the five parameters were firstly estimated simultaneously. Afterwards, a systematic procedure was carried out in order to decide the best combination and functional dependency on the five parameters: initially, an attempt has been made by introducing the temperature term directly on each of the parameters (i.e., for instance, $W_{n,MX} T/T_r$ instead of $W_{n,MX}$), avoiding the need of other constants. From all possible combinations, very poor results were found for the correlation of the ternary data available at two different temperatures. Alternatively, a linear temperature dependency was introduced and after a detailed study over many different possibilities, the best results were found when applying it to $W_{n,MX}$ and $Y_{1,n,MX}^{(0)}$ parameters.

Due to the limited number of experimental data, a mix of those two methodologies was also studied reducing the number of fitted constants. In this way, to one of the parameters a linear temperature dependency was applied while to the other, only the term T/T_r was included. Detailed trial studies indicated that the best results could be found with the $W_{n,MX}$, and one of the $U_{n,MX}$, $V_{n,MX}$ or $Y_{1,n,MX}^{(0)}$ parameters.

Seeking for uniformity, the same temperature dependency was introduced in the three systems studied in this work. A criterion based on the minimum value for the objective function summation over all the systems was selected, and can be written as:

$$W_{n,MX} = q_1 + q_2(T - T_r) \quad (4.21a)$$

$$U_{n,MX} = q_1 T/T_r \quad (4.21b)$$

i.e., a linear temperature dependency on the parameter $W_{n,MX}$ and the term T/T_r in the $U_{n,MX}$ parameter. It should be stressed that with this approach, with one less constant to be fitted, similar accuracy for the simultaneous correlation of the activity coefficient, or solubility data at two different temperatures has been found, when compared to that by applying linear temperature dependency on both $W_{n,MX}$ and $Y_{1,n,MX}^{(0)}$ parameters. In this way, only the results concerning the approach expressed by equations 4.21 will be presented. Table 4.4 lists the parameters estimated together with the RMSD for each type of data and the respective number of data points.

Table 4.4 Model parameters and RMSDs for aqueous KCl solutions with amino acids.

		$W_{n,MX}$	$U_{n,MX}$	$V_{n,MX}$	$Y_{1,n,MX}^{(0)}$	$Y_{1,n,MX}^{(1)}$
Glycine	q_1	-23.996	85.953	-247.32	3.8511	-3034.9
	q_2	-8.9388E-04	-	-	-	-
	RMSD	$\gamma^r : 0.0037 \text{ (} n = 157 \text{)}^a$		Solubility (*): 0.82 g per kg of water		
DL-alanine	q_1	3.3050	99.848	-736.26	-16.632	-2858.5
	q_2	3.0058E-02	-	-	-	-
	RMSD	$\gamma^r : 0.0036 \text{ (} n = 45 \text{)}^a$		Solubility (*): 0.66 g per kg of water		
L-serine	q_1	-47.020	78.130	-202.93	29.124	-1300.8
	q_2	0.12057	-	-	-	-
	RMSD	$\gamma^r : 0.0028 \text{ (} n = 24 \text{)} \text{ (b)}$		Solubility (*): 1.08 g per kg of water		
		$w_{1,n}$			$u_{1,n}$	
		-37.109			14.421	
	RMSD	$a_w = 0.0020 \text{ (} n = 12 \text{)} \text{ (c)}$		$\phi = 0.0064 \text{ (} n = 9 \text{)} \text{ (a)}$		

^a Data sources given in Table 4.2.

(*) This work; (a) Hutchens (1963); (b) Khoshkbarchi et al. (1997); (c) Kuramochi et al. (1997).

4.4.2 RESULTS AND DISCUSSION

Concerning the solubilities of glycine and DL-alanine, the RMSDs are now much lower than the values presented before (section 4.3.2), while for the ratio of the mean ionic activity coefficients of KCl in the presence of the amino acid to those in the absence of the amino acid, the RMSDs are very similar (Table 4.2). The better quality for the correlation of the solubilities can also be seen from Figures 4.1 and 4.2, and a comparison of the correlation results, with or without solubility data, for the ratio of the mean ionic activity coefficients of KCl in the presence of glycine to those in the absence of glycine at three different electrolyte molalities is presented in Figure 4.3. As pointed out from the RMSD values, the performance is, in both cases, very good.

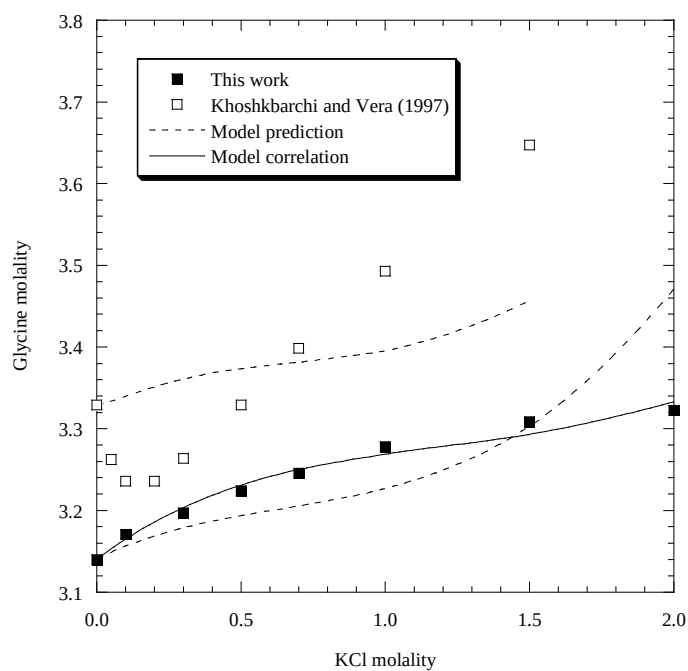


Figure 4.1 Experimental and calculated solubilities of glycine in water/KCl solutions at 298.15 K.

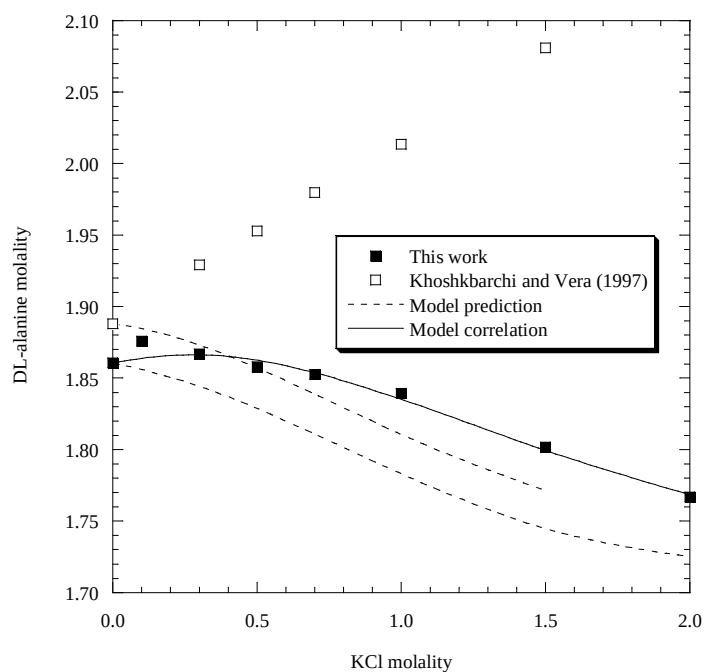


Figure 4.2 Experimental and calculated solubilities of DL-alanine in water/KCl solutions at 298.15 K.

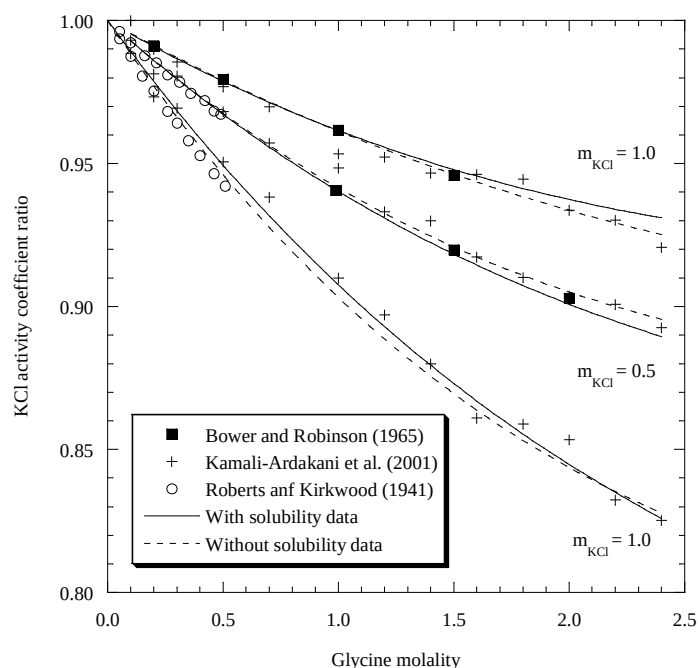


Figure 4.3 Ratio of the mean ionic activity coefficients of KCl in the presence to those in the absence of glycine at 298.15 K: comparison of the model performance with and without solubility data.

For L-serine, the RMSD obtained for the ratio of the mean ionic activity coefficients of KCl in the presence to those in the absence of amino acid is lower, which is certainly related to the fact that the amino acid molality range is narrower than in the previous cases. The good quality of the correlation obtained for the ratio of the mean ionic activity coefficients of KCl in the presence to that in the absence of L-serine at three different electrolyte molalities can be observed in Figure 4.4.

As stated before, one of the objectives was to study the capabilities of the PSC equations to describe amino acid solubility at different temperatures. In this work, the RMSDs found in the calculation of the solubilities of glycine and DL-alanine were 0.82 and 0.66 (g of amino acid/kg of water), respectively. These values represent, for each system, averages of the solubility data used at two different temperatures. Concerning the system containing L-serine the RMSD is slightly larger, 1.08 g of L-serine per kg of water but, as can be seen in Figure 4.5, the quality of the correlation for the solubility of L-serine in water/KCl solutions at 298.15 K is very good. In fact, for that particular system, the change on amino acid solubility with electrolyte molality is much more evident than for the other systems and so, the higher RMSD is only apparent. Figure 4.5 also presents the correlation of glycine solubility in

water/KCl solutions at 323.15 K, and even if the quality of the representation is not so good the results can be considered very satisfactory.

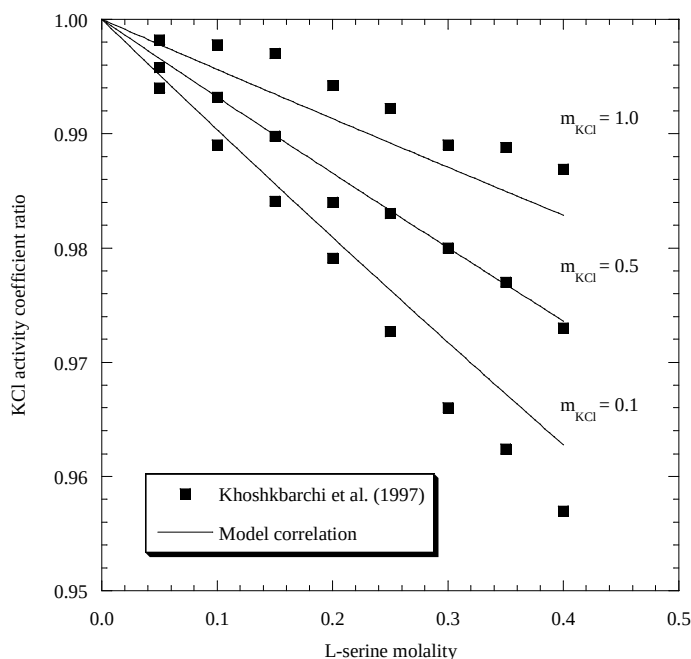


Figure 4.4 Experimental and calculated ratio of the mean ionic activity coefficients of KCl in the presence to those in the absence of L-serine at 298.15 K.

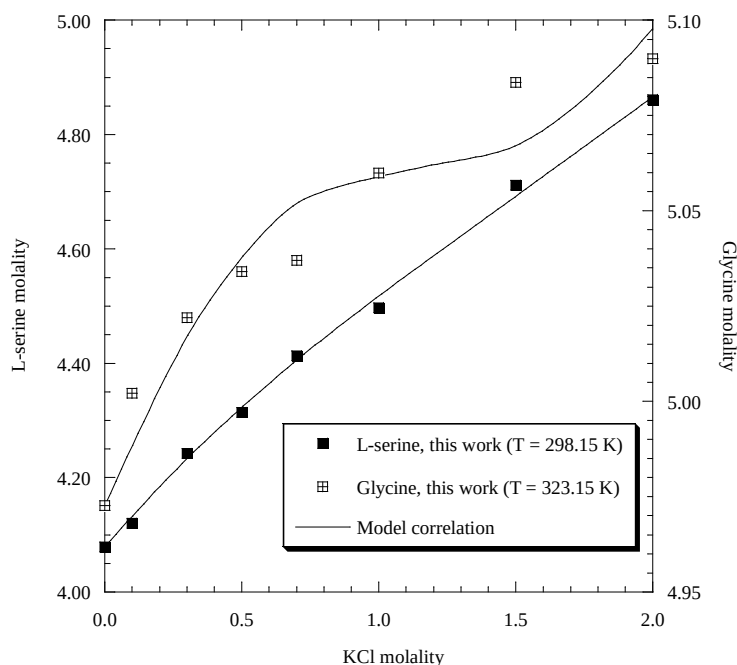


Figure 4.5 Solubilities of glycine or L-serine in water/KCl solutions: comparison between model correlation (—) and the experimental data measured in this work at different temperatures.

Introducing the appropriate temperature dependency on the $W_{n,MX}$ and $U_{n,MX}$ parameters a good representation of the solubilities at different temperatures can be achieved. However, a very important feature of any model is its predictive ability. To evaluate it, a stringent test was carried out. The solubility of DL-alanine in aqueous KCl solutions was also measured at 333.15 K, which is outside the temperature range used for correlation, and the predicted values applying the model proposed in this work were calculated.

Table 4.5 summarizes the experimental and predicted values. It can be easily observed that the deviations increase with the electrolyte molality and the RMSD found is only slightly higher than for correlation; 0.80 g of DL-alanine per kg of water. Despite the extremely good results found in the prediction, the methodology and the model applied in this work should be used with caution for predictive purposes. In fact, very good correlation results could also be found using a linear temperature dependency on $W_{n,MX}$ and $Y_{1,n,MX}^{(0)}$ parameters, but as far as prediction concerns the results can present a much larger RMSD value. One main reason for this lies on the fact that two temperature levels, which are close to each other, without other thermodynamic properties like excess enthalpies or heat capacities, are usually insufficient to find the correct trends for the fitted parameters with temperature. However, the approach presented is still useful as it gives very good indications about the model capabilities on the simultaneous representation of activity coefficient ratios and solubility behavior for water/electrolyte/amino acid systems and, as far as new data are available more correct trends for parameters with temperature can be found. In fact, it should be mentioned that the application of other models like extended UNIQUAC (Breil, 2001), which include directly the temperature effect on the parameters, have shown considerable difficulties for the simultaneous representation of those properties at 298.15 K only.

Table 4.5 Solubilities of DL-alanine (g amino acid/100 g of water) in aqueous KCl solutions at 333.15 K: experimental and predicted values.

KCl molality	Experimental	Predicted
0.000	26.36	26.36
0.100	26.43	26.38
0.500	26.27	26.25
1.000	25.92	25.83
2.000	25.10	24.98

Additionally, the capability of the PSC equations to predict water activity in aqueous amino acid solutions with KCl at 298.15 K was also studied. A comparison between the measured (Pinho, 2008) and predicted water activities in a 1 *m* aqueous KCl solution with amino acids is shown in Figure 4.6. For glycine and DL-alanine the prediction results using the parameters obtained in section 4.3 are also presented. The RMSDs found were 0.0008 (0.0008 without solubility data) for glycine, 0.0004 (0.0007 without solubility data) for DL-alanine and 0.0018 for L-serine system. For higher amino acid molalities in the L-serine system, it is possible to observe evident deviations but, in general prediction results are very good. Also, the results using the parameters obtained in the section 4.3 (systems with glycine and DL-alanine) show a very good agreement.

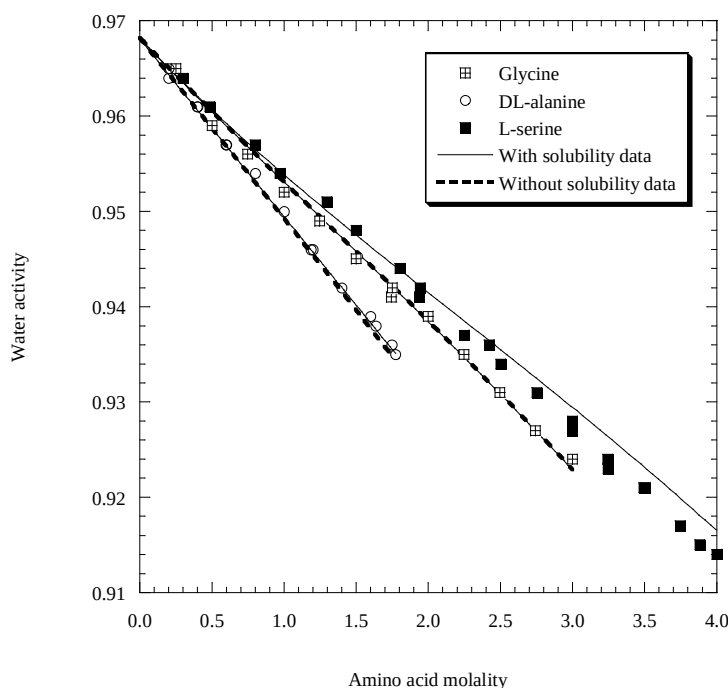


Figure 4.6 Water activity in aqueous 1 *m* KCl solutions containing amino acids at 298.15 K: comparison between model results and the experimental data given by Pinho (2008).

The parameter estimation was based on electrolyte activity coefficient data in the presence of an amino acid, with a maximum KCl concentration equal to 1 *m*, and a maximum of 2.4, 1.6 and 0.4 *m* for glycine, DL-alanine and L-serine, respectively. Therefore, the result found for L-serine is not surprising. The extrapolation of the calculation of water activity in aqueous 1 *m* KCl solutions up to 4 *m* is, perhaps, very difficult (Pinho, 2008).

A similar analysis for the aqueous 3 *m* KCl solutions at 298.15 K is shown in Figure 4.7. As expected, the deviations found between measured and calculated water activity are now very considerable. The RMSDs found were 0.0114 (0.0268 without solubility data) for glycine, 0.0034 (0.0035 without solubility data) for DL-alanine and 0.0064 for L-serine system. Comparing the prediction results with and without solubility data it is possible to verify that no significant changes are obtained for DL-alanine while, for glycine the prediction results without solubility data are far from being in agreement with the measured water activities. For glycine and DL-alanine systems the RMSDs are now an order of magnitude larger than the ones obtained for the aqueous 1 *m* KCl solutions, however the increase observed for L-serine is not so pronounced. It is worth to mention the importance of the measured water activity data that can be very useful for the accurate thermodynamic description of these highly complex systems for wide salt and amino acid molalities.

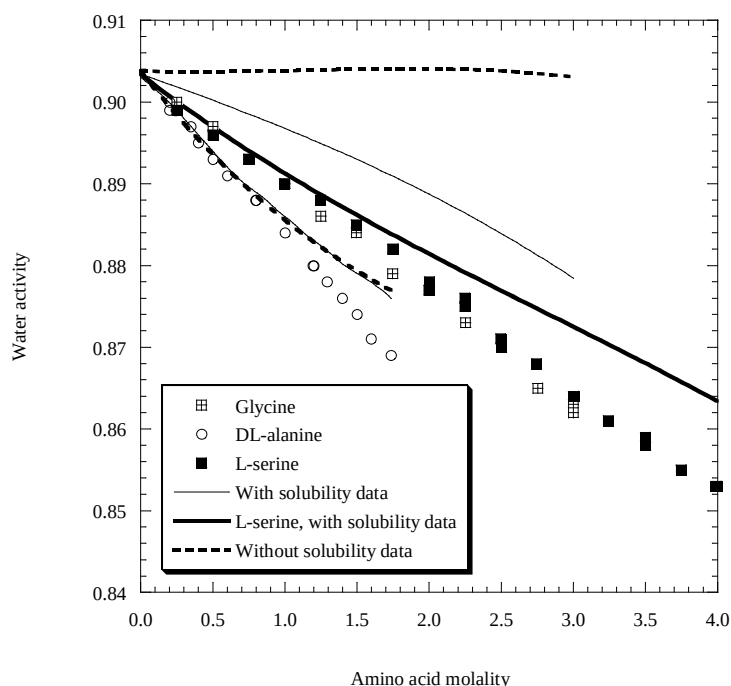


Figure 4.7 Water activity in aqueous 3 *m* KCl solutions containing amino acids at 298.15 K: comparison between model results and the experimental data given by Pinho (2008).

4.5 CONCLUSIONS

The Pitzer-Simonson-Clegg equations were applied to predict the solubility of glycine and DL-alanine in aqueous KCl solutions at 298.15 K using activity coefficient data only. Quantitatively, the predictions are weak, but they made it possible to confirm the solubility trends found experimentally. Nevertheless, it was possible to conclude the usefulness of those equations in the simultaneous correlation of the activity coefficient data and solubility, and their ability to describe the change on amino acid solubility with the temperature. It was also possible to conclude the adequacy of those equations in the simultaneous correlation of the activity coefficient data at 298.15 K and amino acid solubility at two different temperatures (298.15 and 323.15 K). The introduction of a temperature dependency on the $W_{n,MX}$ and $U_{n,MX}$ parameters allowed very satisfactory correlation results: the global root mean square deviations (RMSD) found were 0.0036 for the activity coefficients and 0.87 g of amino acid per kg of water for the solubility data in those ternary systems.

The results calculated for the prediction of DL-alanine solubility in aqueous KCl systems at 333.15 K showed satisfactory agreement (RMSD: 0.80 g of DL-alanine per kg of water). The prediction of the amino acid solubility in aqueous KCl solutions, at least outside the temperature range used for correlation, can give higher deviations than for correlation, and this is intimately related with the chosen temperature dependency for the model parameters. Therefore, it must be carried out with caution. Nevertheless, the model and methodology given are very important to open new paths in the search of appropriate models to describe the behavior of these highly complex systems.

Additionally, the water activity in aqueous amino acid solutions with KCl at 298.15 K was predicted at two different salt molalities. Even though the results encountered for the prediction, for aqueous 1 *m* KCl solutions, were good, this methodology should be used with caution for predictive purposes. The results show very good agreement for experimental data inside the KCl and amino acid molality range used during parameter estimation.

NOMENCLATURE

List of symbols

a	activity
A	Debye-Hückel parameter
B	specific electrolyte parameter
d	density
D	relative permittivity
e	electronic charge ($e = 1.60218 \cdot 10^{-19}$ C)
f	rational activity coefficient
$\hat{f}$	fugacity
g	Gibbs energy, function given by equation 4.12
I	ionic strength
k	Boltzmann constant ($k = 1.38026 \cdot 10^{-23}$ J.K ⁻¹)
m	molality (mol.kg ⁻¹ of solvent)
M	molar mass (kg.mol ⁻¹), cation
MX	electrolyte
N_a	Avogadro's number ($N_a = 6.02380 \cdot 10^{23}$ mol ⁻¹)
N_{solu}	total number of solutes
n	number of data points, mole number
P	model parameters (equation 4.20), pressure
Q	experimental quantity
q_i	constants in equations 4.20 and 4.21, $i = 1$ to 3
R	ideal gas constant ($R = 8.31451$ J.mol ⁻¹ .K ⁻¹)
T	absolute temperature
X	anion
x	mole fraction
w, u	water/amino acid parameters
W, U, V	model parameters
$Y^{(0)}, Y^{(1)}$	model parameters
z	charge number

Greek Letters

α	standard value ($\alpha = 13.0$)
γ	molal activity coefficient
ρ	closest approach distance for the ions
ϵ_0	vacuum permittivity ($\epsilon_0 = 8.85419 \cdot 10^{-12} \text{ C}^2 \cdot \text{J}^{-1} \cdot \text{m}^{-1}$)
ϕ	osmotic coefficient
ν	stoichiometric coefficient

Superscripts

<i>calc</i>	calculated by the model
<i>DH</i>	Debye-Hückel
<i>E</i>	excess property
<i>exp</i>	experimental
<i>r</i>	ratio
<i>S</i>	short-range, solid state
<i>*</i>	unsymmetric
<i>,</i>	solute free basis
<i>o</i>	binary system (absence of electrolyte)

Subscripts

<i>1, w</i>	water
<i>i</i>	component, ions
<i>I</i>	ionic
<i>j</i>	species
<i>k</i>	data point
<i>M</i>	cation
<i>MX</i>	electrolyte
<i>n</i>	amino acid
<i>r</i>	reference
<i>X</i>	anion
<i>x</i>	mole fraction
$\pm$	mean ionic property

Abbreviations

<i>FOBJ</i>	objective function
RMSD	root mean square deviation
PSC	Pitzer-Simonson-Clegg

CHAPTER 5.

MODELLING AMINO ACID SOLUBILITY IN ALKANOL SOLUTIONS (g^E MODELS)

5.1 INTRODUCTION

The thermodynamic modelling of amino acids in mixed solvent systems has been focused in detail in chapter 2. Now that a reliable and consistent database, that includes the new data measured and presented in chapter 3, was built, two distinct formulations are going to be undertaken (in this and the next chapters) for the calculation of the solubilities of the amino acids in pure and mixed solvent systems, as function of the temperature and solvent composition.

In a water/alcohol mixed solvent, the solubility of amino acids varies strongly with the solvent composition; in pure alcohol the solubilities can be three orders of magnitude lower than in pure water. To overcome difficulties arising from this fact, the excess solubility approach has been used as a fundamental tool to understand the influence of various agents on the solubility. The potentialities of the application of the excess solubility approach combined with conventional thermodynamic models, namely g^E models such as the NRTL, the modified NRTL, the modified UNIQUAC and also with the model presented by Gude et al. (1996a,b) to represent the solubility of amino acids in water-alcohol systems are explored in this chapter. The performance of the models used to correlate and predict the solubility data for these systems is studied and compared.

5.2 EXCESS SOLUBILITY APPROACH

In this work, the excess solubility approach was applied with different thermodynamic models to represent the solubilities of amino acids in aqueous aliphatic alcohol solutions at different temperatures. As described in the following sections, the thermodynamic models chosen were the NRTL (Renon and Prausnitz, 1968), the modified NRTL (Vetere, 2000), the modified UNIQUAC (Peres and Macedo, 1996, 1997) and the one proposed by Gude et al. (1996a,b).

As could be observed in chapter 3, in a water/alcohol mixed solvent system the solubility of amino acids varies strongly with the solvent composition; in pure alcohols the solubility can be three orders of magnitude lower than in pure water. To overcome difficulties arising from this fact, the excess solubility approach can be of great importance to understand the influence of various agents on the solubility (Gude et al., 1996a,b; Orella and Kirwan, 1989, 1991). According to Gude et al. (1996a,b) the excess solubility (x_{aa}^E) of an amino acid in a mixed solvent solution constituted by N solvents is defined as:

$$\ln x_{aa}^E \equiv \ln x_{aa,mix} - \sum_{i=1}^N x'_i \ln x_{aa,i} \quad (5.1)$$

where $x_{aa,mix}$ and $x_{aa,i}$ are the saturated solute mole fractions in the mixed solvent and in the pure solvent i , respectively, and x'_i is the mole fraction of the solvent i in amino acid free basis.

Choosing the standard state of the solute as the pure fused amino acid at the system temperature and pressure, the chemical potential of the solute at the standard state is independent of the solvent composition. Thus, introducing the solid-liquid equilibrium conditions and assuming pure solid phase, the excess solubility can be written as:

$$\ln x_{aa}^E \equiv -\ln f_{aa,mix} + \sum_{i=1}^N x'_i \ln f_{aa,i} \quad (5.2)$$

being $f_{aa,mix}$ and $f_{aa,i}$ the rational activity coefficients of the amino acid in saturated solutions of the mixed solvent or in pure solvent i , respectively. As noticed by Cohn and Edsall (1943) the mole fractions of amino acids in saturated solutions are relatively low and

so the activity coefficients in saturated solutions are very similar to the infinite dilution activity coefficients (f^∞). Therefore, the excess solubility can be approximated to:

$$\ln x_{aa}^E \equiv -\ln f_{aa,mix}^\infty + \sum_{i=1}^N x'_i \ln f_{aa,i}^\infty \quad (5.3)$$

5.2.1 NRTL MODEL

Combining the expression for the activity coefficient of the amino acid according to the NRTL equation, with equation 5.3, the expression for the excess solubility (x_{aa}^E) of a solute in a mixed solvent solution constituted by N solvents can be expressed as:

$$\ln x_{aa}^E = \sum_{i=1}^N (\tau_{i,aa} + G_{aa,i} \tau_{aa,i}) x'_i - \frac{\sum_{i=1}^N \tau_{i,aa} G_{i,aa} x'_i}{\sum_{i=1}^N G_{i,aa} x'_i} - \sum_{i=1}^N \frac{x'_i G_{aa,i}}{\sum_{j=1}^N G_{ji} x'_j} \left[\tau_{aa,i} - \frac{\sum_{j=1}^N \tau_{ji} G_{ji} x'_j}{\sum_{j=1}^N G_{ji} x'_j} \right] \quad (5.4)$$

with $G_{ij} = \exp(-\alpha_{ij} \tau_{ij} / RT)$, being τ_{ij} is the NRTL binary interaction parameter between species i and j , and α_{ij} is the non-randomness parameter. Therefore, for each pair of components there are three parameters, τ_{ij} , τ_{ji} and α_{ij} . In order to reduce the number of unknown parameters it is assumed that $\tau_{i,aa} = \tau_{aa,i}$ and the non-randomness parameter of each solvent with different solutes is considered to be a constant value. Since the parameters between solvent species, obtained from low pressure vapor-liquid equilibrium data, can be found in the Dechema Chemistry Data Series (Gmehling et al., 1981) only the parameters between each solvent and the solute (amino acid) remain to be estimated.

5.2.2 MODIFIED NRTL MODEL

During 2000, Vetere proposed a simple modification of the NRTL equation, which consisted in the introduction of the ratio of the molar volumes of the pure compounds as a multiplying factor to the binary parameters (G_{ij}). The suitability of this modification was evaluated by Vetere (2000) on the correlation and/or prediction of the vapor-liquid equilibrium of several systems characterized by strong non-idealities, namely organic aqueous mixtures and mixtures of non-polar compounds in alcohols, and it showed to be more reliable than the original NRTL equation (Vetere, 2000, 2004).

Applying this model to a mixture constituted by N solvents, the expression of the excess solubility can be easily obtained:

$$\ln x_{aa}^E = \sum_{i=1}^N \left(\tau_{i,aa} + \frac{V_{aa}}{V_i} G_{aa,i} \tau_{aa,i} \right) x'_i - \frac{\sum_{i=1}^N \tau_{i,aa} \frac{V_i}{V_{aa}} G_{i,aa} x'_i}{\sum_{i=1}^N \frac{V_i}{V_{aa}} G_{i,aa} x'_i} - \sum_{i=1}^N \frac{x'_i \frac{V_{aa}}{V_i} G_{aa,i}}{\sum_{j=1}^N \frac{V_j}{V_i} G_{ji} x'_j} \left[\tau_{aa,i} - \frac{\sum_{j=1}^N \tau_{ji} \frac{V_j}{V_i} G_{ji} x'_j}{\sum_{j=1}^N \frac{V_j}{V_i} G_{ji} x'_j} \right] \quad (5.5)$$

where V_i is the molar volume of the pure substance i . Here, the adjustable parameters are also τ_{ij} , τ_{ji} and the non-randomness parameter α_{ij} . Following the same conditions assumed for the NRTL model, the parameters left to be estimated are the binary parameter $\tau_{i,aa} = \tau_{aa,i}$ and $\alpha_{i,aa}$. Fixing the non-randomness parameters in the modified NRTL model to the values used for the NRTL model, only the binary interaction parameter between the amino acid and the solvents remain unknown.

5.2.3 MODIFIED UNIQUAC MODEL

Three UNIQUAC-based activity coefficient models were developed by Peres and Macedo (1997) to describe the vapor–liquid and the solid–liquid equilibria of aqueous solutions containing one or two sugars, as well as the solid-liquid equilibria of one sugar in mixed solvent mixtures at different temperatures. The modified UNIQUAC model was the only one to give an accurate representation for all those types of equilibria.

Combining the expression for the activity coefficient of the amino acid by the modified UNIQUAC equation (Peres and Macedo, 1996, 1997) with the excess solubility approach, it is possible to obtain the following expression for the excess solubility of the amino acid in a mixture of N solvents:

$$\ln x_{aa}^E = \sum_{i=1}^N x'_i \left[\ln \left(\frac{r_{aa}^{2/3}}{r_i^{2/3}} \right) - \left(\frac{r_{aa}^{2/3}}{r_i^{2/3}} \right) \right] - \ln \frac{r_{aa}^{2/3}}{\sum_{i=1}^N x'_i r_i^{2/3}} + \frac{r_{aa}^{2/3}}{\sum_{i=1}^N x'_i r_i^{2/3}} - q_{aa} \left[\sum_{i=1}^N x'_i (\ln \tau_{i,aa} + \tau_{aa,i}) - \ln \left(\sum_{i=1}^N \theta'_i \tau_{i,aa} \right) + \sum_{i=1}^N \frac{\theta'_i \tau_{aa,i}}{\sum_j \theta'_j \tau_{ji}} \right] \quad (5.6)$$

with $\tau_{ij} = \exp(-a_{ij}/T)$, being a_{ij} the UNIQUAC interaction parameter between species i and j , which is temperature dependent according to:

$$a_{ij} = a_{ij}^o + a_{ij}^t (T - 298.15) \quad (5.7)$$

In equation 5.6, r_i and q_i are, respectively, the volume and the surface area parameters of component i , and θ'_i is the molecular area fraction of component i defined as:

$$\theta'_i = \frac{x'_i q_i}{\sum_{j=1}^N x'_j q_j} \quad (5.8)$$

As for both NRTL and modified NRTL equations, in order to reduce the number of parameters to be estimated, it was assumed that $\tau_{i,aa} = \tau_{aa,i}$.

5.2.4 MODEL BY GUDE ET AL. (1996)

A simple excess Gibbs energy model with a single amino acid specific parameter was successfully used by Gude et al. (1996a,b) to correlate the partition coefficients and solubilities of seven α -amino acids in aqueous alkanol solutions at 298.15 K. This model is the sum of a combinatorial term (Flory-Huggins theory) and a Margules residual expression. The excess solubility for a system containing N solvents and a single solute is given by (Gude et al., 1996a,b):

$$\ln x_{aa}^E = \ln r' - \sum_{j=1}^N x'_j \ln r_j + r_{aa} \left[\frac{1}{r'} - \sum_{j=1}^N \frac{x'_j}{r_j} \right] + \sum_{j=1}^N \sum_{i \neq j}^N [A_{ji} x'_j x'_i (1 + C_{j,i,aa})] \quad (5.9)$$

where A_{ji} is the interaction parameter between the solvents i and j , $C_{j,i,aa}$ is the ternary interaction parameter which has a constant value for each amino acid in different solvent systems, and $r' = \sum_j x'_j r_j$. Since the A_{ji} parameters between solvents are known (Gude et al., 1996b), there is one parameter left to estimate, $C_{j,i,aa}$.

5.3 PARAMETERS ESTIMATION

A major difference between the approaches concerns the number of parameters needed to be calculated: the model proposed by Gude et al. (1996a,b) requires only a single amino acid specific parameter; while NRTL, the modified NRTL and the modified UNIQUAC models require the same number of estimated parameters: for an amino acid for which solubility data is available in n aqueous-alkanol systems the number of parameters to be determined is $n + 1$.

For each system, the parameters were estimated minimizing the following objective function ($FOBJ$):

$$FOBJ = \sum_k \left[\frac{\left(x_{aa,mix} / x_{aa,w} \right)_k^{calc} - \left(x_{aa,mix} / x_{aa,w} \right)_k^{exp}}{\left(x_{aa,mix} / x_{aa,w} \right)_k^{exp}} \right]^2 \quad (5.10)$$

where $x_{aa,mix}$ and $x_{aa,w}$ are the saturated solute mole fractions in the mixed solvent and in pure water, respectively, and $x_{aa,mix} / x_{aa,w}$ is the relative solubility, which is the ratio between the solubilities of the amino acid in the mixed solvent and in pure water, and the superscripts *exp* and *calc* mean experimental and calculated quantities, respectively.

A database on amino acid solubilities data in aqueous alkanol solutions was established to allow the estimation of the parameters required by the models considered. The solubility data used includes the experimental results obtained in this work, as well as those selected from the open literature there are all collected in Table 5.1.

Table 5.1 Database on amino acid solubility data in aqueous alkanol solutions: experimental temperature range (first row), number of data points (second row), data sources (third row).

Amino acid	Methanol	Ethanol	1-Propanol	2-Propanol	1-Butanol
Glycine	298.15 K	298.15-333.15 K	298.15-333.15 K	298.15-333.15 K	298.15 K
	6	51 (32)*	17 (8)*	15 (6)*	7
	(b)	(a, d)	(e)	(e)	(c)
DL-alanine	NA	298.15-333.15 K	298.15-333.15 K	298.15-333.15 K	NA
		28 (18)*	18 (18)*	18 (18)*	
		(a)			
L-isoleucine	NA	298.15-333.15 K	298.15-333.15 K	298.15-333.15 K	298.15 K
		19 (19)*	28 (19)*	28 (19)*	6
			(e)	(e)	(c)
L-threonine	NA	298.15-333.15 K	298.15-333.15 K	298.15-333.15 K	NA
		18 (18)*	18 (18)*	18 (18)*	
L-serine	NA	298.15-333.15 K	298.15-333.15 K	298.15-333.15 K	298.15 K
		18 (18)*	16 (16)*	17 (17)*	6
					(c)

(a) Cohn et al. (1934); (b) Gekko (1981); (c) Gude et al. (1996b); (d) Nozaki and Tanford (1971); (e) Orella and Kirwan (1991). NA – Not available. (*) In brackets number of data points obtained in this work.

5.3.1 NRTL MODEL

The NRTL parameters between the solvent species listed in Table 5.2 were used for the calculations. They were obtained from low pressure vapor-liquid equilibrium data, and can be found in the Dechema Chemistry Data Series (Gmehling et al., 1981). The non-randomness parameters between the amino acids and the solvents were fixed during the minimization process at the values presented in Table 5.3. It should be noticed, once more, that the value is the same for each solvent with different solutes. The remaining parameters to be estimated were obtained correlating the solubility data for each amino acid in the different aqueous-alkanol solutions. Due to the existence of measured solubility data at temperatures different from 298.15 K, it was possible to study the capabilities of this methodology for the description of the temperature influence on the solubilities. To do so, a temperature dependence on the parameter $\tau_{aa,i}$ (i is ethanol, 1-propanol and 2-propanol) was introduced according to:

$$\tau_{aa,i} = \tau_{aa,i}^o + \tau_{aa,i}^t (T - 298.15) \quad (5.11)$$

where T is the absolute temperature.

The parameters obtained from the correlation of the experimental data measured in this work are given in Table 5.3.

Table 5.2 NRTL parameters (cal.mol⁻¹) between water (1) and alcohols (2).

	Methanol	Ethanol	1-Propanol	2-Propanol	1-Butanol
	$\alpha = 0.2994$	$\alpha = 0.1830$	$\alpha = 0.5081$	$\alpha = 0.2879$	$\alpha = 0.4447$
τ_{12}	845.2062	814.903+2.0078T	1636.572	1569.294	2633.695
τ_{21}	-253.8802	-697.613+0.9765T	500.3962	-26.279	504.0381

Table 5.3 NRTL $\tau_{aa,i}^o$ (cal.mol⁻¹) and $\tau_{aa,i}^t$ (cal.mol⁻¹.K⁻¹) parameters.

Amino acid	Water $\alpha_{aa,i} = 0.05$	Methanol $\alpha_{aa,i} = 0.00$	Ethanol $\alpha_{aa,i} = 0.02$	1-Propanol $\alpha_{aa,i} = 0.45$	2- Propanol $\alpha_{aa,i} = 0.45$	1-Butanol $\alpha_{aa,i} = 0.10$
Glycine	6823.1	5476.3	6388.9 -25.45 ^a	6644.2	1294.2	428.71
DL-alanine	9259.0		7704.4 -23.76 ^a	917.22 -2.384 ^a	7907.1 15.80 ^a	
L-isoleucine	16880.		14041. -36.68 ^a	1557. -3.86 ^a	18034. 16.70 ^a	4613.
L-threonine	17290.		15273. -32.29 ^a	1711. -2.09 ^a	1911. -2.99 ^a	
L-serine	11589.		9442. -16.33 ^a	904.5 0.59 ^a	12264. -8.89 ^a	2211.

^a $\tau_{aa,j}^t$ in accordance to equation 5.11.

5.3.2 MODIFIED NRTL MODEL

Before the application of the methodology presented for the modified NRTL model, it is necessary to obtain the interaction parameters between the solvents in accordance to the modification introduced by Vetere (2000). They were estimated from low pressure vapor-liquid equilibrium data taken from the Dechema Chemistry Data Series (Gmhelning et al., 1981), minimizing the following objective function (F):

$$F = \sum_k \left[\left(\frac{f_1^{\text{exp}} - f_1^{\text{calc}}}{f_1^{\text{exp}}} \right)^2 + \left(\frac{f_2^{\text{exp}} - f_2^{\text{calc}}}{f_2^{\text{exp}}} \right)^2 \right] \quad (5.12)$$

where f is the rational activity coefficient of water (1) or alcohol (2), and the superscripts *exp* and *calc* are the experimental and calculated activity coefficients, respectively. Table 5.4 lists the estimated parameters between solvent species together with the average relative deviation (ARD) for the activity coefficients.

As already mentioned, the non-randomness parameters in the modified NRTL model were also fixed during the estimation process, and their values are those used for the NRTL model (Table 5.3). A temperature dependence was also introduced in the amino acid/alcohol (ethanol, 1-propanol or 2-propanol) parameter, according to equation 5.11. The molar volumes of the pure solvents were taken from the literature (DIPPR), while the pure amino acid molar volumes in the liquid state were totally unknown, even at 298.15 K, for most amino acids. In order to obtain a uniform approximation for the amino acid molar volumes, the standard partial molar volumes of amino acids, at 298.15 K, in pure water, given in the extensive review by Zhao (2006) were adopted. They are presented in Table 5.5. The values of the estimated parameters are also compiled in Table 5.6.

Table 5.4 Modified NRTL parameters τ_{ij}^o (cal.mol⁻¹) and τ_{ij}^t (cal.mol⁻¹.K⁻¹) between water (1) and alcohols (2).

	Methanol	Ethanol	1-Propanol	2-Propanol	1-Butanol
	$\alpha = 0.2994$	$\alpha = 0.1830$	$\alpha = 0.5081$	$\alpha = 0.2879$	$\alpha = 0.4447$
τ_{12}	-129.1	-2829.	379.8	-1524.	1195.
	-8.313 ^a	-13.71 ^a	-7.581 ^a	-16.88 ^a	-9.790 ^a
τ_{21}	366.9	2596.	638.1	1461.	521.3
	5.078 ^a	14.22 ^a	3.478 ^a	14.63 ^a	2.652 ^a
ARD(%)	$\gamma_1 : 0.176$	$\gamma_1 : 0.047$	$\gamma_1 : 2.597$	$\gamma_1 : 0.162$	$\gamma_1 : 3.055$
	$\gamma_2 : 0.240$	$\gamma_2 : 0.090$	$\gamma_2 : 4.016$	$\gamma_2 : 0.457$	$\gamma_2 : 4.794$

^a τ_{ij}^t in accordance to equation 5.11.

Table 5.5 Standard partial molar volumes of amino acids (cm³.mol⁻¹).

Amino acid	Glycine	DL-alanine	L-isoleucine	L-threonine	L-serine
Molar volume	43.18	60.48	105.71	76.81	60.56

Table 5.6 Modified NRTL $\tau_{aa,i}^o$ (cal.mol⁻¹) and $\tau_{aa,i}^t$ (cal.mol⁻¹.K⁻¹) parameters.

Amino acid	Water	Methanol	Ethanol	1-Propanol	2-Propanol	1-Butanol
Glycine	-1514.	-1607.	2459. 2.87 ^a	-784.4	-295.3	-2981.
DL-alanine	-1824.		2947. 16.95 ^a	-1097. -6.11 ^a	-1050. 24.56 ^a	
L-isoleucine	217220.		217201. -8.72 ^a	25996. 7.03 ^a	218396. 16.74 ^a	118052.
L-threonine	215389.		215565. -8.18 ^a	216005. 1.12 ^a	25838. 7.27 ^a	
L-serine	-2826.		515.7 -2.13 ^a	297.5 1.68 ^a	1024. -0.34 ^a	-4798.

^a $\tau_{aa,j}^t$ in accordance to equation 5.11.

5.3.3 MODIFIED UNIQUAC MODEL

As before, for the modified NRTL model, at a first stage it was necessary to estimate the interaction parameters between the solvents. They were also obtained from low pressure vapor-liquid equilibrium data taken from Dechema Chemistry Data Series (Gmehling et al., 1981). They are presented in Table 5.7, as well as the ARD for the activity coefficients. The parameters were estimated minimizing the objective function (F) given by equation 5.12.

The structural parameters (r_i and q_i), which are given in Table 5.8, were calculated from UNIFAC group-contribution method (Reid et al., 1987). Again, in order to reduce the number of parameters to be estimated, it was assumed that $\tau_{i,aa} = \tau_{aa,i}$. The interaction parameters amino acid/water or methanol or 1-butanol were considered temperature independent ($a_{aa,1}^t = 0.0$). The estimated interaction parameters obtained are listed in Table 5.9.

Table 5.7 Modified UNIQUAC parameters a_{ij}^o (K) and a_{ij}^t between water (1) and alcohols (2).

	Methanol	Ethanol	1-Propanol	2-Propanol	1-Butanol
a_{12}	220.4	196.0	281.9	205.0	384.8
	0.465 ^a	0.705 ^a	0.498 ^a	0.606 ^a	0.436 ^a
a_{21}	-45.80	87.64	165.3	174.4	152.1
	0.171 ^a	0.396 ^a	0.034 ^a	-0.040 ^a	0.118 ^a
ARD(%)	$\gamma_1 : 0.049$	$\gamma_1 : 0.115$	$\gamma_1 : 0.609$	$\gamma_1 : 0.095$	$\gamma_1 : 1.114$
	$\gamma_2 : 0.069$	$\gamma_2 : 0.194$	$\gamma_2 : 1.226$	$\gamma_2 : 0.193$	$\gamma_2 : 2.724$

^a a_{ij}^t in accordance to equation 5.7.**Table 5.8** Structural parameters (r_i and q_i).

	r_i	q_i		r_i	q_i
Water	0.9200	1.400	Glycine	2.6705	2.460
Ethanol	2.1055	1.972	DL-alanine	3.3441	2.996
1-Propanol	2.7799	2.512	L-isoleucine	5.3665	4.412
2-Propanol	2.7791	2.508	L-serine	4.1174	3.888
1-Butanol	3.4543	3.052	L-threonine	4.7910	4.424

Table 5.9 Modified UNIQUAC parameters $a_{aa,i}^o$ (K) and $a_{aa,i}^t$.

Amino acid	Water	Methanol	Ethanol	1-Propanol	2-Propanol	1-Butanol
Glycine	4112.8	4138.7	4222.6	4221.5	4208.4	3678.6
			-6.200 ^a			
DL-alanine	2731.8		2855.6	2841.9	2933.8	
			-9.57 ^a	-5.88 ^a	-2.51 ^a	
L-isoleucine	3113.		3554.	3783.	3800.	2701.
			2.50 ^a	3.51 ^a	4.16 ^a	
L-threonine	3294.		3451.	3871.	3811.	
			7.02 ^a	1.81 ^a	4.07 ^a	
L-serine	428.8		3554.	3783.	3800.	219.9
			2.50 ^a	3.51 ^a	4.16 ^a	

^a $a_{aa,i}^t$ in accordance to equation 5.7.

5.3.4 MODEL OF GUDE ET AL. (1996)

The alcohol-water interaction parameters (A_{ji}) used in this work are given in Table 5.10 and are the ones reported by Gude et al. (1996b), while the UNIQUAC volume parameters are given in Table 5.8. Similarly to the previous models, a linear temperature dependence on the parameter $C_{j,i,aa}$ was introduced according to equation 5.13, and the capabilities of the model for the description of the temperature influence on the solubilities studied. The ternary interaction parameters obtained from correlation of the experimental data are summarized in Table 5.11.

$$C_{j,i,aa} = C_{j,i,aa}^o + C_{j,i,aa}^t (T - 298.15) \quad (5.13)$$

Table 5.10 The alcohol-water interaction parameters (A_{ji}).

	Methanol	Ethanol	1-Propanol	2- Propanol	1-Butanol
A_{ji}	0.59	1.55	2.68	2.25	3.15

Table 5.11 Ternary interaction parameters ($C_{j,i,aa}$).

Amino acid (aa)	$C_{j,i,aa}^o$	$C_{j,i,aa}^t$
Glycine	-1.3890	0.0339
DL-alanine	-1.2910	0.0181
L-isoleucine	1.9530	0.0092
L-threonine	0.5480	0.0154
L-serine	0.7731	-0.0123

5.4 RESULTS AND DISCUSSION

Table 5.12 lists the ARDs obtained with the different models in the correlation of the solubilities, together with the number of data points (NDPs). As can be observed, the results

are very reasonable for all amino acids in the several aqueous-alkanol systems. In general, the modified NRTL or NRTL models combined with the excess solubility approach give the best quantitative fit of the solubility data with global ARD of 12.0% and 12.2%, respectively. The model proposed by Gude et al. (1996a,b), with a single amino acid specific parameter, and the modified UNIQUAC equation, showed global ARDs of 16.2% and 15.1%, respectively.

Figure 5.1 shows a comparison between the experimental relative solubilities of four amino acids in the aqueous system of ethanol (298.15 K) and the results of the correlation based on the NRTL and modified NRTL models. It can be seen that both models are able to give a satisfactory representation of the data for L-serine, glycine and DL-alanine. However, for L-isoleucine, at high alcohol composition, the solubility is underestimated with both models.

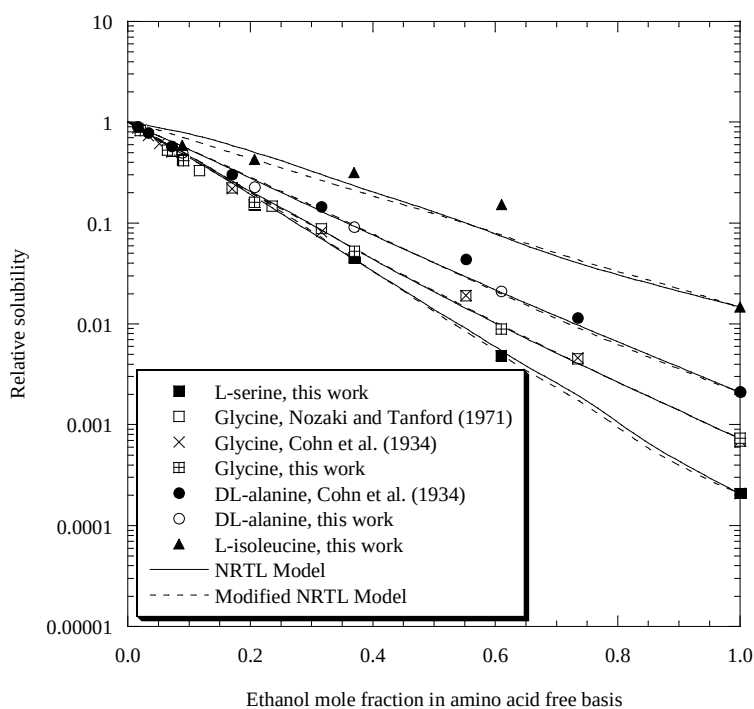


Figure 5.1 Relative solubilities of amino acids in water/ethanol solutions at 298.15 K.

In the Figure 5.2, the correlation results for of L-threonine, L-isoleucine and DL-alanine solubilities in aqueous 1-propanol solutions, at 298.15 K, with NRTL and modified UNIQUAC models is shown. Although the results obtained with the NRTL model are reasonably good, a better fit of the experimental data is obtained applying the modified UNIQUAC model, with special attention for the solubility of L-isoleucine in the water rich region.

Table 5.12 Average relative deviations (%).*

Amino acid	Model	Methanol	Ethanol	1-Propanol	2-Propanol	1-Butanol	Average
L-isoleucine (NDP=81)	(I)	NA	21.5	16.2	13.7	5.8	17.0
	(II)	NA	24.2	11.3	13.7	3.0	14.5
	(III)	NA	12.3	18.2	13.8	11.2	14.8
	(IV)	NA	12.8	12.1	18.1	11.3	15.4
L-serine (NDP=57)	(I)	NA	11.0	10.4	15.3	3.4	11.8
	(II)	NA	11.6	14.2	12.0	6.5	12.6
	(III)	NA	12.0	13.0	15.7	3.3	12.4
	(IV)	NA	18.1	14.8	17.9	11.4	17.2
L-threonine (NDP=54)	(I)	NA	16.1	15.7	17.6	NA	16.4
	(II)	NA	9.9	10.3	15.7	NA	12.0
	(III)	NA	10.2	10.6	11.8	NA	10.9
	(IV)	NA	16.3	13.3	14.8	NA	14.8
Glycine (NDP=96)	(I)	5.0	7.2	15.2	12.1	2.2	8.9
	(II)	6.4	6.9	18.2	8.2	3.8	8.9
	(III)	9.8	9.2	23.1	44.3	2.8	16.7
	(IV)	3.2	7.5	19.1	13.5	56.6	13.7
DL-alanine (NDP=64)	(I)	NA	8.8	8.4	8.2	NA	8.6
	(II)	NA	9.5	19.5	14.4	NA	13.7
	(III)	NA	9.5	13.2	39.8	NA	19.0
	(IV)	NA	20.7	26.2	23.2	NA	23.0

* For each system $ARD = 100 / NDP \sum_{k=1}^{NDP} \left| \left(x_{aa,k}^{calc} - x_{aa,k}^{exp} \right) / x_{aa,k}^{exp} \right|$. NA – Not available

(I) NRTL; (II) Modified NRTL; (III) Modified UNIQUAC; (IV) Gude et al. (1996a,b).

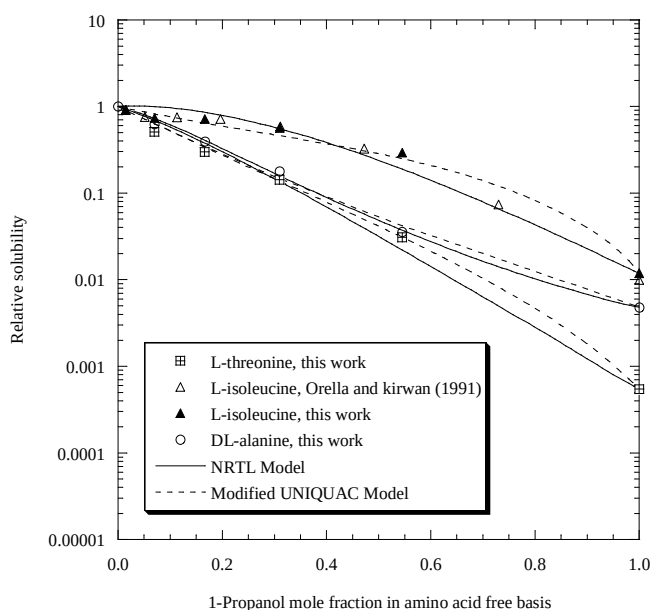


Figure 5.2 Relative solubilities of amino acids in water/1-propanol solutions at 298.15 K.

Some comparisons between the performances of the modified NRTL and modified UNIQUAC models are given in Figure 5.3 for the solubility of the amino acids in 2-propanol aqueous system also at 298.15 K. As can be observed, the results with the two models are fairly good in all solvent composition range for L-serine and L-threonine. However, the description of the L-isoleucine solubility detains our attention. Like observed in the aqueous 1-propanol system (Figures 5.2), the L-isoleucine solubility data in the water rich region is inaccurately represented.

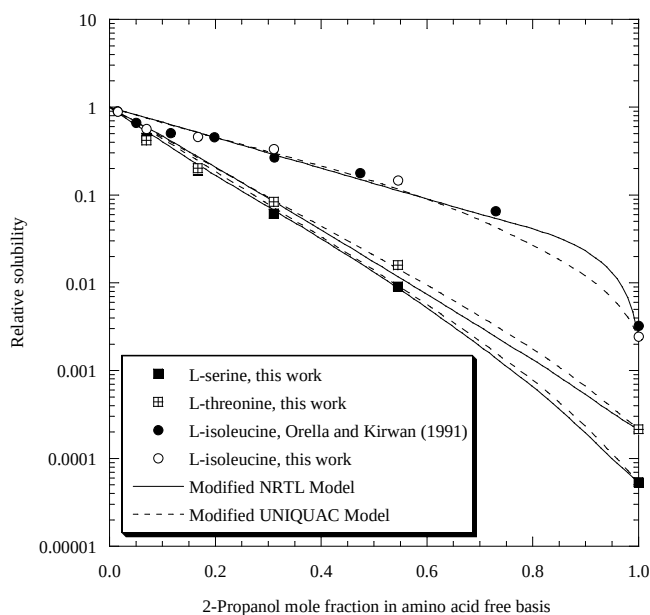


Figure 5.3 Relative solubilities of amino acids in water/2-propanol solutions at 298.15 K.

In Figure 5.4, the influence of the temperature on the relative solubility of L-isoleucine in the aqueous 1-propanol systems is shown. The model proposed by Gude et al. (1996a,b) gives a good description of the solubility data at 298.15 K for the water rich region only. For the higher temperature studied (333.15 K) the NRTL model is very successful and it gives a very good quantitative description of the equilibrium data in the water rich region, while the model proposed by Gude et al. (1996a,b) fails. In an attempt to overcome this limitation of the model proposed by Gude et al. (1996a,b), a temperature dependence of the alkanol-water interaction parameter (A_{ji}) was also introduced. However, no significant improvement was observed when compared with the results obtained using the temperature dependence on the ternary parameter $C_{j,i,aa}$ only.

The data measured by Gude et al. (1996b) for the solubilities of amino acids in water/1-butanol solutions were also included in the correlation. For these systems the correlation using the NRTL, modified NRTL and modified UNIQUAC equations is very good (Table 5.12). These systems exhibit liquid-liquid equilibrium and the solubility data used is confined for the alcohol rich region. In Figure 5.5 it is possible to observe the quality of the correlation achieved using the NRTL and the modified NRTL models.

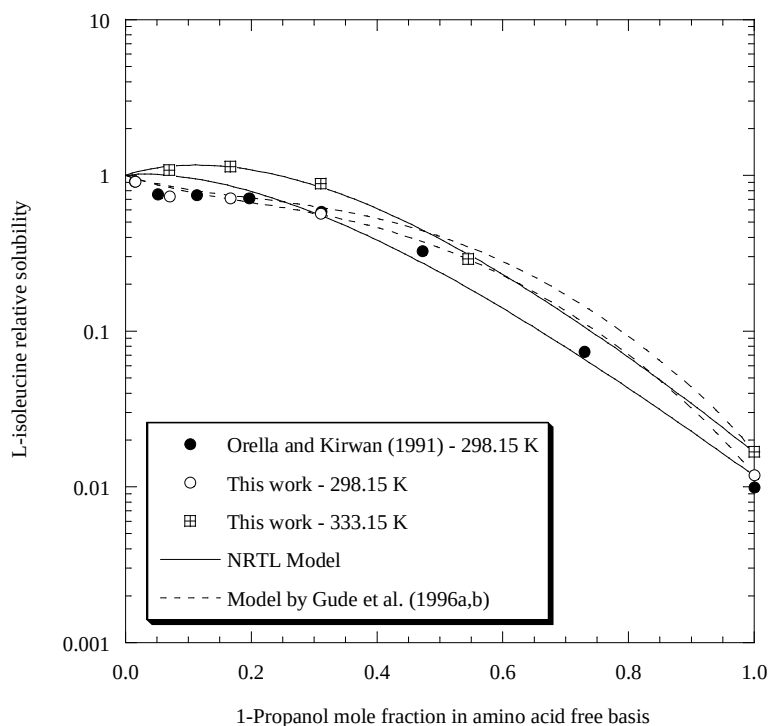


Figure 5.4 Relative solubilities of L-isoleucine in water/1-propanol solutions at different temperatures.

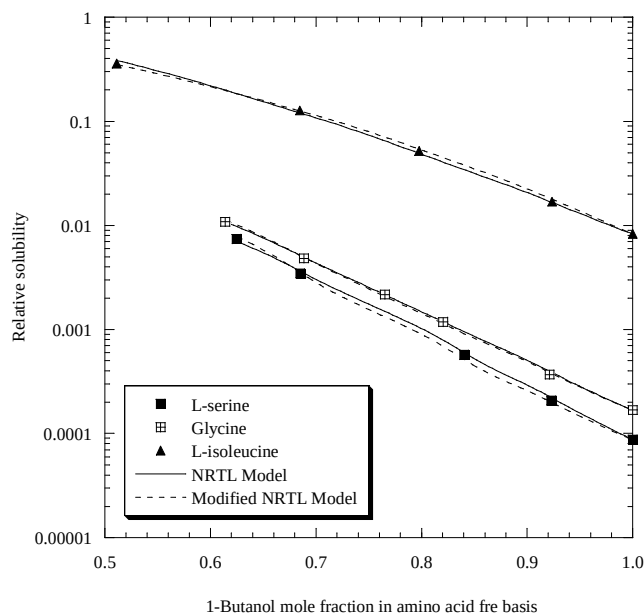


Figure 5.5 Relative solubilities of amino acids in water/1-butanol solutions at 298.15 K. Data from Gude et al. (1996b).

A comparison between the NRTL model and the model suggested by Orella and Kirwan (1991) is given in Figure 5.6 for the solubility of glycine in 1-propanol/water and 2-propanol/water mixtures at 298.15 K. A better correlation is observed with the NRTL model, which presents an ARD of 15.2 and 12.1%, while that for the model of Orella and Kirwan (1991) shows deviations of 22.4 and 19.4%, respectively, for those particular systems with the same number of regressed parameters.

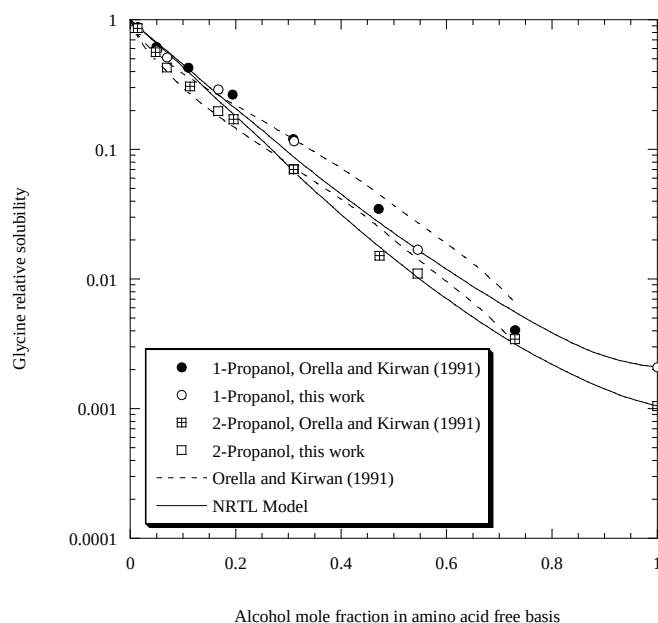


Figure 5.6 Glycine relative solubilities in water/alcohol solutions at 298.15 K. Comparison with the model by Orella and Kirwan (1991).

A very important feature of any model is its predictive ability. Solubility equilibria of threonine enantiomers in water/ethanol mixtures, with exception for the solubility in pure alcohol, were measured in the temperature range between 286.15 K and 319.15 K by Sapoundjiev et al. (2006). Since the knowledge of the solubility in pure solvents is essential for the methodology proposed in this work, and it is not available for pure ethanol, those data were not included in the correlation, but they were used to test the ability of the studied models to predict the solubility at different temperatures. The solubility of L-threonine in pure ethanol was extrapolated and the values used to predict the solubility at 283.15 K and 303.15 K. As can be seen from Figure 5.7, at 303.15 K the results obtained with the modified NRTL model are very good. The average relative deviation found for predictions with the modified NRTL equation are 14.7% at 283.15 K and 3.6% at 303.15 K. Since the ARD found for the correlation of L-threonine solubility in the aqueous alcohol systems applying the modified UNIQUAC model is very similar to the ARD found for the modified NRTL model, similar results were also expected for prediction; however this was not verified and the ARD found is 46.6% at 283.15 K and 11.3% at 303.15 K. Using the model proposed by Gude et al. (1996a,b) the results for prediction are also not good.

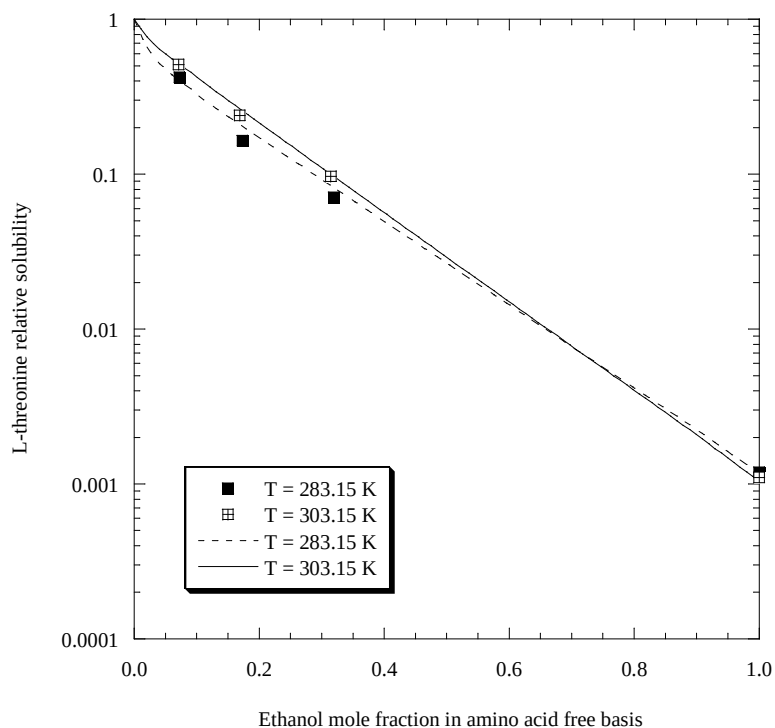


Figure 5.7 Modified NRTL predictions of the relative solubilities of L-threonine in water/ethanol solutions. Data from Sapoundjiev et al. (2006).

Dunn and Ross (1938) published solubility data of glycine and DL-alanine in water/ethanol solutions at different temperatures, but did not measure the solubility in pure ethanol. For correlation purposes, like mentioned before, this makes the data useless since the methodology proposed here involves the knowledge of the solubility in pure solvents. However, data compiled from this work between 298.15 K and 333.15 K for the solubilities of those amino acids in pure ethanol was also extrapolated and those values used for the prediction in the mixed solvent systems at 273.15 K and 338.15 K. Even if the calculations are very sensitive to the values found by extrapolation, the ARD found for those predictions given by the modified NRTL model were 24% and 9.4%, respectively, at 273.15 K and 338.15 K. The predictions can be seen in Figure 5.8, where it is evident the much better results achieved at the higher temperature. Clearly, the fact that the lowest temperature included in the correlation is 298.15 K advises some caution when predicting results to temperature values outside the temperature range used for correlation.

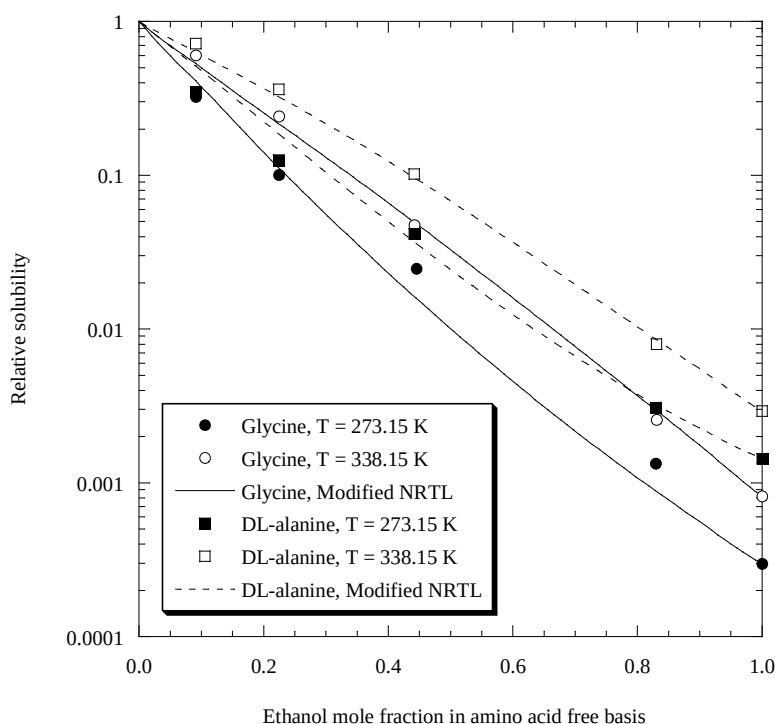


Figure 5.8 Modified NRTL predictions of the relative solubilities of glycine and DL-alanine in water/ethanol solutions. Data from Dunn and Ross (1938).

5.5 CONCLUSIONS

Theoretical work was focused on the application of the excess solubility approach with four different models: the NRTL model, a modified NRTL model, a modified UNIQUAC model and a model proposed by Gude et al. (1996a,b). The NRTL, the modified NRTL and the modified UNIQUAC models with the same number of parameters give a reasonable representation of the solubility of the amino acids in water-alcohol systems. Despite the simplicity of the model by Gude et al. (1996a,b), the results of the correlation are also very satisfactory. Their performance (correlation) showed global ARDs of 12.2%, 12.0%, 15.1% and 16.2%, respectively, while their application for the prediction of solubilities in water/ethanol mixed solvent mixtures, at different temperatures, showed ARDs of 16.3%, 14.6%, 27.3%, and 22.0%, respectively. Regardless of the fact that the model by Gude et al. (1996a,b) requires less regressed parameters, the improvement obtained using the other models gives a strong support to their use in the correlation of amino acid solubilities in mixed solvents. Concerning the influence of temperature on the solubility of the amino acids, the success of the correlations was not so good, evidencing some loss of accuracy. Nevertheless, the predictions obtained for water/ethanol systems may be considered very acceptable.

It is known that conventional thermodynamic models present serious difficulties to account accurately for the hydrophobic effects. Still, the excess solubility approach combined with the modified NRTL model can satisfactorily correlate and predict the amino acids solubilities in the different aqueous alcohol systems at the temperature range studied in this work.

As pointed out, two distinct formulations, for the calculation of the solubilities of the amino acids in pure and mixed solvent systems, as a function of the temperature and solvent composition, are proposed. In the next chapter, an equation of state is used to describe the same systems and the influence of other interactions like association is analyzed.

NOMENCLATURE*List of symbols*

a	UNIQUAC interaction parameter
A	binary interaction parameter in the Gude et al. (1996a,b) model
C	ternary interaction parameter in the Gude et al. (1996a,b) model
f	rational activity coefficient
g	Gibbs energy
G	NRTL parameter
N	number of solvents in solution
q	area parameter
r	volume parameter
R	ideal gas constant
T	absolute temperature (K)
V	molar volume ($\text{cm}^3 \cdot \text{mol}^{-1}$)
x	mole fraction

Greek Letters

α	non-randomness parameter
τ	NRTL or UNIQUAC parameter
θ	molecular area fraction

Subscripts

aa	amino acid
i,j,n	any species
k	experimental data point
mix	solvent mixture
$1, w$	water
2	alcohol

Superscripts

<i>calc</i>	calculated by the model
<i>exp</i>	experimental
<i>E</i>	excess property
<i>'</i>	solute free
∞	infinite dilution
<i>o</i>	reference
<i>t</i>	dependent temperature parameter

Abbreviations

ARD	average relative deviation
DIPPR	Design Institute for Physical Property (data base)
FOBJ, F	objective function
NA	Not available
NDP	number of data points
NRTL	Non-Random Two Liquid
UNIFAC	Universal Quasi Chemical Functional Group Activity Coefficient
UNIQUAC	Universal Quasi Chemical

CHAPTER 6.

MODELLING AMINO ACID SOLUBILITY IN ALKANOL SOLUTIONS (PC-SAFT EoS)

6.1 INTRODUCTION

For the thermodynamic modelling of amino acids in mixed solvent systems two different formulations were proposed. In the previous chapter, the application of the excess solubility approach combined with conventional thermodynamic models such as the NRTL, the modified NRTL, the modified UNIQUAC equation, and also the model presented by Gude et al. (1996a,b) was explored. In this chapter, the recently developed equation of state, the Perturbed-Chain Statistical Associated Fluid Theory (PC-SAFT EoS) (Gross and Sadowski, 2001, 2002) is presented. Amino acid pure-component parameters are fitted to the densities, activity and osmotic coefficients, vapor pressures and water activity of their aqueous solutions. For each system amino acid/solvent, one temperature independent binary parameter is required. Then, the potentialities of the model to predict the solubility in ternary mixtures, using only information from the binary systems, without the addition or refitting of model parameters will detain our attention. The estimation of the binary amino acid/solvent parameters will be discussed. Initially, the binary parameters amino acid/alcohol are fitted to the solubility of the amino acid in the pure alcohol, and on a second stage they are estimated from the solubility of the amino acid in the solvent mixtures.

This EoS was used by Fuchs et al. (2006) to model the solubility of glycine and DL-alanine in aqueous and alcohol solutions and the results of the prediction in water-alcohol mixtures corresponds well to literature data. After reproducing their results, a comparison of the two works will be performed, and some conclusions drawn.

6.2 THEORETICAL BACKGROUND

Based on the phase equilibrium conditions for a pure solid and a fluid phase, the following equation can be written:

$$f_s^{liq}(T, P) f_s^L(T, P, n) x_s^L = f_s^{solid}(T, P) \quad (6.1)$$

where x_s^L represents the mole fraction of the solute in the liquid phase, f_s^L is the symmetric activity coefficient of solute in the liquid phase, f_s^{liq} and f_s^{solid} are the reference state fugacities of the solute in the liquid and solid phases at the system pressure and temperature, respectively. The relation between the reference state fugacities can be calculated according to:

$$\ln \frac{f_s^{liq}(T, P)}{f_s^{solid}(T, P)} = \frac{\Delta H_o}{RT_o} \left(\frac{T_o}{T} - 1 \right) - \frac{\Delta C_p}{R} \left(\frac{T_o}{T} - 1 \right) + \frac{\Delta C_p}{R} \ln \frac{T_o}{T} \quad (6.2)$$

where ΔH_o corresponds to the change in enthalpy upon melting, ΔC_p is the difference of the heat capacity between the pure liquid and the pure solid, being this difference regarded as temperature independent, and T_o is the melting temperature of the pure solute (Prausnitz et al., 1999). The symmetric activity coefficient (f_s^L) is given by:

$$f_s^L = \frac{\hat{\phi}_s(T, P, n)}{\phi_s(T, P)} \quad (6.3)$$

being $\hat{\phi}_s(T, P, n)$ and $\phi_s(T, P)$ the fugacity coefficients of the solute in the mixture and as a pure component, respectively. In this work, the fugacity coefficients are calculated using the PC-SAFT EoS (Gross and Sadowski, 2001, 2002).

Therefore, the solubility of a substance at atmospheric pressure can be given by the following equation:

$$x_s^L = \frac{1}{f_s^L} \exp \left[-\frac{\Delta H_o}{RT_o} \left(\frac{T_o}{T} - 1 \right) + \frac{\Delta C_p}{R} \left(\frac{T_o}{T} - 1 \right) - \frac{\Delta C_p}{R} \ln \frac{T_o}{T} \right] \quad (6.4)$$

The use of PC-SAFT EoS and equation 6.4 are fully described in the following sections.

6.3 MODEL DESCRIPTION

A modified Statistical Associating Fluid Theory (SAFT) was developed by Gross and Sadowski (2000, 2001) by using the hard-chain system as the reference system for the dispersion term and by extending the perturbation theory of Barker and Henderson (1967a,b) to chain molecules (Tumakaka et al., 2005). Because a hard-chain fluid serves as a reference, rather than spherical molecules as in the former SAFT version, the proposed model was referred as perturbed-chain SAFT (PC-SAFT).

The molecular model underlying the PC-SAFT equation of state is depicted by Tumakaka et al. (2005). Molecules are assumed to be chains of freely joined spherical segments. The segments may possess association sites, exhibiting specific short-range interactions (like hydrogen bondings) and also carry partial charges (dipolar and quadrupolar interactions) (Tumakaka et al., 2005).

In this work, the PC-SAFT EoS is used as given in detail by Gross and Sadowski (2001, 2002). The residual Helmholtz energy (A^{res}) of the systems is considered as a sum of different contributions:

$$A^{res} = A^{hc} + A^{disp} + A^{assoc} \quad (6.5)$$

where A^{hc} accounts for the repulsion, A^{disp} , accounts for attractions and A^{assoc} denotes the contribution of the association interactions (Gross and Sadowski, 2001). The detailed expressions required for the individual terms in equation 6.5 are summarized in Appendix D.

The PC-SAFT EoS requires three pure-component parameters; the segment number, m ; the segment diameter, σ ; and the dispersion energy, ε/k for non-associating molecules, and two additional pure-component parameters; the association energy, $\varepsilon^{A_{B_i}}$; and the association volume, $\kappa^{A_{B_i}}$ for associating molecules.

The hard-chain reference fluid consists of a chain of molecules having no attractive interactions, being defined by two pure component parameters, the segment number and the segment diameter. In the reference and dispersion term, the conventional Berthelot-Lorentz combining rules for the binary mixture properties are applied and the correction of the

dispersion-energy parameter for the mixture is given by the introduction of one constant temperature independent binary parameter (k_{ij}):

$$\sigma_{ij} = \frac{1}{2}(\sigma_i + \sigma_j) \quad (6.6)$$

$$\varepsilon_{ij} = (1 - k_{ij})\sqrt{\varepsilon_i \varepsilon_j} \quad (6.7)$$

Additionally to the three pure-component parameters mentioned for non-associating molecules, there are two more required for the description of the association interactions between the association site A_i and B_i of a pure-component i : the association energy, $\varepsilon^{A_i B_i}$; and the association volume, $\kappa^{A_i B_i}$. The strength of cross-associating interactions between two associating substances is described by applying simple mixing and combining rules (Wolbach and Sandler, 1998). Those rules are applied without any adjustable correction parameter and are written as:

$$\varepsilon^{A_i B_j} = \frac{1}{2}(\varepsilon^{A_i B_i} + \varepsilon^{A_j B_j}) \quad (6.8)$$

$$\kappa^{A_i B_j} = \sqrt{\kappa^{A_i B_i} \kappa^{A_j B_j}} \left[\frac{\sqrt{\sigma_{ii} \sigma_{jj}}}{\frac{1}{2}(\sigma_{ii} + \sigma_{jj})} \right]^3 \quad (6.9)$$

The association term depends also on the choice of the association scheme, i.e., the number and type of association sites for the associating compounds. A schematic explanation of the association schemes is provided by Huang and Radosz (1990).

6.4 ESTIMATION OF AMINO ACID PC-SAFT PARAMETERS

In this work, amino acids were treated as non-associating molecules and since amino acids exist only as solids under normal conditions, the three pure-component parameters required were fitted to all binary aqueous mixture data (densities, activities and osmotic coefficients, vapor pressures and water activities) compiled in our database.

To characterize the association of water, a two association site model (2B model) was considered. Although a four-site model would reflect better the physics of the water

molecules, a two-site approach yields better agreement between model and reality (Gross and Sadowski, 2002). Besides that, using only two association sites instead of four, decreases the computational time.

The water pure component PC-SAFT parameters used were taken from Fuchs et al. (2006) and are given in Table 6.1. A temperature dependent segment diameter, given by equation 6.10, where T is the absolute temperature, was introduced by the authors to improve the description of the water densities at low temperatures. Mixtures with only one associating substance do not require mixing rules for the association term (Gross and Sadowski, 2002).

$$\sigma = 2.7927 + 10.10 \exp(-0.01775T) - 1.417 \exp(-0.01146T) \quad (6.10)$$

Table 6.1 Pure component PC-SAFT parameters for water (Fuchs et al., 2006).

	segment number m	segment diameter σ	energy Parameter ε/k	energy parameter (association) $\varepsilon^{A_i B_i}$	association volume $\kappa^{A_i B_i}$
water	1.2047	Eq. 6.10	353.95	2425.67	0.045

To fit the pure component PC-SAFT parameters for each amino acid, the objective function (*FOBJ*) chosen was the sum of squared relative deviation:

$$FOBJ = \sum_k \left[\frac{(Q)_k^{calc} - (Q)_k^{exp}}{(Q)_k^{exp}} \right]^2 \quad (6.11)$$

Q means thermodynamic property, namely density, activity and osmotic coefficients, vapor pressure, and water activity in aqueous solutions of the amino acid, for all experimental data points k . The superscripts *exp* and *calc* mean experimental and calculated quantities, respectively.

Table 6.2 presents the average relative deviations (ARD) (%) obtained for the different binary aqueous mixture data, number of data points, experimental temperature range and data sources. The small ARDs obtained indicate the accuracy of the correlation and, considering the wide temperature range used for the regression of density data, the ARDs for this property

are remarkably small. Some correlation results for binary aqueous amino acid (glycine and DL-alanine) data are illustrated in section 6.10. Generally, all the thermodynamic properties; densities, activities and osmotic coefficients, vapor pressures, and water activities in aqueous solutions of amino acid, are reproduced by the PC-SAFT EoS in very good agreement with the experimental data. It is worthwhile to mention that the literature survey shows a considerable lack of information for some amino acids: for L-isoleucine, only densities in aqueous solutions were found.

The estimated parameters for the amino acids and the respective standard deviation (SD) are given in Table 6.3. To have a good description of all thermodynamic properties, a binary interaction parameter (k_{ij}) for each amino acid/water system was introduced, which is listed in Table 6.4 for each pair. The estimation of k_{ij} followed an iterative procedure, first k_{ij} was fixed and the three pure component PC-SAFT parameters estimated, then the k_{ij} was changed, and the parameters refitted. To follow a reliable procedure and to obtain reasonable sets of parameters the calculated density for the pure amino acid in a hypothetical liquid state should be of the same order of magnitude of the crystal density. The calculated densities (d_{calc}) and the available density of the pure crystal (d_{crystal}), at 298.15 K, are also given in Table 6.3. The calculated densities are lower than the density of the pure crystal, with special attention to the one obtained for glycine with a higher deviation. For L-isoleucine, since the number of experimental data points is very limited, the standard deviation of the parameters were very high so, the segment number was fixed and only the segment diameter and energy parameters were estimated.

Table 6.2 Experimental data used to estimate the pure amino acid PC-SAFT parameters: average relative deviation (ARD)* for correlation (first row), number of data points (NDPs, second row), experimental temperature range (third row), data source (fourth row).

Amino acid	Density	Vapor pressure	Water activity	Activity coefficient	Osmotic coefficient	Total
Glycine	0.10	0.39	0.03	5.25	—	1.64
	149	14	183	151		497
	278 – 318 K	298 K	298 K	298 K		
	(g, i, j, k, r, s)	(h)	(d, k, l, m, o, p)	(d, m, o, p) [§]		
DL-alanine	0.08	0.36	0.03	—	0.29	0.12
	162	13	99		67	341
	278 – 318 K	298 K	298 K		298 K	
	(b, g, i, j, k, s)	(h)	(k, l, n, o, p)		(n, o, p)	
L-serine	0.06	0.47	0.07	—	0.34	0.12
	78	13	46		9	146
	278 – 328 K	298 K	298 K		298 K	
	(e, s)	(h)	(f, h, l)		(f)	
L-threonine	0.05	NA	0.003	—	0.20	0.08
	60		24		24	108
	288 – 328 K		298 K		298	
	(a, e, t)		(q)		(q)	
L-isoleucine	0.05	NA	NA	NA	NA	0.05
	106					106
	278 – 328 K					
	(c, g)					

(a) Banipal et al. (2007); (b) Dalton and Schmidt (1933); (c) Duke et al. (1994); (d) Ellerton et al. (1964); (e) Hakin et al. (1994); (f) Hutchens et al. (1963); (g) Kikuchi et al. (1995); (h) Kuramochi et al. (1997); (i) Lark et al. (2004); (j) Matsuo et al. (2002); (k) Ninni and Meirelles (2001); (l) Pinho (2008); (m) Richards (1938); (n) Robinson (1952); (o) Romero and González (2006); (p) Smith and Smith (1937b); (q) Smith and Smith (1940a); (r) Soto et al. (1998c); (s) Yan et al. (1999); (t) Yuan et al. (2006). NA – Not available.

[§] Calculated.

$$* \text{ARD} = 100 / \text{NDP} \sum_{k=1}^{\text{NDP}} \left| (Q_k^{\text{calc}} - Q_k^{\text{exp}}) / Q_k^{\text{exp}} \right|$$

Table 6.3 Pure component PC-SAFT parameters for amino acids.

Amino acid	Segment number		Segment diameter		Energy parameter		d_{crystal}	d_{calc}
	m	SD	σ	SD	ε/k	SD		
Glycine	5.0503	2.4E-2	2.27	7.8E-3	204.81	1.0E+0	1.607 (a)	1.413
DL-alanine	4.3623	6.9E-3	2.65	5.2E-3	226.02	1.5E-1	1.424 (a,b)	1.418
L-serine	5.2266	3.3E-3	2.48	4.1E-3	167.54	2.1E-1	NA	1.537
L-threonine	5.3468	4.7E-3	2.66	1.2E-3	218.91	7.3E-2	NA	1.559
L-isoleucine	3.0000	—	3.62	6.6E-3	257.26	3.2E+0	1.2 (c)	1.199

(a) Cohn et al. (1934); (b) Merck Index; (c) Zumstein and Rousseau (1989).

NA (Not available)

Table 6.4 Binary interaction PC-SAFT parameters of amino acid/water systems.

	Glycine	DL-alanine	L-serine	L-threonine	L-isoleucine
Water	-0.10	-0.10	-0.12	-0.10	-0.03

6.5 SOLUBILITY AND THE ESTIMATION OF HYPOTHETICAL MELTING PROPERTIES

Since amino acids decompose before melting, there is a great lack of data on melting properties. Therefore, to apply equation 6.4, melting properties were treated as adjustable parameters, as hypothetical properties with no physical meaning, and were fitted to the experimental solubility data of the amino acid in water at different temperatures. Figures 6.1 to 6.5 show the calculated solubility curves of the amino acids in water, where x^L is the amino acid mole fraction. The symbols represent experimental data and the curves were calculated with the PC-SAFT EoS. The solubility of the 5 amino acids in pure water can be described with a very good accuracy. For L-serine, the experimental data presents some scattering, especially at higher temperatures, and for this reason the ARD is much higher than the one obtained for the other amino acids as can be observed in Table 6.5. As mentioned above, the solubility of the amino acid at atmospheric pressure was calculated according to equation 6.4, where the fugacity coefficients of the substance in the mixture and as a pure substance were calculated using the PC-SAFT equation of state. The estimated hypothetical properties and the respective SD are also given in Table 6.5.

Reducing the number of adjustable parameters, and since there was no difference in the ARD values, the influence of the change in heat capacity was neglected, except for L-isoleucine where its estimation was mandatory to obtain the desired accuracy. The objective function used to fit the hypothetical melting properties was the same as presented before (equation 6.11) where *Prop* corresponds to the solubilities of the amino acid in water (x_s^L).

Table 6.5 Hypothetical fusion properties for amino acids.

Amino acid	Enthalpy of melting		Melting temperature		Difference in the heat capacity		Solubility data source	ARD* (%)
	ΔH_o [kJ.mol ⁻¹]		T_o [K]		ΔC_p [kJ.mol ⁻¹ .K ⁻¹]			
	SD		SD		SD			
Glycine	21.97	1.8E-1	489.78	2.4E0	neglected		(a, g)	1.68
DL-alanine	15.98	3.2E-2	581.72	1.0E0	neglected		(a, b, g)	0.33
L-serine	24.54	9.8E-1	375.28	3.7E0	neglected		(c, d, e, g)	8.19
L-threonine	17.72	1.6E-1	637.12	5.8E0	neglected		(f, g)	1.45
L-isoleucine	11.27	8.9E-1	621.28	3.7E+1	0.073	6.1E-3	(g, h)	1.08

(a) Dalton and Schmidt (1933); (b) Dunn et al. (1933); (c) Hutchens (1976); (d) Jin and Chao (1992); (e) Luk and Rousseau (2006); (f) Profir and Matsuoka (2000); (g) This work; (h) Zumstein and Rousseau (1989).

$$* \text{ARD} = 100 / \text{NDP} \sum_{k=1}^{\text{NDP}} \left| \left(x_{aa,k}^{\text{calc}} - x_{aa,k}^{\text{exp}} \right) / x_{aa,k}^{\text{exp}} \right|$$

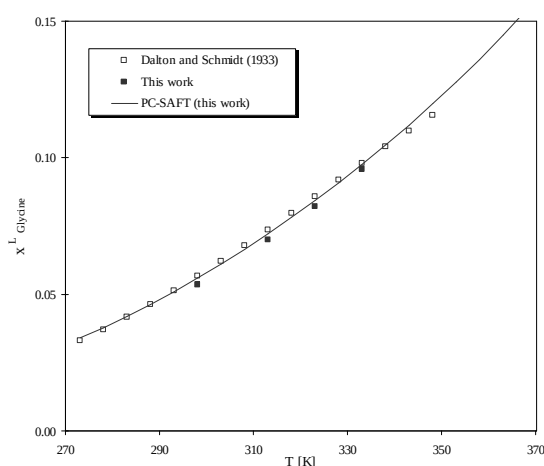


Figure 6.1 Solubilities of glycine in water at different temperatures.

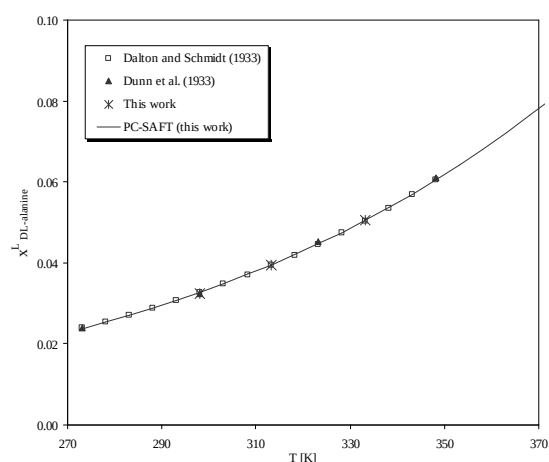


Figure 6.2 Solubilities of DL-alanine in water at different temperatures.

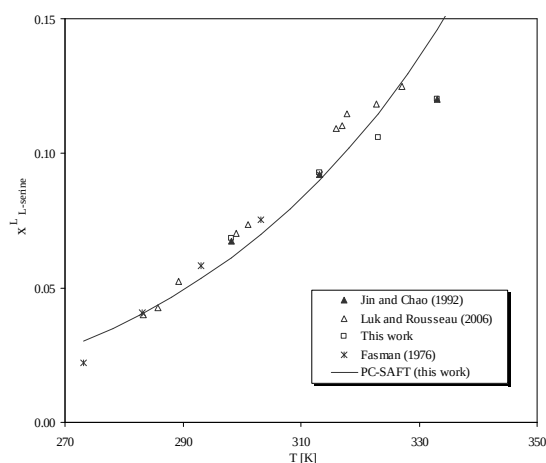


Figure 6.3 Solubilities of L-serine in water at different temperatures.

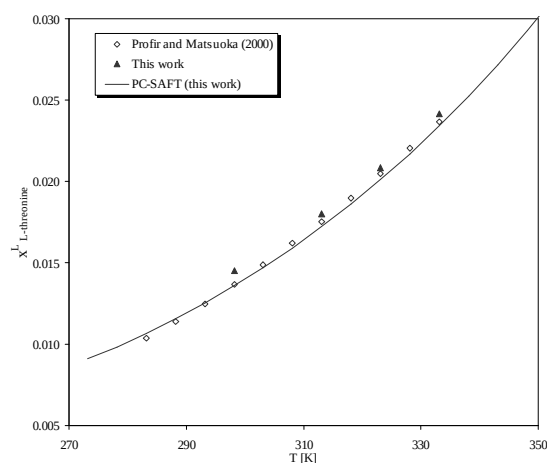


Figure 6.4 Solubilities of L-threonine in water at different temperatures.

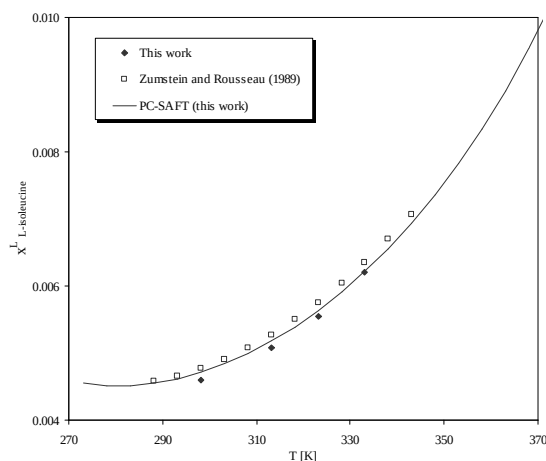


Figure 6.5 Solubilities of L-isoleucine in water at different temperatures.

6.6 MODELLING AMINO ACID SOLUBILITIES IN PURE ALCOHOLS

To model the solubility of the amino acids in pure alcohols the methodology applied by Fuchs et al (2006) was followed. To characterize the association of the alcohols, two association sites were assigned (2B model). The pure PC-SAFT parameters for the alcohols were taken from Fuchs et al. (2006) and are given in Table 6.6, while the amino acid parameters have been summarized before (Table 6.3). For each binary amino acid/alcohol systems a binary parameter k_{ij} was introduced to correlate the solubility data. These parameters are given in Table 6.7.

Since the solubility temperature dependence of glycine, DL-alanine, L-serine and L-threonine, Figures 6.6 to 6.9, respectively, is not correctly described; the binary parameters (amino acid-alcohols) were fitted to the solubility data at 298.15 K only. For L-isoleucine (Figure 6.10) the correlation results for the solubilities in pure alcohols is much better than for the other amino acids.

Table 6.6 Pure component PC-SAFT parameters for alcohols (Fuchs et al., 2006).

	Segment number m	Segment diameter σ	Energy parameter ε/k	Energy parameter (association) $\varepsilon^{A_i B_i}$	Association volume $k^{A_i B_i}$
Ethanol	2.3827	3.18	198.24	2653.38	0.032
1-Propanol	2.9997	3.25	233.40	2276.78	0.015
2-Propanol	3.0929	3.21	208.42	2253.90	0.025

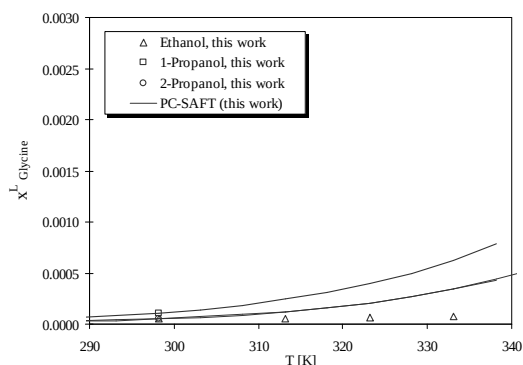


Figure 6.6 Solubilities of glycine in different pure alcohols (k_{ij} adjusted to the pure alcohol).

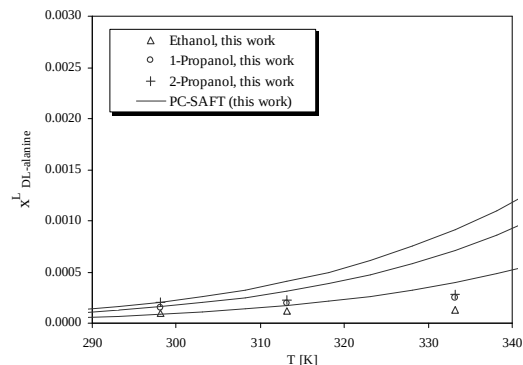


Figure 6.7 Solubilities of DL-alanine in different pure alcohols (k_{ij} adjusted to the pure alcohol).

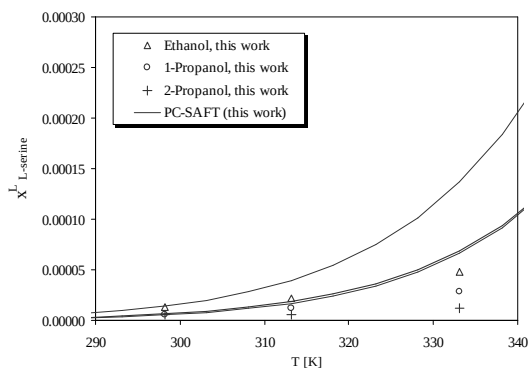


Figure 6.8 Solubilities of L-serine in different pure alcohols (k_{ij} adjusted to the pure alcohol).

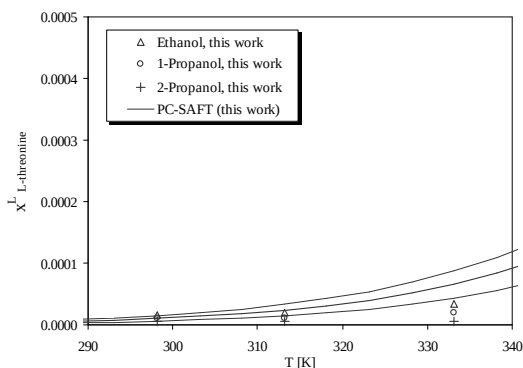


Figure 6.9 Solubilities of L-threonine in different pure alcohols (k_{ij} adjusted to the pure alcohol).

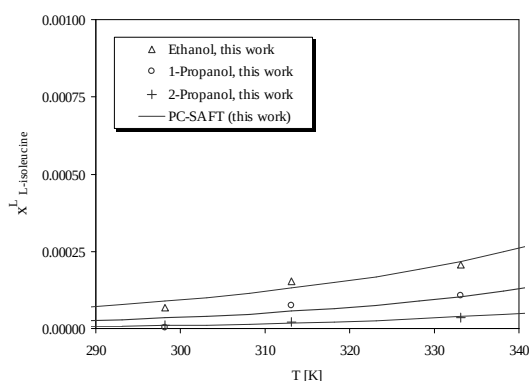


Figure 6.10 Solubilities of L-isoleucine in different pure alcohols (k_{ij} adjusted to the pure alcohol).

6.7 MODELLING AMINO ACID SOLUBILITIES IN MIXED SOLVENTS

The amino acids solubilities in solvent mixtures were predicted using the pure components parameters for the amino acids and solvents, as well as the binary amino acid/solvent parameters estimated so far. The binary PC-SAFT parameters of water/alcohol systems used were the ones reported by Fuchs et al. (2006) and presented in Table 6.8. The solubilities of the amino acids in different water-alcohol mixtures were predicted using only information from the binary systems without any additional parameters. Figures 6.11a to 6.15a show the predicted solubilities of the considered amino acids in different water/alcohol mixtures (ethanol, 1-propanol and 2-propanol), where the ratio of alcohol (2) and water (1) in the equilibrium solutions is given by x_2 / x_1 . Since the solubility in pure alcohol is very small, the solubility axis was extended for better visibility of the experimental data and model predictions at high alcohol concentrations. The temperature dependency is in good agreement with the experimental data. However, it was observed that the predicted solubilities, for all the systems, are always below the experimental data.

Following a different methodology, the binary amino acid/alcohol parameters were, after, treated as an adjustable parameters not to the solubilities in pure alcohols but to the solubilities in the mixed solvent systems. The new binary amino acid/alcohol parameters are summarized in Table 6.7. Figures 6.11b to 6.15b show the correlated solubilities of the considered amino acids in different alcohol-water mixtures using the new fitted binary amino acid/alcohol parameters. These new results are now in much better agreement with the

experimental data. However, the amino acids solubilities in pure alcohols became poorly represented.

For the solubility of the amino acids glycine, DL-alanine and L-threonine, in the pure solvents and in various alcohol-water mixtures similar performances were observed. As mentioned before, for the different ratios of alcohol to water, the predicted solubilities are below to the experimental data (Figures 6.11a, 6.12a and 6.14a, respectively). Now, the correlation results at medium solvent ratios of the different alcohols - shown in Figures 6.11b, 6.12b and 6.14b, respectively - are in very good agreement with the literature data. Using a binary amino acid/alcohol parameter for each system, adjusted to the mixed solvent system, the description of the amino acid solubility in mixed solvent systems was greatly improved.

The solubility of L-serine in several alcohol-water mixtures is shown in Figures 6.13a and b. Using the binary parameter k_{ij} estimated from the solubility data in pure alcohol, large divergences can be found for the predictions at low alcohol ratios (e.g. $x_2/x_1 = 0.098$, for aqueous ethanol mixtures and $x_2/x_1 = 0.075$, for aqueous 2-propanol mixtures, see Figure 6.13a). Adjusting the k_{ij} to the mixed solvent systems there is a considerable quantitative improvement (Figure 6.13b). The same calculations were performed for the solubility of L-isoleucine in aqueous mixtures of ethanol, 1-propanol and 2-propanol (Figures 6.15a and b). The predicted solubilities, as shown in Figure 6.15a, were not reasonable, but when the binary parameter k_{ij} is adjusted to the ternary system data the solubilities for high alcohol ratios show much better agreement with the experimental data.

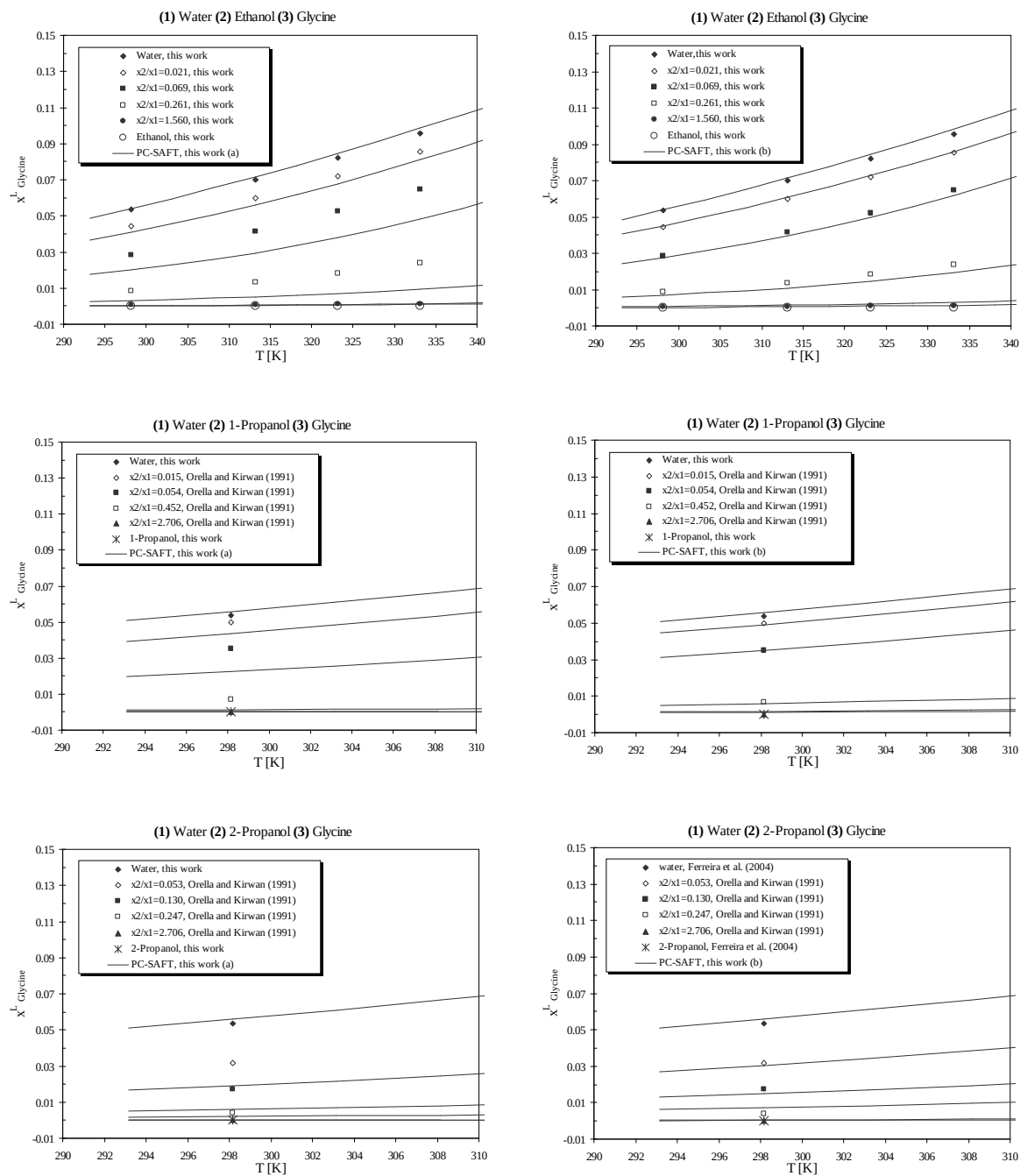
Table 6.7 Binary interaction PC-SAFT parameters of amino acid/alcohol systems.

	k_{ij}					
	Ethanol		1-Propanol		2-Propanol	
Glycine	0.22	0.15*	0.21	0.12*	0.24	0.14*
DL-alanine	0.18	0.12*	0.17	0.11*	0.16	0.13*
L-serine	0.35	0.23*	0.38	0.21*	0.40	0.20*
L-threonine	0.18	0.12*	0.20	0.11*	0.22	0.12*
L-isoleucine	0.31	0.26*	0.31	0.22*	0.38	0.25*

* k_{ij} adjusted to the mixed solvent systems.

Table 6.8 Binary interaction PC-SAFT parameters of water/alcohol systems (Fuchs et al., 2006).

	k_{ij}		
	Ethanol	1-Propanol	2-Propanol
Water	-0.0382	-0.017	-0.044

**Figure 6.11** Solubilities of glycine in various alcohol-water mixtures: PC-SAFT (a) prediction, (b) correlation.

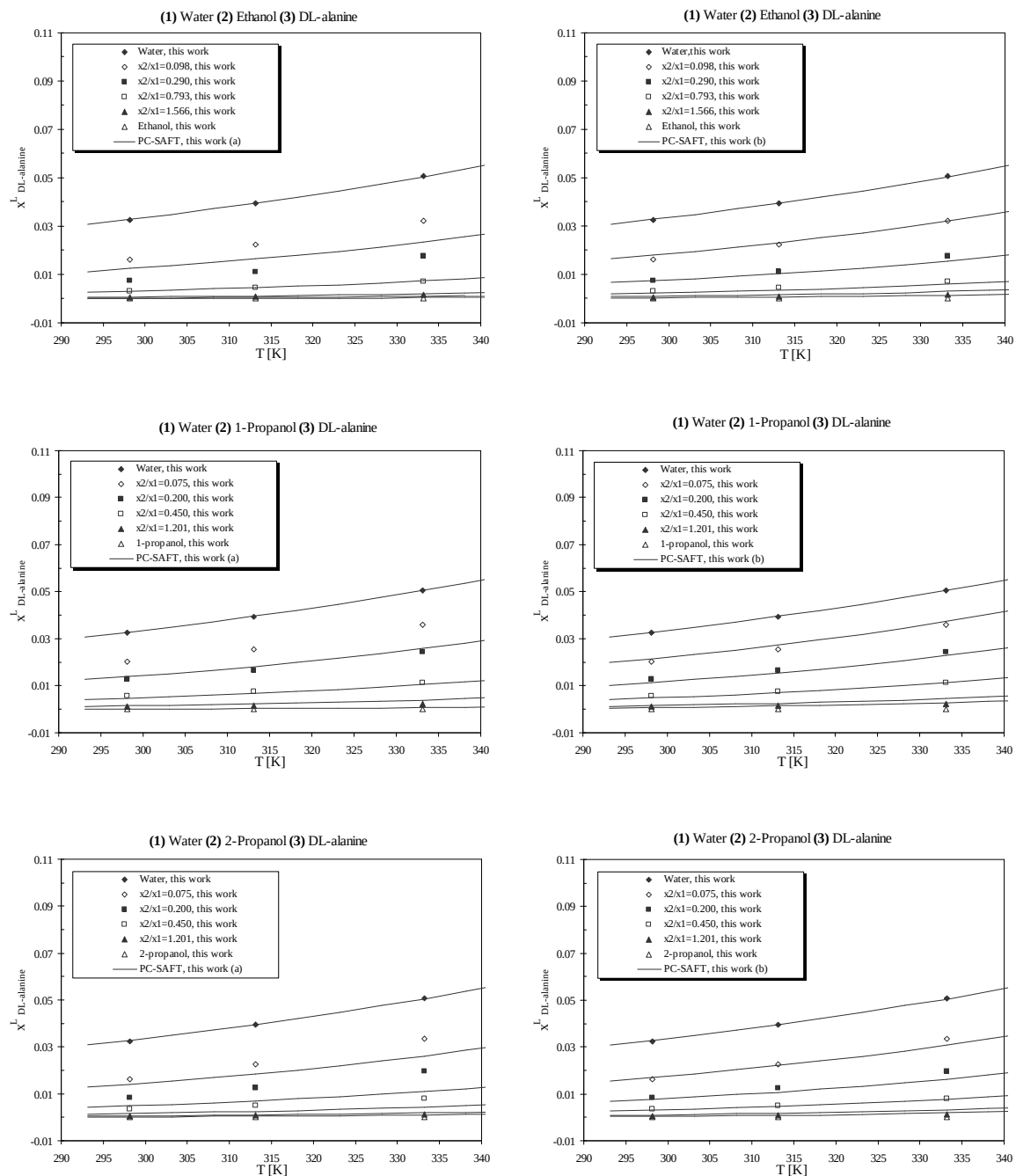


Figure 6.12 Solubilities of DL-alanine in various alcohol-water mixtures: PC-SAFT (a) prediction, (b) correlation.

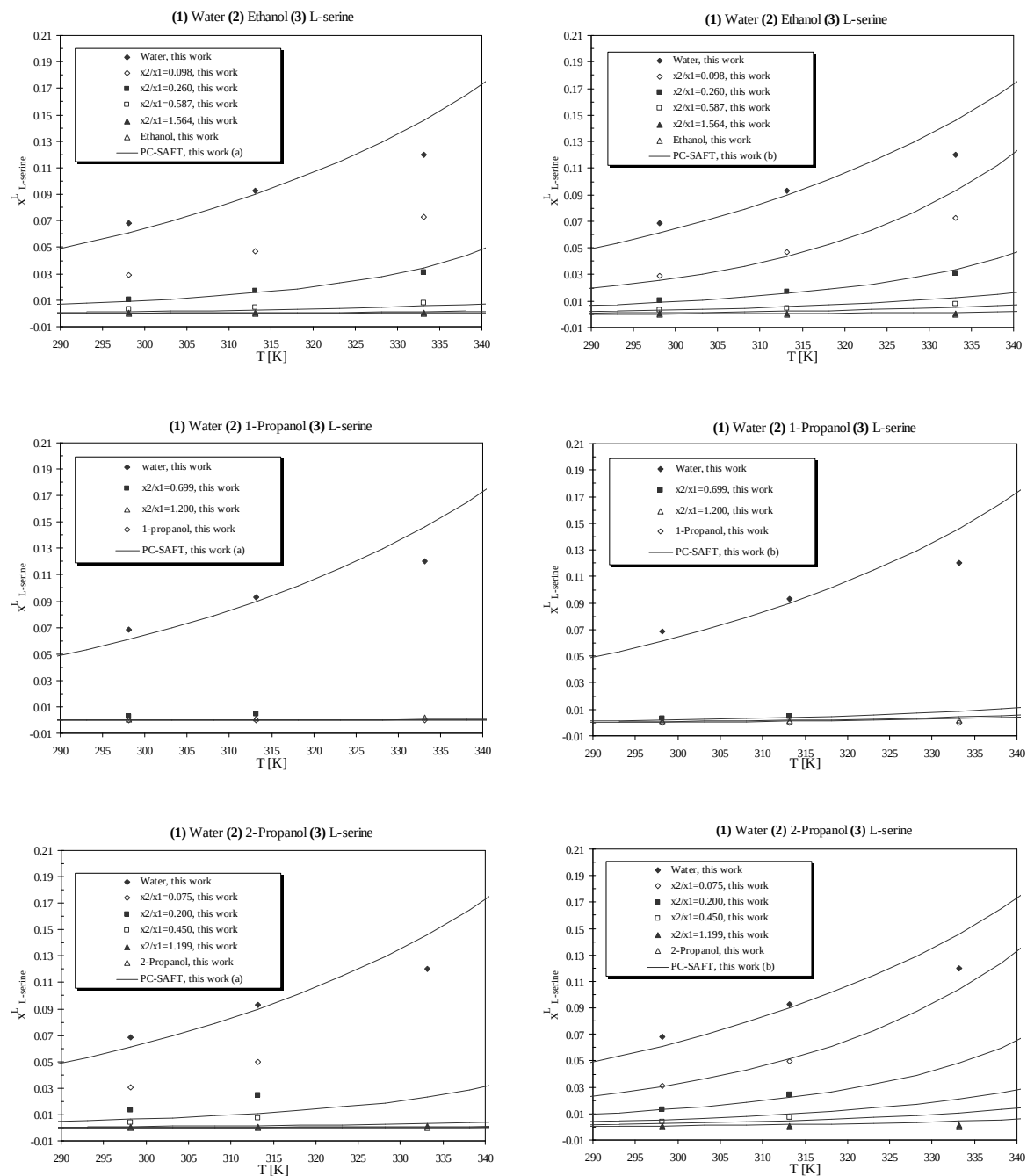


Figure 6.13 Solubilities of L-serine in various alcohol-water mixtures: PC-SAFT (a) prediction, (b) correlation.

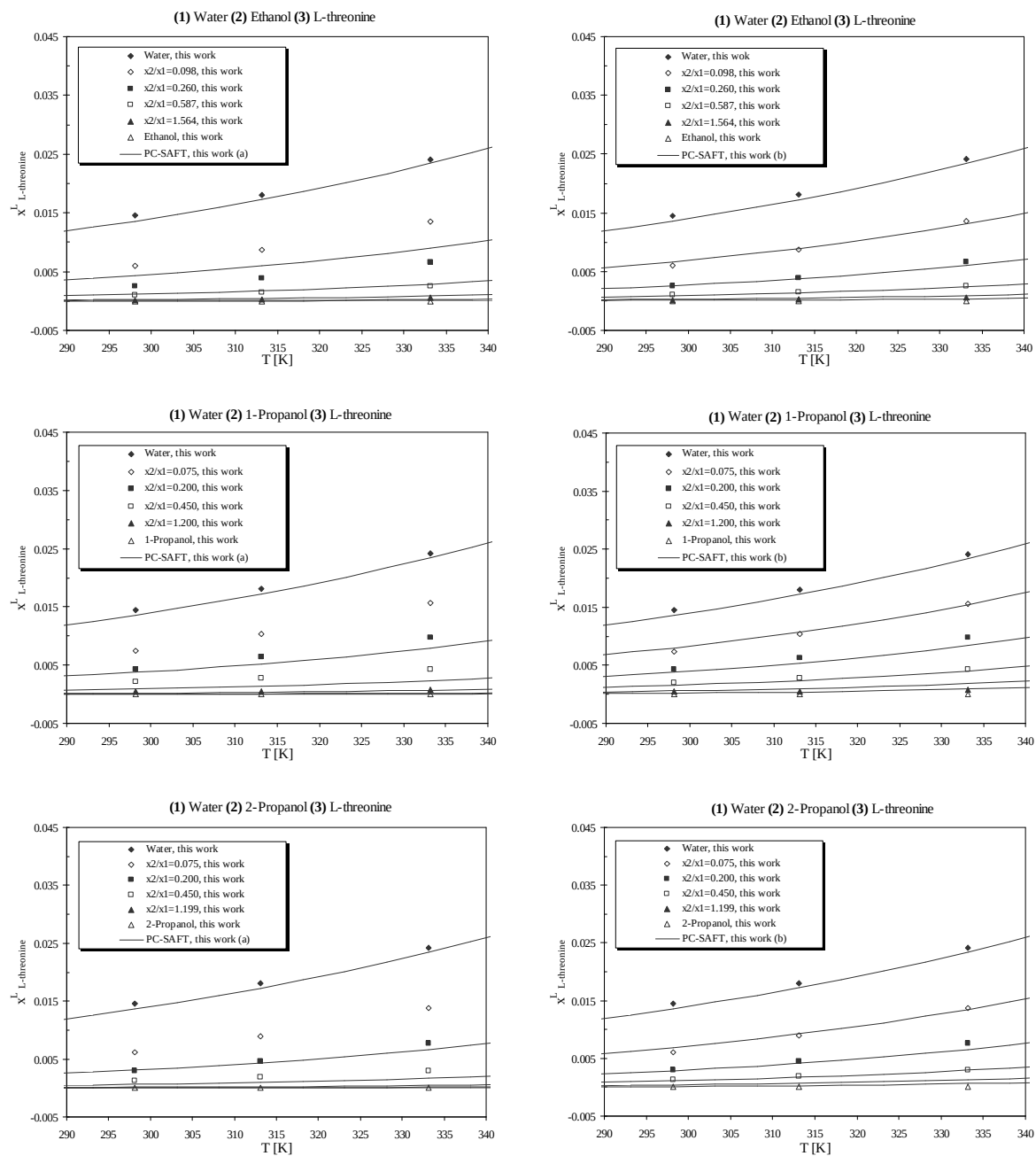


Figure 6.14 Solubilities of L-threonine in various alcohol-water mixtures: PC-SAFT (a) prediction, (b) correlation.

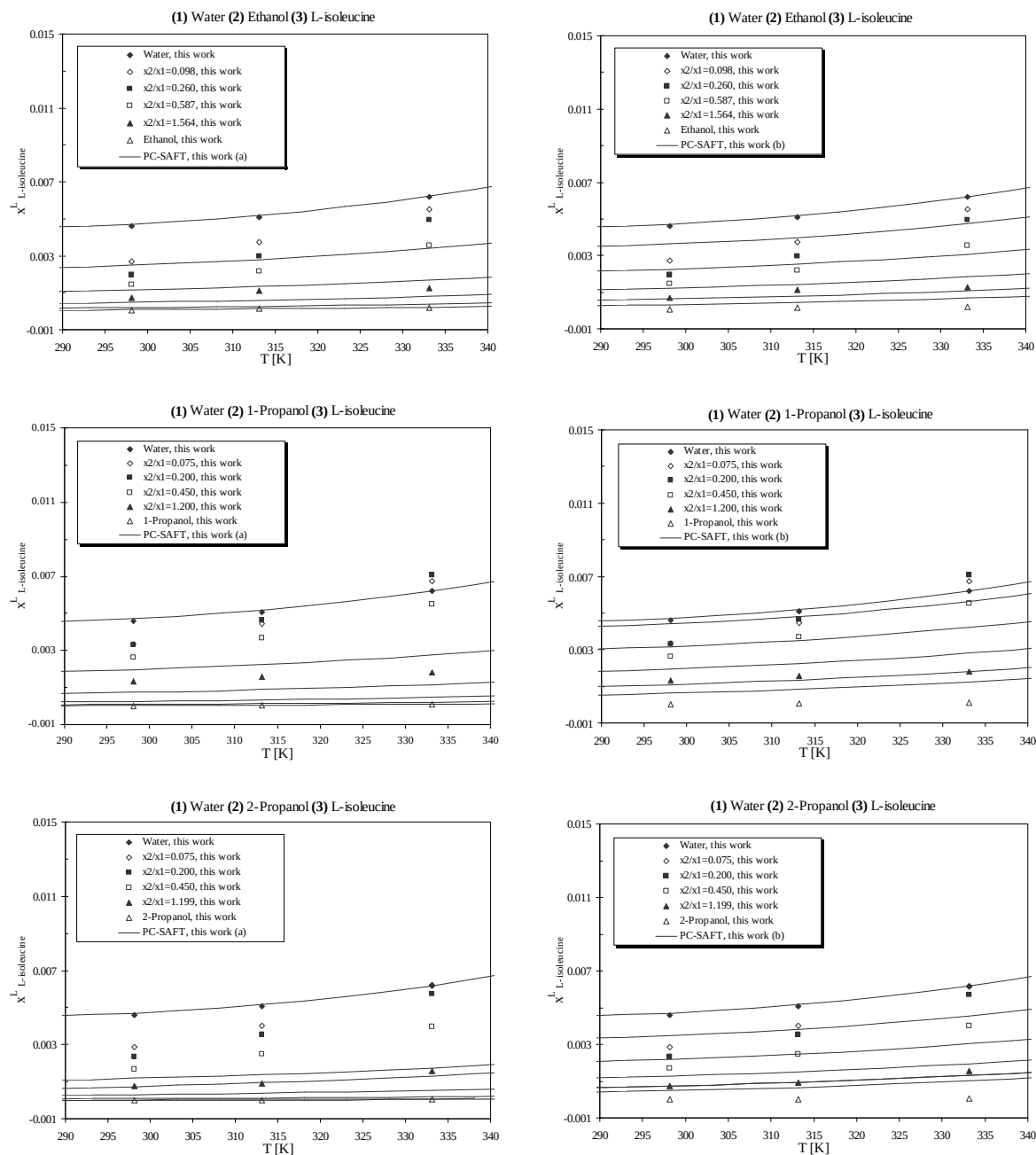


Figure 6.15 Solubilities of L-isoleucine in various alcohol-water mixtures: PC-SAFT (a) prediction, (b) correlation.

6.8 PARAMETERS ESTIMATION - DISCUSSION

A database on amino acid (glycine, DL-alanine, L-serine, L-threonine and L-isoleucine) properties in aqueous solution, namely, densities, vapor pressures, osmotic coefficients, activity coefficients and water activities, was built to allow the estimation of the amino acid pure PC-SAFT parameters (see Table 6.2). Since the number of experimental data points is very limited it was necessary to reduce the number of parameters to be estimated, and the amino acids were considered as non-associating molecules. A binary parameter (k_{ij}) was fixed and the three PC-SAFT parameters estimated. To have a consistent procedure, the calculated density (d_{calc}) of the pure amino acid in a hypothetical liquid state should be of the same order of magnitude as the density of the pure crystal. To explain the importance of this procedure DL-alanine will be used as example. In Table 6.9, three different sets of parameters are given as well as the respective calculated density (d_{calc}) and the ARD; that value for each thermodynamic property are also presented in Table 6.10. Using a $k_{ij} = -0.15$ the description of the osmotic coefficients of DL-alanine solutions are improved (Figure 6.16) while the other thermodynamic properties show similar ARD (Table 6.10), but the calculated density is lower than the density of the pure crystal (Table 6.3). The parameters obtained for the three sets were used to calculate the hypothetical melting properties. The enthalpy and temperature of melting of the pure amino acid were adjusted without any constrain and the results are displayed in Table 6.11. As mentioned before, these properties were treated as hypothetical properties with no physical meaning and were adjusted to get a good fit of the experimental solubility data. The calculated DL-alanine symmetric activity coefficients in pure water, at saturated conditions, using the different sets of parameters were plotted in Figure 6.17. The solubility is described with the same accuracy and the hypothetical properties are of the same order of magnitude. However, some difference can be observed for the calculated symmetric activity coefficients. Since there is no available information for the temperature influence in the symmetric activity coefficient of the amino acid the preference goes to the one that gives a reasonable density for the hypothetical pure amino acid in the liquid state, in this case it corresponds to the set of parameters obtained with a $k_{ij} = -0.10$. The same methodology was followed for all the other amino acids. This criterion becomes even more useful when the number of the experimental data points available decreases, e.g. L-isoleucine; only

experimental densities could be found in the literature, so the density of the pure crystal was also included in the estimation of the pure PC-SAFT parameters.

Table 6.9 PC-SAFT parameters for DL-alanine.

Amino acid	Set	k_{ij}	Segment number m	Segment diameter σ	Energy parameter ε/k	ARD (%)	d_{calc}
DL-alanine	1	-0.06	3.3195	2.9282	292.3921	0.15	1.463
	2	-0.10	4.3623	2.6506	226.0170	0.12	1.418
	3	-0.15	4.9178	2.5526	174.3154	0.10	1.276

Table 6.10 Average relative deviation (%) obtained for the different thermodynamic property using different PC-SAFT parameters for DL-alanine.

Thermodynamic property	k_{ij}		
	-0.06	-0.10	-0.15
Densities	0.08	0.08	0.07
Vapor pressure	0.36	0.36	0.36
Water activity	0.03	0.03	0.02
Osmotic coefficient	0.47	0.29	0.21

Table 6.11 Hypothetical properties for DL-alanine.

Amino acid	Set	Enthalpy of melting		Melting temperature		Difference in the heat capacity ΔC_p [kJ.mol ⁻¹ .K ⁻¹]	Solubility data source	ARD (%)
		ΔH_o [kJ.mol ⁻¹]		T_o [K]				
DL-alanine	1	11.35	4.8E-2	635.98	2.8E0	neglected	(a, b, c)	0.49
	2	15.98	3.2E-2	581.72	1.0E0	neglected		0.33
	3	20.64	4.1E-2	514.04	6.8E-1	neglected		0.39

(a) Dalton and Schmidt (1933); (b) Dunn et al. (1933); (c) This work.

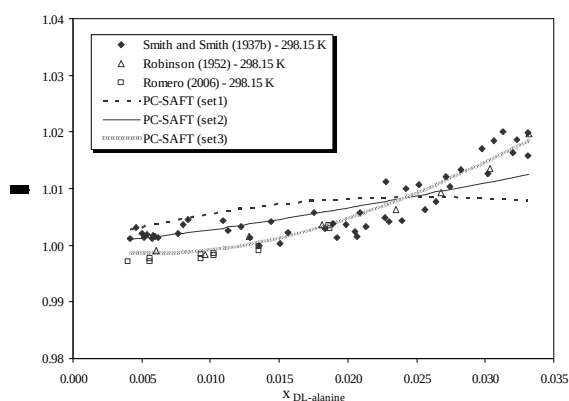


Figure 6.16 The osmotic coefficients in aqueous DL-alanine solutions at 298.15 K.

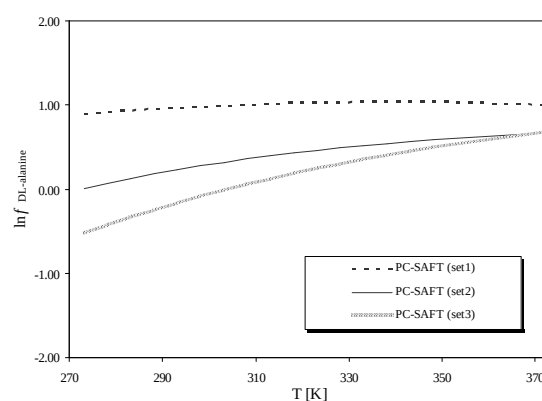


Figure 6.17 Symmetric activity coefficients in aqueous DL-alanine solutions at different temperatures (saturated conditions).

As already mentioned, for glycine, the calculated density presents higher deviation to the density of the pure crystal, but the melting temperature estimated is in very good agreement with the experimental value given in DIPPR (509.4 K).

Concerning the amino acid solubilities in pure solvents, the solubility in pure water was reproduced with accuracy but the description in pure alcohols was not so accurate. This is easy to understand since the melting properties were treated as adjustable parameters in the modelling of the solubility of amino acids in pure water. However, the possibility of fitting those melting properties to the experimental solubility data of the amino acids in pure alcohol instead, was also considered. The solubility of L-serine in ethanol is here considered as an example. The hypothetical melting properties obtained were $\Delta H_o = 32.27 \text{ kJ.mol}^{-1}$; $T_o = 408.76 \text{ K}$ and $\Delta C_p = 0.433 \text{ kJ.mol}^{-1}.\text{K}^{-1}$. As observed in Figure 6.18 (dashed line) the solubility of this amino acid in the alcohol is now correctly described. The pure PC-SAFT parameters used for L-serine and ethanol were the ones given in Tables 6.3 and 6.6, respectively; a $k_{ij} = 0.38$ was necessary to correlate the solubility data quantitatively. When these hypothetical melting properties were used to calculate the solubility in water, the description became very poor (Figure 6.19, dashed line). This result can indicate that the crystal phases of the pure substance may not be identical in both solvents.

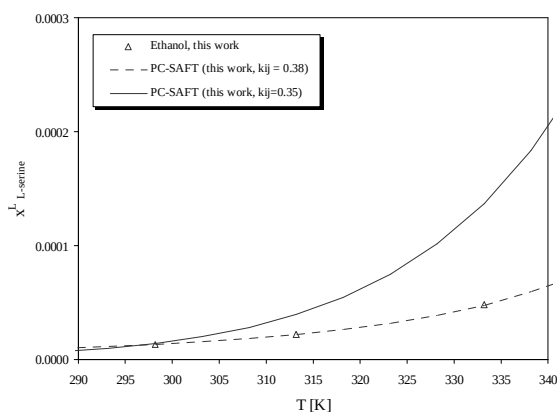


Figure 6.18 Solubilities of L-serine in pure ethanol (k_{ij} adjusted to the pure alcohol).

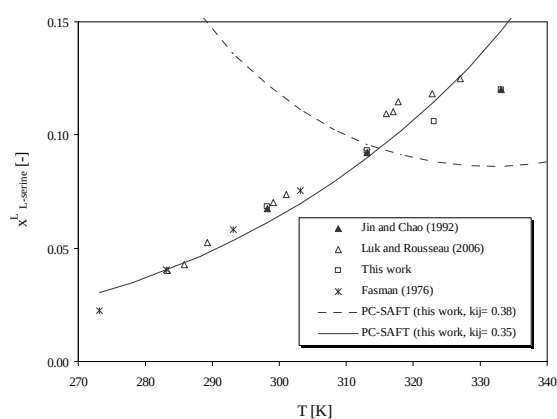


Figure 6.19 Solubilities of L-serine in water at different temperatures (saturated conditions).

Concerning the ternary mixtures, the solubility of the amino acid at different alcohol/water ratios was predicted using only the parameters estimated from the binary systems. Since the PC-SAFT predictions were always inferior to the respective experimental data (Figures 6.11a to 6.15a) the binary parameters for the amino acid-alcohol systems were refitted. In this way, the dependency of the solubility on the solvent composition for the ternary systems was improved (Figures 6.11b to 6.15b) but, as consequence, the calculated solubilities in pure alcohols were penalized.

The solubility of the L-isoleucine in alcohol-water mixtures shows a particular behavior, especially for the system with 1-propanol at 333.15 K (Figure 6.15). At this temperature, the solubility, expressed in mole fraction, is superior to the solubility in pure water. For this system, the correlations obtained with the PC-SAFT EoS were only in agreement with experimental data for higher alcohol ratios. The same was observed for the other alcohol systems studied: large deviations were obtained for the medium and low solvent ratios.

In a new attempt to improve the results obtained, with especial attention to the systems with L-isoleucine, association was considered also for the amino acids. Two different types of association sites (of equal strength), each of them having two sites were assumed to characterize the association of amino acids (Fuchs et al., 2006). The results obtained for L-isoleucine in aqueous 1-propanol solutions will be presented. The five pure PC-SAFT parameters required for an associating molecule were estimated and are listed with the respective SD in Table 6.12. The PC-SAFT parameters for water are those presented before.

A $k_{ij} = -0.03$ for the interaction water/amino acid was considered. The experimental data used in the correlation was the same used before (see Table 6.2), and the order of magnitude of the standard deviation was, as expected, very high. The calculated density was 1.200 and the average relative deviation 0.05%. The estimated hypothetical melting properties were $\Delta H_o = 8.54 \text{ kJ.mol}^{-1}$; $T_o = 592.90 \text{ K}$ and $\Delta C_p = 0.054 \text{ kJ.mol}^{-1}.\text{K}^{-1}$ with an ARD equal to 1.14%. A $k_{ij} = 0.31$ was necessary to describe the solubility of the amino acid in pure 1-propanol. Using these new parameters the solubility of L-isoleucine in different 1-propanol aqueous mixtures was predicted and the results presented in Figure 6.20. The prediction results are not as good as the ones obtained for the amino acids as non-associating molecules. So, even if similar results to the ones presented before, with no association, were obtained for the binary systems, the association did not improve the prediction results, and the number of parameters to be estimated increased.

Table 6.12 Pure component PC-SAFT parameters for L-isoleucine (associating substance).

Amino acid	Segment number		Segment diameter		Energy parameter		Energy parameter (association)		Association volume	
	m	SD	σ	SD	$\mathcal{E}/k$	SD	$\epsilon^{A_i B_i}$	SD	$k^{A_i B_i}$	SD
L-isoleucine	3.5385	7.8E+1	3.41	2.7E+1	229.21	3.0E+3	1109.25	1.4E+5	0.001	4.5E-1

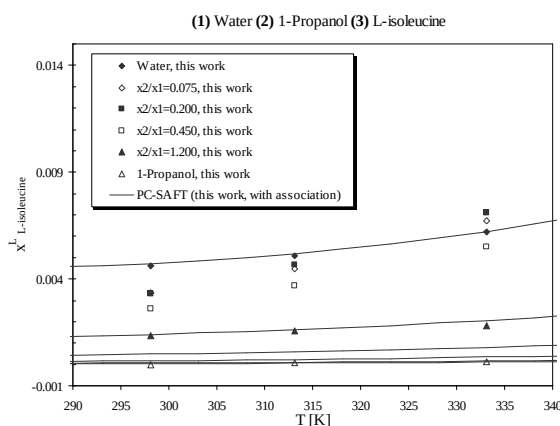


Figure 6.20 Solubilities of L-isoleucine in various 1-propanol-water mixtures (PC-SAFT EoS prediction, amino acid considered as an associating molecule).

6.9 PC-SAFT PARAMETERS BY FUCHS ET AL. (2006)

To characterize the association of amino acids, Fuchs et al. (2006) considered two different types of association sites, each of them having two sites and both types were assumed to be of equal strength. Only vapor-liquid equilibrium data and densities of aqueous solutions were used by Fuchs et al. (2006) to calculate the amino acid parameters. The parameters estimated by Fuchs et al. (2006) are summarized in Table 6.13.

Table 6.13 Pure component PC-SAFT parameters for amino acids given by Fuchs et al. (2006).

Amino acid	Segment number m	Segment diameter σ	Energy parameter ε/k	Energy parameter (association) $\varepsilon^{A_i B_i}$	Association volume $\kappa^{A_i B_i}$	d_{calc}
Glycine	3.7900	2.61	320.00	1539.53	0.025	1.623
DL-alanine	2.6408	3.28	386.26	2797.72	0.029	1.504

For the other associating components (water and alcohols) two association sites (2B model) are assigned. Water and alcohols pure-component PC-SAFT parameters used by Fuchs et al. (2006) are the ones given in Tables 6.1 and 6.6, respectively.

When the solubility of the amino acid in pure solvents was considered, one constant (temperature independent) binary parameter k_{ij} for each binary solute/solvent system was introduced to correlate the solubility data. The binary PC-SAFT parameters of amino acid/solvent systems are shown in Table 6.14.

Table 6.14 Binary interaction PC-SAFT parameters of amino acids/solvent systems given by Fuchs et al. (2006).

	k_{ij}			
	Water	Ethanol	1-Propanol	2-Propanol
Glycine	-0.0665	0.054	0.053	0.058
DL-alanine	-0.0598	0.015	0.007	-0.02

The estimated hypothetical melting properties are also listed in Table 6.15. Those were treated as adjustable parameters and fitted to the slope of the amino acid solubility in water (Fuchs et al., 2006). To get reasonable values, the calculated enthalpy of melting should not deviate

more than 16% from the enthalpy of melting calculated by the method proposed by Marrero and Gani (2001). The estimated enthalpies of melting using that group-contribution method were 28.4 kJ.mol^{-1} for glycine, and 25.9 kJ.mol^{-1} for DL-alanine (Fuchs et al., 2006).

Table 6.15 Hypothetical melting properties given by Fuchs et al. (2006).

Amino acid	Enthalpy of melting	Melting temperature	Difference in the
	$\Delta H_o [\text{kJ.mol}^{-1}]$	$T_o [\text{K}]$	heat capacity $\Delta C_p [\text{kJ.mol}^{-1}.\text{K}^{-1}]$
glycine	24.1	565.00	neglected
DL-alanine	21.90	963.22	neglected

6.9.1 CRITICAL ANALYSIS

In this section, densities, vapor pressures, amino acid activity coefficients, water activities and solubilities in aqueous solutions are calculated using the PC-SAFT parameters given by Fuchs et al. (2006) and the results investigated. The five pure-component PC-SAFT parameters for associating substances, the binary PC-SAFT parameters of amino acid/water systems, and the hypothetical melting properties were presented before. The calculations are shown in Figures 6.21 to 6.25 for glycine and Figures 6.26 to 6.30 for DL-alanine. The solid lines, which corresponds to the calculations of Fuchs et al. (2006) with a $k_{ij} = 0.0$, show, generally, a good agreement with the experimental data. However, Figures 6.25 (glycine) and 6.30 (DL-alanine), demonstrates that with a $k_{ij} = 0.0$ the solubility of the amino acids in pure water is poorly correlated (solid lines) so, the introduction of a k_{ij} was necessary to have a good agreement with the experimental solubility data (dashed lines). That binary parameter has a very minor effect on the correlation of densities (Figures 6.21 and 6.26), but a pronounced one on the calculated vapor pressures, activity coefficients and water activities; for these properties the agreement with experimental data is not so good as with a $k_{ij} = 0.0$. In fact, to quantitatively represent solubility data, though the estimation of a k_{ij} parameter, Fuchs et al. (2006) overestimate considerably the values for the osmotic and activity coefficients, that in some cases show an inverse trend to the experimental observations.

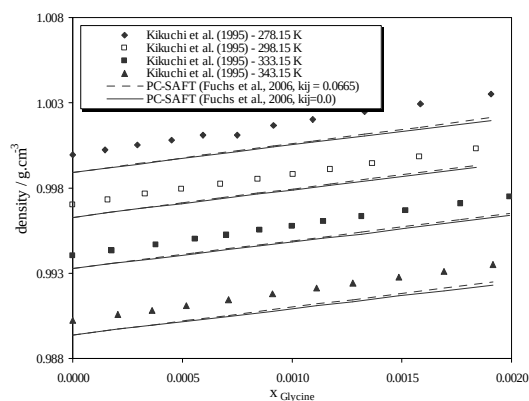


Figure 6.21 Densities of aqueous glycine solutions at different temperatures.

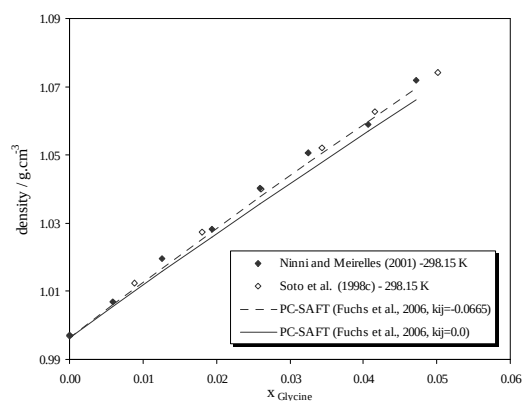


Figure 6.22 Vapor pressures in aqueous glycine solutions.

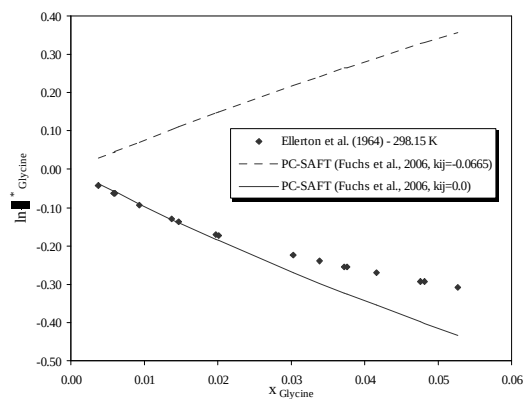


Figure 6.23 Unsymmetric activity coefficients in aqueous glycine solutions.

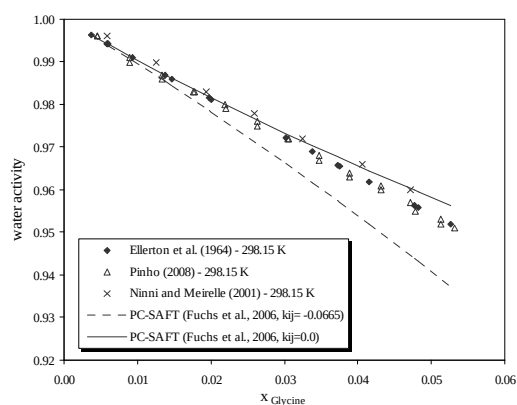


Figure 6.24 Water activities in aqueous glycine solutions.

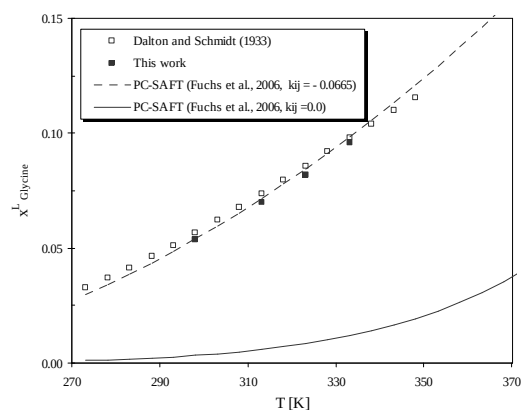


Figure 6.25 Solubilities of glycine in water at different temperatures.

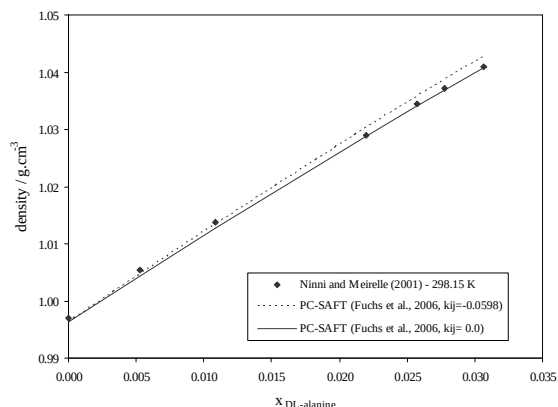
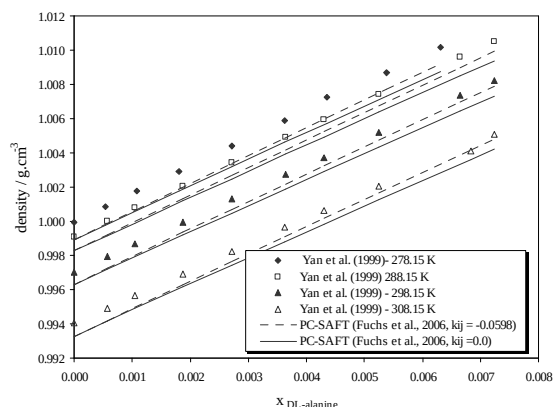


Figure 6.26 Densities of aqueous DL-alanine solutions at different temperatures.

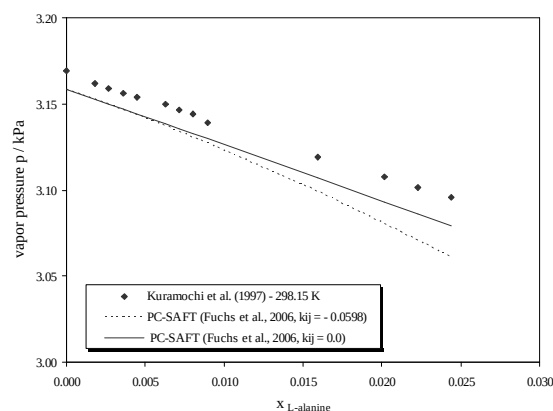


Figure 6.27 Vapor pressures in aqueous L-alanine solutions.

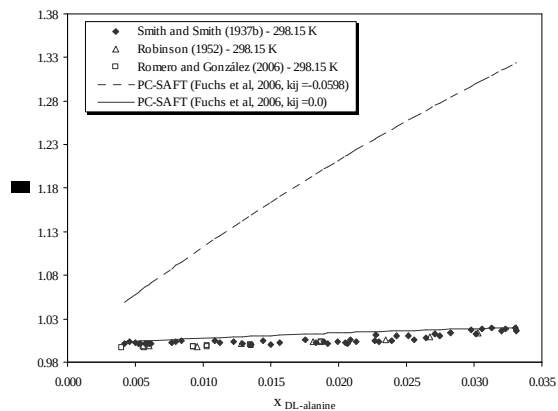


Figure 6.28 Osmotic coefficients in aqueous DL-alanine solutions.

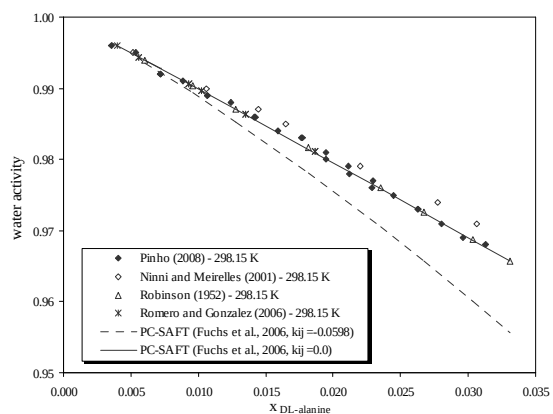


Figure 6.29 Water activities in aqueous DL-alanine solutions.

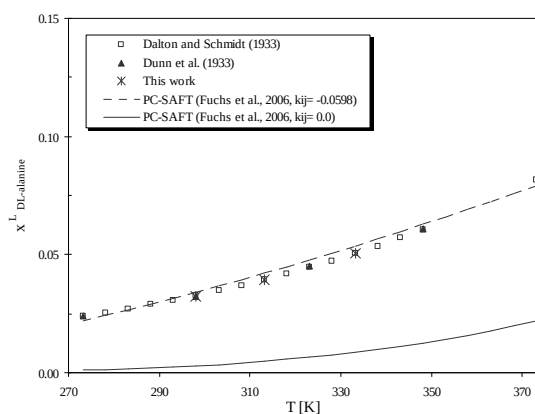


Figure 6.30 Solubilities of DL-alanine in water at different temperatures.

Although the solubility of glycine and DL-alanine in water was described with accuracy, for the alcohols, the model calculates steeper slopes of the solubility curves (Fuchs et al., 2006). The authors suggested that the correlation results could be improved using temperature dependent binary parameters. However, since their main goal was to use the pure-component and binary parameters to predict the solubility of amino acids in mixed solvent systems without fitting any additional parameter, the k_{ij} was fitted to the solubility data at 298.15 K.

6.9.2 PREDICTION OF AMINO ACID SOLUBILITIES IN MIXED SOLVENTS

As mentioned before, based on the modelling of the solubility in pure solvents, Fuchs et al. (2006) predicted the solubility in mixed solvents (ternary systems) without fitting any additional parameters. The results obtained for glycine and DL-alanine are displayed in the next section and compared with the ones developed in this work. Predictions and experimental data are in fair agreement. The authors state divergences at medium solvent ratios for ethanol and at higher solvent ratios for 1-propanol and 2-propanol for systems with glycine and deviations at medium solvent ratios for aqueous mixtures of alcohols for the systems with DL-alanine.

6.10 PC-SAFT PARAMETERS – A COMPARISON OF THE RESULTS OF THIS WORK AND THOSE OF FUCHS ET AL. (2006)

The results obtained in this work and the ones achieved by Fuchs et al. (2006) are now compared and discussed. In this study, glycine and DL-alanine were treated as non-associating molecules and their pure PC-SAFT parameters were refitted to get a better representation of all the water-amino acid solution data. Besides vapor-liquid equilibrium data and densities used by Fuchs et al. (2006), activity coefficients, osmotic coefficients and water activities in aqueous solutions of the amino acid were also considered.

In Figures 6.31 to 6.35, the results from this work (solid line) are compared with the ones obtained by Fuchs et al. (2006) with a $k_{ij} = -0.0665$ (dashed line) for the different thermodynamic properties of aqueous glycine solutions. Correlated densities (Figures 6.31)

and solubility in pure water (Figures 6.35) show the same accuracy. The other thermodynamic properties are reproduced with a much better agreement using the parameters of this work. The improvement is very pronounced for the unsymmetric molal activity coefficients (Figure 6.33).

Similar behavior was observed when comparing the results obtained for DL-alanine (Figures 6.36 to 6.40). Calculated vapor pressures, osmotic coefficients and water activities in aqueous DL-alanine solutions using the parameters obtained in this work are in fair agreement with the experimental data. The correlation results for densities and solubilities show similar deviations to those obtained using DL-alanine parameters by Fuchs et al. (2006) with a $k_{ij} = -0.0598$.

When the calculated densities are compared, it is possible to verify that the ones calculated using the Fuchs et al. (2006) parameters are superior to the density of the pure crystal with deviations of 1.0% and 5.5% for glycine and DL-alanine, respectively. In this work, the densities calculated are inferior to the density of the pure crystal and the deviations found were 12% and 0.4% for glycine and DL-alanine, respectively.

As mentioned in the previous sections the melting properties were treated as adjustable parameters in both works but with one difference; in this work they were estimated without any constrain while Fuchs et al. (2006) used a group-contribution method to have a reasonable range for the hypothetical enthalpy of melting and let the hypothetical temperature of melting to be freely estimated. The values obtained for those properties are very similar for glycine but not for DL-alanine. Regarding the melting temperature, an experimental value for glycine was found in the literature 509.4 K (DIPPR, 1998). Comparing the experimental value to the ones given in Tables 6.5 and 6.15 the deviations found are 4 and 11%, respectively. For DL-alanine, no melting temperature was found however, as observed by Fuchs et al. (2006) the estimated melting temperature for glycine was in a reasonable range while the one for DL-alanine was very high, almost reaches the melting temperature of ionic compounds. In this work the estimated melting temperatures for glycine and DL-alanine are much more reasonable.

Regarding the modelling of amino acids solubilities in pure alcohol none of the works are successful. When the binary amino acid/alcohol parameters were treated as adjustable

parameters to the solubility in pure alcohol, the results obtained were comparable to the ones presented by Fuchs et al. (2006) (Figures 6.41a and 6.42a). When the binary amino acid/alcohol parameters were treated as adjustable parameters not to the solubility in pure alcohol but to the solubility in the mixed solvent system, the solubility in pure alcohol became much worse (Figures 6.41b and 6.42b).

Figures 6.43 and 6.44 show the predicted solubilities of glycine and DL-alanine, in various water-alcohol mixtures, given by Fuchs et al. (2006), which are compared with the results calculated in this work using the binary parameters amino acid/alcohol adjusted to the mixed solvents systems. It is possible to verify that the divergences at medium solvent ratios of ethanol for glycine and for aqueous mixtures of ethanol, 1-propanol and 2-propanol for the DL-alanine, reported by Fuchs et al. (2006) were improved. The modelling of the solubility of DL-alanine in aqueous 2-propanol systems (this work) shows a much better agreement with experimental data and similar deviations to those observed for the other systems. Nevertheless, at high solvent ratios, and pure alcohols the results present larger deviations, for both amino acids. Table 6.16 lists the root mean square deviation (RMSD) for each alcohol system and the number of data points.

Recently, Cameretti and Sadowski (2008) have extended their findings for serine, proline and valine. Since they calculated the solubility of a different serine stereoisomer (DL-serine), it is not possible to present any comparison for this amino acid.

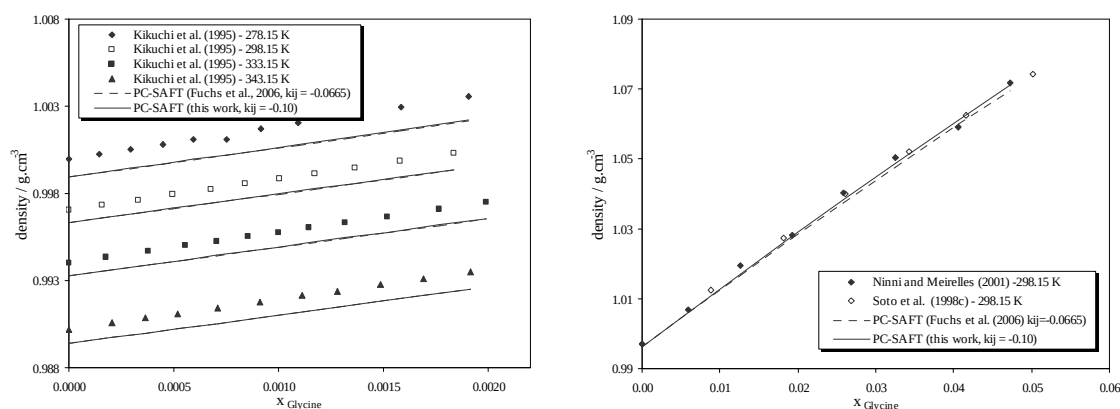


Figure 6.31 Densities of aqueous glycine solutions at different temperatures.

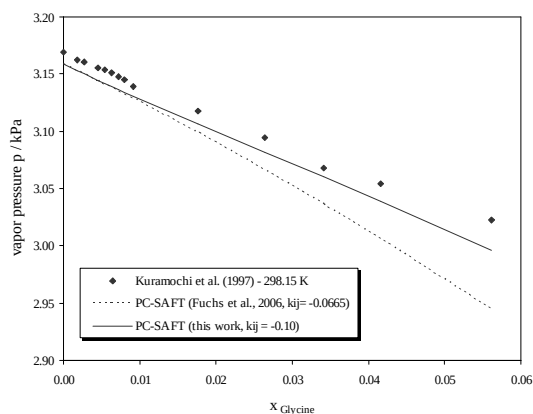


Figure 6.32 Vapor pressures in aqueous glycine solutions.

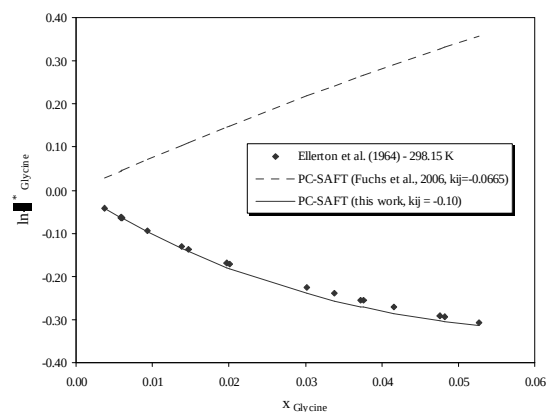


Figure 6.33 Unsymmetric activity coefficients in aqueous glycine solutions.

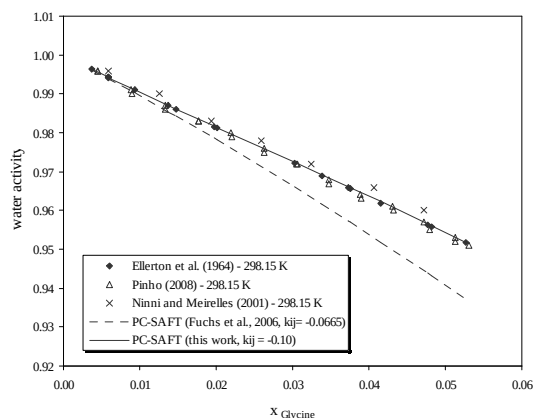


Figure 6.34 Water activities in aqueous glycine solutions.

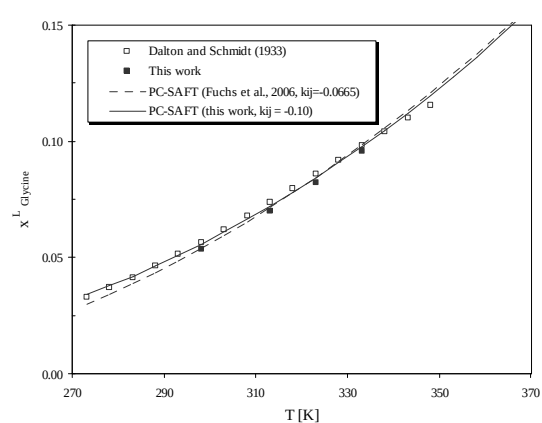


Figure 6.35 Solubilities of glycine in water at different temperatures.

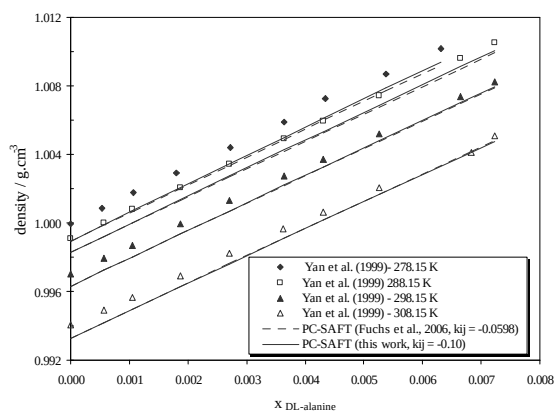
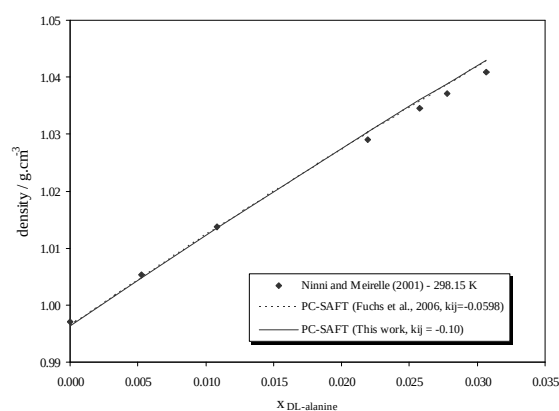


Figure 6.36 Densities of aqueous DL-alanine solutions at different temperatures.



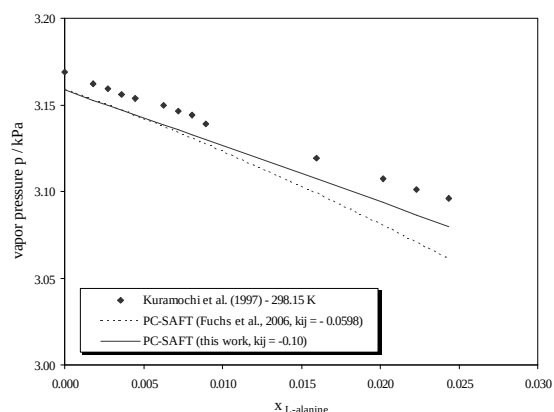


Figure 6.37 Vapor pressures in aqueous L-alanine solutions.

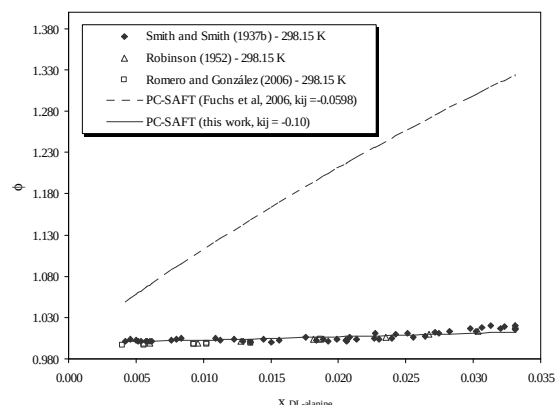


Figure 6.38 Osmotic coefficients in aqueous DL-alanine solutions.

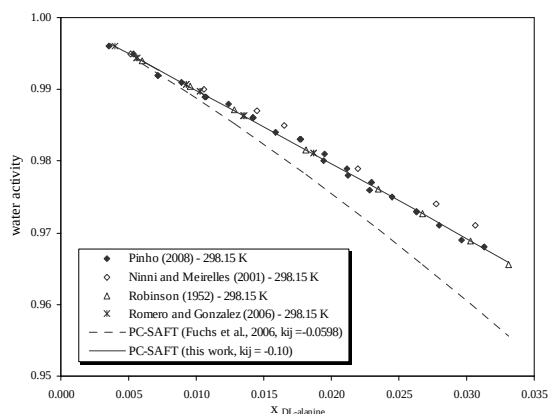


Figure 6.39 Water activities in aqueous DL-alanine solutions.

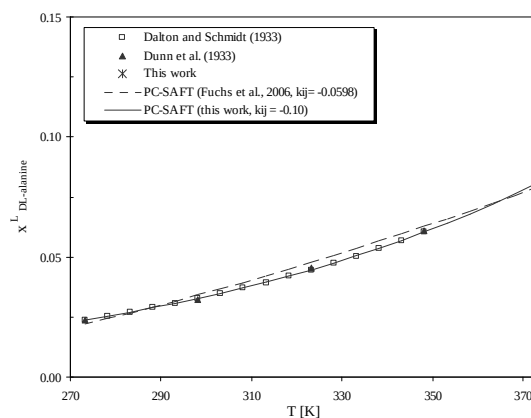


Figure 6.40 Solubilities of DL-alanine in water at different temperatures.

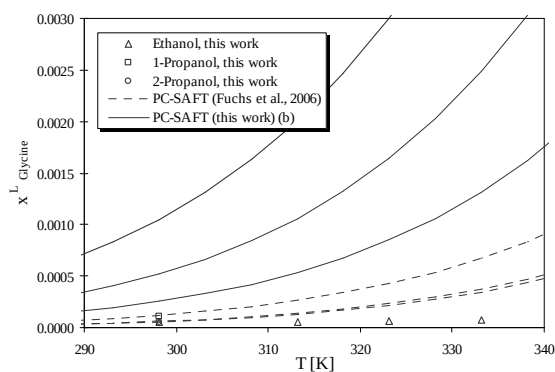
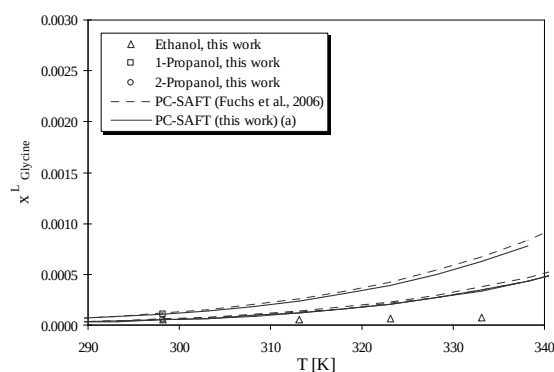


Figure 6.41 Solubilities of glycine in different pure alcohols: (a) k_{ij} adjusted to the pure solvent, (b) k_{ij} adjusted to the mixed solvent.

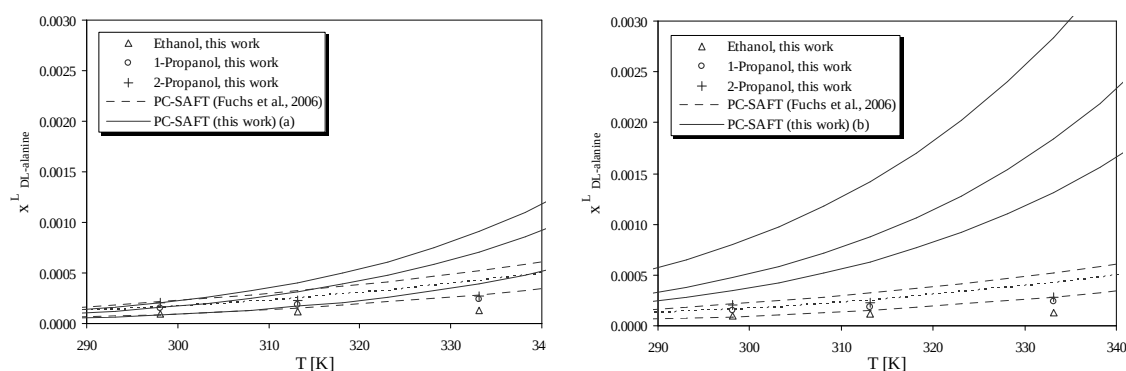


Figure 6.42 Solubilities of DL-alanine in different pure alcohols: (a) k_{ij} adjusted to the pure solvent, (b) k_{ij} adjusted to the mixed solvent.

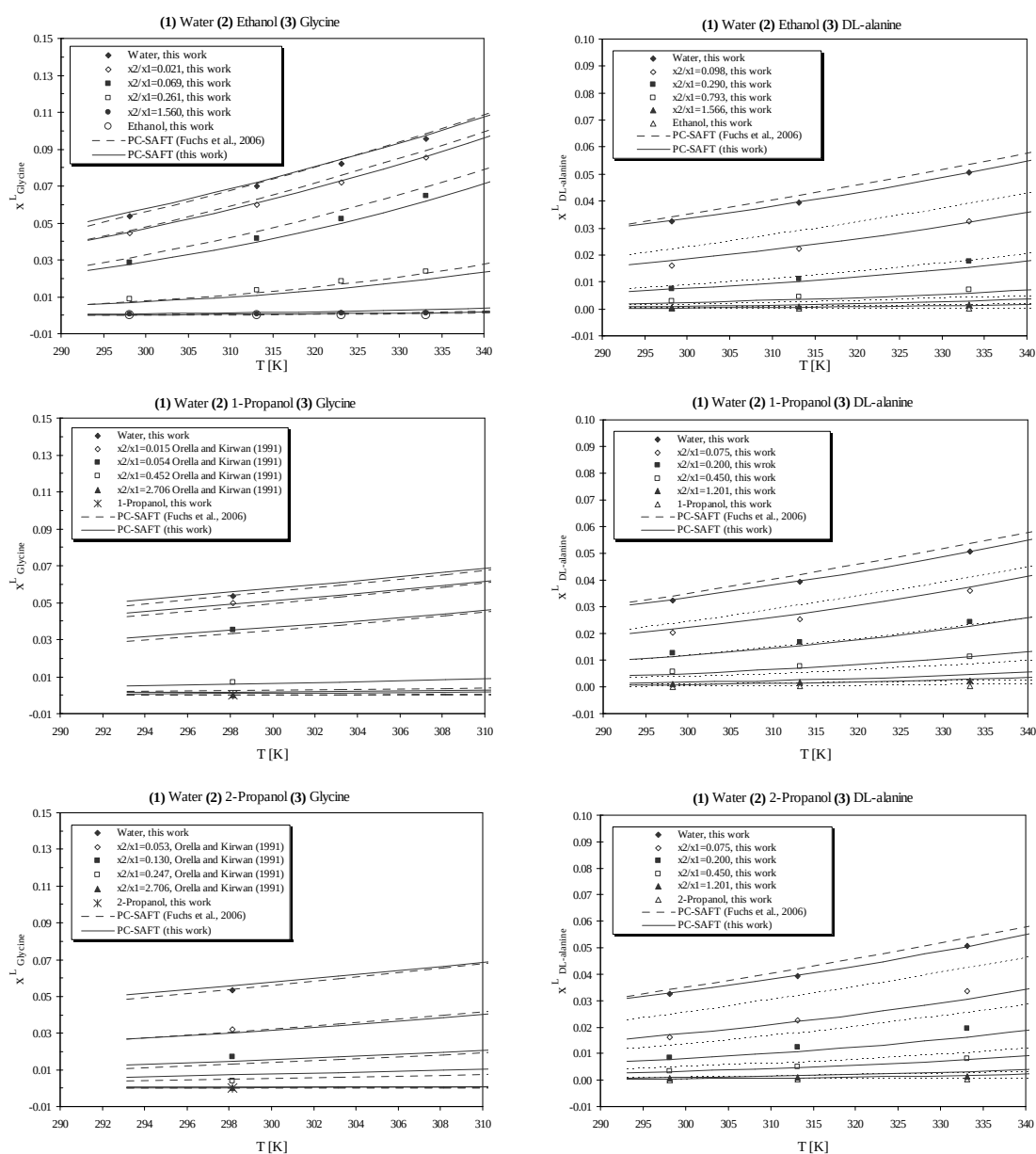


Figure 6.43 Solubilities of glycine in various alcohol-water mixtures.

Figure 6.44 Solubilities of DL-alanine in various alcohol-water mixtures.

Table 6.16 RMSD for each alcohol system with glycine and DL-alanine.

		Ethanol		1-Propanol		2-Propanol	
Glycine	This work	0.0019	(n = 24)	0.0013	(n = 6)	0.0018	(n = 6)
	Fuchs et al. (2006)	0.0023		0.0022		0.0027	
DL-alanine	This work	0.0009	(n = 18)	0.0013	(n = 18)	0.0013	(n = 18)
	Fuchs et al. (2006)	0.0029		0.0024		0.0044	

6.11 EQUATION OF STATE VERSUS g^E MODELS

Two approaches have been developed for the description of thermodynamic properties of amino acids in pure and mixed solvent solutions: g^E models and an equation of state. In chapter 5, the g^E models were only applied to calculate solubilities; while in this chapter, densities, activity and osmotic coefficients, vapor pressures, water activities of amino acid aqueous solutions were also calculated using the EoS.

Applying the excess solubility approach combined with the g^E models the knowledge of the solubility in pure solvents is essential. The parameters required by the models considered were obtained correlating the solubility data for each amino acid in different water-alcohol systems and then used to predict the solubility in the same mixtures at different temperatures.

The amino acids in the PC-SAFT EoS are modelled as chains of spherical segments characterized by their diameter and dispersion energy when interacting with another segment of the same type. In this approach, the knowledge of the amino acid fusion properties is essential to calculate the solubility and since amino acids decompose before melting there is a great lack of data on the melting temperatures and enthalpies. Therefore, those properties were treated as adjustable parameters.

Concerning the number of parameters required by the g^E models it is possible to state: the model proposed by Gude et al. (1996a,b) requires a ternary parameter for an amino acid in a particular solvent system; while NRTL, modified NRTL and modified UNIQUAC models require the same number of estimated parameters; for an amino acid for which solubility data is available in n aqueous-alkanol systems the number of parameters to be determined is $n + 1$. The PC-SAFT EoS benefits from the pure component parameters and only one constant

(temperature independent) binary parameter k_{ij} for each solute/solvent system has to be introduced. The modelling of the solubility of the amino acids in water-alcohol solutions over wide ranges of concentrations and temperatures can be performed using the information from the binary systems only.

Since the two approaches are very distinct the direct comparison of both performances is not reasonable. The limitations observed for each one as well as the important results achieved were presented in chapters 5 and 6. Both approaches are demanding and the obtained results (correlation or prediction) are quite acceptable.

6.12 CONCLUSIONS

The recently developed equation of state, the Perturbed Chain SAFT model (Gross and Sadowski, 2001, 2002) was applied to model the solubilities of glycine, DL-alanine, L-serine, L-threonine and L-isoleucine in pure water, pure alcohols (ethanol, 1-propanol and 2-propanol) and in mixed solvent systems. The amino acids were treated as non-associating molecules and the pure component parameters were identified by fitting simultaneously the densities, activity and osmotic coefficients, vapor pressures, water activities of their aqueous solutions. One binary parameter was necessary for each system to correct the dispersive interactions. Good correlation results were obtained.

The hypothetical melting properties were treated as adjustable parameters and were estimated fitting the solubility curves in water. Even though the model was able to accurately correlate the solubility of the amino acids in water, the correlation results for the solubility in pure alcohols were not so satisfactory.

The solubilities in mixed solvent systems were predicted using the pure component and binary parameters without fitting any additional parameters. With the exception of L-isoleucine systems, the prediction results were reasonable. Fitting the binary parameter for the pair amino acid/alcohol to the solubility in the mixed solvent system instead, the description of the solubility in the mixed solvent systems was clearly improved.

In this work the results given by Fuchs et al. (2006) were reproduced using also the PC-SAFT equation of state. In their study the amino acids were considered as associating substances and the five pure-component PC-SAFT parameters were fitted using only experimental densities and vapor pressures. When the solubility of the amino acid in pure solvents was considered, one constant (temperature independent) binary parameter k_{ij} for each binary solute/solvent system was introduced to correlate the solubility data quantitatively. The binary parameter amino acid/water has a very minor effect on the correlation of the densities but a pronounced effect on the correlated vapor pressures, activity coefficients and water activities; for those properties the agreement with experimental data is not so good as with a $k_{ij} = 0.0$.

The glycine and DL-alanine PC-SAFT parameters were refitted and the comparison with the results given by Fuchs et al. (2006), using the k_{ij} , presented. For densities, and solubility in pure water there are very minor differences between the two correlations. The other thermodynamic properties are reproduced with a much better agreement using the parameters proposed here.

In this work, and considering the amino acids as non-associating molecules the number of estimated parameters was reduced. The model performance, regarding the modelling of amino acid solubilities in pure water and in solvent mixtures, was kept, and very good improvements were observed specially for the description of the unsymmetric amino acid activity coefficients. Attempts to introduce association parameters did not improve the correlations.

NOMENCLATURE

List of symbols

A	Helmholtz free energy
C_p	heat capacity ($\text{J.mol}^{-1}.\text{K}^{-1}$)
d	density (g.cm^{-3})
f	fugacity, rational activity coefficient
g	Gibbs energy
H	enthalpy (J.mol^{-1})
k	binary interaction parameter
m	segment number
n	mole number, number of data points
P	pressure (Pa)
Q	thermodynamic property
R	ideal gas constant ($\text{J.mol}^{-1}.\text{K}^{-1}$)
T	absolute temperature (K)
x	mole fraction

Greek Letters

γ	molal activity coefficient
Δ	property difference
ε / k	energy parameter, dispersion
ε	association energy
κ	association volume
σ	segment diameter
ϕ	osmotic coefficient
φ	fugacity coefficient

Subscripts

1	water
2	alcohol
calc	calculated

crystal	crystal
i, j	any species
k	experimental data point
s	solute
o	pure substance, melting property

Superscripts

A, B	association sites
<i>assoc</i>	association
<i>calc</i>	calculated by the model
<i>disp</i>	dispersion
E	excess property
<i>exp</i>	experimental
<i>hc</i>	hard chain
L, liq	liquid phase
<i>res</i>	residual
<i>solid</i>	solid phase
*	unsymmetric

Abbreviations

ARD	Average Relative Deviation
DIPPR	Design Institute for Physical Property (data base)
EoS	Equation of State
<i>FOBJ</i>	objective function
NA	not available
NDP	number of data points
NRTL	Non-Random Two Liquid
PC-SAFT	Perturbed-Chain SAFT
RMSD	Root mean square deviation
SAFT	Statistical Associating Fluid Theory
SD	Standard deviation
UNIQUAC	Universal Quasi Chemical

CHAPTER 7.

CONCLUSIONS

7.1 MAIN CONCLUSIONS

Biotechnological production of amino acids is nowadays a prospect market due to major successes in cost effective production and isolation of amino acids products. For the design, optimization and scale-up of the separation processes the knowledge of solid-liquid data is of extreme importance.

Although it is possible to find in literature a considerable number of both experimental and modelling work concerning amino acid studies in pure water, the situation is not the same for aqueous amino acid solutions with a salt or an alcohol where there is still a great lack of information. A great majority of the measurements were only carried out at 298.15 K and the number of studied systems is very limited to very few solvents/salts and/or conditions, and rather old, leaving some doubts about their quality. It was then evident the absolute need to carry out further measurements in order to extend the experimental database already available.

The solubility of the amino acids (glycine, DL-alanine, L-serine, L-isoleucine, L-threonine) has been measured in the systems water/alcohol (ethanol, 1-propanol and 2-propanol) and water/electrolyte [KCl and $(\text{NH}_4)_2\text{SO}_4$] in the temperature range between 298.15 K and 333.15 K. Analytical methods were chosen to perform the measurements: concerning the systems with electrolytes the analytical gravimetric method was always applied. The solid content of the solutions containing alcohols was measured differently depending on the alcohol mass fraction in amino acid free basis in the mixed solvent (w'_{alcohol}). When $w'_{\text{alcohol}} < 0.8$, the gravimetric method was applied; at higher alcohol concentrations

($w'_{alcohol} \geq 0.8$), the spectrophotometric ninhydrin method was used for the quantitative determination of the extremely low solubility of the amino acids. In the gravimetric method, each experimental point is an average of at least three different measurements obeying the following criterion: the quotient $2s/\text{solubility} \times 100$ should be lower than 0.2, where s is the standard deviation within a set of different experimental results. Using the spectrophotometric ninhydrin method, each experimental solubility data verifies the following criteria: the quotient $2s/\text{solubility} \times 100$ is lower than 10%, for L-threonine or L-serine solubility values inferior to 1×10^{-5} (mass fraction), and lower than 6% for the other solubility values. For L-isoleucine, that quotient is lower than 8% for solubility values inferior to 1×10^{-4} , and lower than 4% for the remaining solubilities.

Due to the already mentioned lack of solid-liquid equilibrium data, a quantitative comparison with literature data was not always possible but, when compared, generally the experimental results showed to be in high agreement. For the aqueous KCl system with glycine and DL-alanine at 298.15 K some discrepancies were found. The Pitzer-Simonson-Clegg equations were applied to predict the solubility of those systems using activity coefficient data only. Quantitatively, those predictions are weak, but they made it possible to confirm the solubility trend found experimentally and the reliability of the solubility data measured in this work.

For the majority of the systems for which no literature data is available, the accuracy tests implemented proved the success and high reproducibility of the experimental techniques used and also the quality of the experimental data.

The systematized experimental study developed in this work contributed, in some cases; to duplicate the number of experimental data points available and, to study new systems, while the temperature and composition studied was extended. The use of the available experimental information from the open literature together with the new measurements has been fundamental for the validation and development of thermodynamic models.

Thermodynamic models: g^E models (Wilson, modified Wilson, NRTL, electrolyte NRTL, UNIQUAC, modified UNIQUAC and UNIFAC models) and equations of state (simplified perturbation theory, simplified perturbed-hard-sphere model; a hydrogen-bonding lattice-fluid equation of state, PC-SAFT) were reviewed and their capabilities to correlate and/or predict the thermodynamic properties of the amino acids in aqueous systems containing alcohol or

electrolytes briefly discussed. A full comparison between the different approaches was not practicable; however it was possible to verify that despite the success they all exhibit some limitations. The development of thermodynamic models for correlation and prediction of those systems is still a growing challenge, where equations of state appear as attractive alternatives to the g^E models.

Solubility data obtained in this work and activity coefficient data collected from literature were used to study the ability of the Pitzer-Simonson-Clegg equations in the thermodynamic description of the ternary systems water-KCl with glycine, DL-alanine or L-serine at different temperatures. The introduction of a temperature dependency on the $W_{n,Mx}$ and $U_{n,Mx}$ parameters allowed very satisfactory correlation results: the global root mean square deviations found were 0.0036 for the activity coefficients and 0.87 g of amino acid per kg of water for the solubility data in those ternary systems. Satisfactory results were obtained for the prediction of DL-alanine solubility in aqueous KCl system at 333.15K (RMSD: 0.80 g of DL-alanine per kg of water) but highly dependent on the chosen temperature dependency for the model parameters, suggesting care when extending solubility calculations to temperatures outside the temperature range used in the correlation. Also the water activity in aqueous amino acid solutions with KCl must be predicted with caution when the KCl and amino acid molalities are extended.

Two different approaches have been proposed to correlate and/or predict the solubility of the amino acids in water-alcohol systems: the application of the excess solubility approach with four different models [NRTL, modified NRTL, modified UNIQUAC models and the model proposed by Gude et al. (1996a,b)], and the recently developed thermodynamic equation of state, PC-SAFT.

Experimental data was reasonably correlated with the thermodynamic approach defined by the combination of the excess solubility approach with a simple excess Gibbs energy model. Concerning the number of parameters needed to be estimated, the NRTL, the modified NRTL and the modified UNIQUAC models require the same number of estimated parameters; for an amino acid for which solubility data is available in n aqueous-alkanol systems the number of parameters to be determined is $n + 1$, while the model proposed by Gude et al. (1996a,b) has a single amino acid specific parameter. For the methodology proposed, the knowledge of the solubility in pure solvents is fundamental. Despite the known difficulties of the conventional

thermodynamic models to account accurately for the hydrophobic effects, in general, the correlation results are fairly good in all solvent composition range. The excess solubility approach combined with the modified NRTL model can satisfactorily correlate and predict the equilibrium data with global average relative deviation of 12.0% and 14.6%, respectively. The correlation performance with the NRTL, modified UNIQUAC equations and the model proposed by Gude et al. (1996a,b) showed global ARDs of 12.2, 15.1 and 16.2%, respectively, while their application in the prediction showed global ARDs of 14.6, 22.0 and 27.3%, respectively. Concerning the influence of temperature on the solubility of the amino acids some lack of precision can be found and the success of the correlations it is not always evident. Regarding predictions, some caution is advised when predicting results to temperature values outside the temperature range used for correlation since the ARD found are not so acceptable.

The PC-SAFT EoS was applied to model the solubilities of glycine, DL-alanine, L-serine, L-threonine and L-isoleucine in pure water, pure alcohols (ethanol, 1-propanol and 2-propanol) and in mixed solvent systems. The three pure component parameters for the non-associating component, the amino acids, were fitted to the densities, activity and osmotic coefficients, vapor pressures, and water activities of their aqueous solutions. Only one temperature independent binary parameter is required for each system. Then, the solubilities of amino acids in pure and in mixed solvent systems can be calculated assuming a pure solid phase and knowing the hypothetical melting properties. Those properties were fitted to experimental data in order to accurately describe the solubilities in pure water. The model can accurately describe the solubility of the amino acids in water but the correlation results for the solubility in pure alcohols were not so satisfactory. The solubility in mixed solvents (ternary systems) was predicted based on the modelling of the solubility in pure solvents, without any additional fitting of the parameters; with the exception of the L-isoleucine, and the results were reasonable. Fitting the binary parameter amino acid/alcohol not to the solubility in pure alcohol, but to the solubility in the mixed solvent system the description of the solubility in the mixed solvent systems was clearly improved and the results in fair agreement with the experimental data for all mixture compositions.

During 2006, Fuchs and collaborators considered the amino acids as associating substances and the 5 pure component PC-SAFT parameters for the amino acids were fitted using only

experimental densities and vapor pressures. To correlate the solubility data quantitatively, one constant (temperature independent) binary parameter k_{ij} for each binary solute/solvent system was introduced. After, the solubility of amino acids in water-alcohol mixtures (ternary systems) was predicted without fitting any additional parameters. The results of the prediction correspond well to literature data. The results given by Fuchs et al. (2006) were reproduced and important considerations were found. The binary parameter necessary to correlate the solubility data quantitatively has a very minor effect on the correlation of densities; however, the consequence on the correlated vapor pressures, activity coefficients and water activities is very obvious. The values for the osmotic and activity coefficients were overestimate and for glycine an inverse trend to the experimental observations is even obtained.

Considering the amino acids as non-associating molecules the number of estimated parameters was reduced and the model performance, regarding the modelling of amino acid solubilities in pure water is comparable, while the description of the osmotic and unsymmetric amino acid activity coefficients is greatly improved. For the ternary systems the lower root mean square deviation obtained for each alcohol system indicates the success of the methodology applied in this work.

The satisfactory results obtained in this work (both experimental and modelling work) will undoubtedly contribute for the understanding of more complex systems like those containing proteins, peptides or antibiotics, and certainly provide new insights for applications in the industry.

7.2 SUGGESTIONS FOR FUTURE WORK

A systematic experimental study on the solubility of several amino acids in aqueous solution with or without a salt, or an alcohol, was developed in order to contribute for the fulfilment of the lack of information found in these fields. Naturally, the database of solid-liquid equilibrium data is far from being complete so, it is fundamental to continue the experimental work increasing the number of studied systems, as well as the conditions of temperature, pH and ionic strength. Specifically, a huge lack of information still remains for the solubility of amino acids in aqueous electrolyte solutions.

One particular suggestion for future work is the study of the unpredicted phenomenon observed for the amino acids L-serine, and glycine, in aqueous alkanol (1-propanol and 2-propanol) solutions. The solubilities of the L-serine in the pure solvents and in the miscible composition range of the systems were measured, but the partition coefficients of the amino acids and the composition of each phase were not subject of this study.

Concerning the modelling work, further work could include the application of an equation of state, the ePC-SAFT developed by Cameretti et al. (2005) that combines the PC-SAFT EoS by Gross and Sadowski (2001) and the Debye-Hückel contribution, to describe the thermodynamic properties of the amino acids in aqueous electrolyte solutions.

REFERENCES

- Akhtar, Y., Volumetric and Viscometric Behaviour of Amino Acids in Aqueous Metal Electrolytes Solutions at 308 K, *Fluid Phase Equilib.*, **2007**, 258, 125.
- Amin, A. S.; Zaky, M.; El-Beshbeshy, A. M., Colorimetric Estimation of Melatonin in Pharmaceutical Formulations, *Mikrochim. Acta*, **2000**, 135, 81.
- Araki, K.; Ozeki, T. (2003). Kirk-Othmer Encyclopedia of Chemical Technology, John Wiley & Sons, New Jersey.
- Archer, D. G., Thermodynamic Properties of the KCl + H₂O System, *J. Phys. Chem. Ref. Data*, **1999**, 28, 1.
- Archer, D. G.; Wang, P., The Dielectric Constant of Water and Debye-Hückel Limiting Law Slopes, *J. Phys. Chem. Ref. Data*, **1990**, 19, 371.
- Banipal, T. S.; Kaur, D.; Banipal, P. K., Apparent Molar Volumes and Viscosities of Some Amino Acids in Aqueous Sodium Acetate Solutions at 298.15 K, *J. Chem. Eng. Data*, **2004**, 49, 1236.
- Banipal, T. S.; Kaur, D.; Banipal, P. K.; Singh, G., Thermodynamic and Transport Properties of L-Serine and L-Threonine in Aqueous Sodium Acetate and Magnesium Acetate Solutions at T = 298.15 K, *J. Chem. Thermodyn.*, **2007**, 39, 371.
- Barker, J. A.; Henderson, D., Perturbation Theory and Equation of State for Fluids: The Square-Well Potential, *J. Chem. Phys.*, **1967a**, 47, 2856.

- Barker, J. A.; Henderson, D., Perturbation Theory and Equation of State for Fluids. II. A Successful Theory of Liquids, *J. Chem. Phys.*, **1967b**, 47, 4714.
- Bouchard, A.; Hofland, G. W.; Witkamp, G.-J., Solubility of Glycine Polymorphs and Recrystallization of β -Glycine, *J. Chem. Eng. Data*, **2007**, 52, 1626.
- Bower, V. E.; Robinson, R. A., Thermodynamics of the Ternary System: Water-Glycine-Potassium Chloride at 25 °C from Vapor Pressure Measurements, *J. Res. Natl. Bur. Stand.*, **1965**, 69, 131.
- Breil, M. P. (2001). Thermodynamics, Experimental, and Modeling of Aqueous Electrolyte and Amino Acid Solutions. Ph.D. Thesis, DTU, Lyngby, Denmark.
- Breil, M. P.; Mollerup, J. M.; Rudolph, E. S.; Ottens, M.; van der Wielen, L. A. M., Densities and Solubilities of Glycylglycine and Glycyl-L-Alanine in Aqueous Electrolyte Solutions, *Fluid Phase Equilib.*, **2004**, 215, 221.
- Brown, M. G.; Rousseau, R. W., Effect of Sodium Hydroxide on the Solubilities of L-Isoleucine, L-Leucine, and L-Valine, *Biotechnol. Prog.*, **1994**, 10, 253.
- Cameretti, L. F.; Sadowski, G., Modeling of Aqueous Amino Acid and Polypeptide Solutions with PC-SAFT, *Chem. Eng. Process.*, **2008**, 47, 1018.
- Cameretti, L. F.; Sadowski, G.; Mollerup, J. M., Modeling of Aqueous Electrolyte Solutions with Perturbed-Chain Statistical Associated Fluid Theory, *Ind. Eng. Chem. Res.*, **2005**, 44, 3355.
- Carey, F. A. (2003). Organic Chemistry, 5th Ed., International Edition, McGraw-Hill, New York.
- Carta, R., Solubilities of L-Cystine, L-Tyrosine, L-Leucine, and Glycine in Sodium Chloride Solutions at Various pH Values, *J. Chem. Thermodyn.*, **1998**, 30, 379.
- Carta, R., Solubilities of L-Cystine, L-Tyrosine, L-Leucine, and Glycine in their Water Solutions, *J. Chem. Eng. Data*, **1999**, 44, 563.

Carta, R.; Tola, G., Solubilities of L-Cystine, L-Tyrosine, L-Leucine, and Glycine in Aqueous Solutions at Various pHs and NaCl Concentrations, *J. Chem. Eng. Data*, **1996**, 41, 414.

Charmolue, H.; Rousseau, R. W., L-Serine Obtained by Methanol Addition in Batch Crystallization, *AIChE J.*, **1991**, 37, 1121.

Chen, C.-C.; Zhu, Y.; Evans, L. B., Phase Partitioning of Biomolecules: Solubilities of Amino Acids, *Biotechnol. Prog.*, **1989**, 5, 111.

Chen, S.-P.; Gao, S.-L.; Yang, X.-W.; Shi, Q.-Z., Preparation and Thermochemistry of Complexes of Zinc Nitrate with Threonine, *Russ. J. Coord. Chem.*, **2004**, 30, 566.

Chung, Y.-M.; Vera, J. H., Activity Coefficients of the Peptide and the Electrolyte in Ternary Systems Water+Glycylglycine +NaCl, +NaBr, +KCl and +KBr at 298.2 K, *Biophys. Chem.*, **2001**, 92, 77.

Chung, Y.-M.; Vera, J. H., Activity of the Electrolyte and the Amino Acid in the Systems Water + DL, α -Aminobutyric Acid + NaCl, +NaBr, +KCl, and +KBr at 298.2 K, *Fluid Phase Equilib.*, **2002**, 203, 99.

Clegg, S. L.; Pitzer, K. S., Thermodynamics of Multicomponent, Miscible, Ionic Solutions: Generalized Equations for Symmetrical Electrolytes, *J. Phys. Chem.*, **1992**, 96, 3513.

Cohn, E. J.; Edsall, J. T. (1943). *Proteins Amino Acids and Peptides as Ions and Dipolar Ions*, Reinhold Publishing Corporation, New York.

Cohn, E. J.; McMeekin, T. L.; Edsall, J. T.; Weare, J. H., Studies in the Physical Chemistry of Amino Acids, Peptides and Related Substances. II. The Solubility of α -Amino Acids in Water and in Alcohol-Water Mixtures, *J. Am. Chem. Soc.*, **1934**, 56, 2270.

Cohn, E. J.; McMeekin, T. L.; Ferry, J. D.; Blanchard, M. H., Studies in the Physical Chemistry of Amino Acids, Peptides and Related Substances. XII. Interactions between Dipolar Ions in Aqueous Solution, *J. Phys. Chem.*, **1939**, 43, 169.

Conio, G.; Curletto, L.; Patrone, E., On the Temperature Coefficient of the Solubility of Some Glycyl Peptides in Water-Ethanol Mixtures, *J. Biol. Chem.*, **1973**, 248, 5448.

CRC Handbook of Chemistry and Physics, 87th Ed., 2006-2007.

Dalton, J. B.; Schmidt, C. L. A., The Solubilities of Certain Amino Acids in Water, the Densities of their Solutions at Twenty Five Degrees, and the Calculated Heats of Solution and the Partial Molal Volumes, *J. Biol. Chem.*, **1933**, 103, 549.

Dalton, J. B.; Schmidt, C. L. A., The Solubilities of Certain Amino Acids and Related Compounds in Water, the Densities of their Solutions at Twenty Five Degrees, and the Calculated Heats of Solution and the Partial Molal Volumes, *J. Biol. Chem.*, **1935**, 109, 241.

Dehghani, M. R.; Modarress, H.; Monirifar, M., Measurements and Modeling of Mean Activity Coefficients of NaBr and Amino Acid in (Sodium Bromide + Potassium Phosphate + Glycine + Water) System at T (298.15 and 308.15) K, *J. Chem. Thermodyn.*, **2005**, 37, 1305.

Dehghani, M. R.; Modarress, H.; Monirifar, M., Measurements and Modeling of Mean Activity Coefficients of Aqueous Mixed Electrolyte Solution Containing Glycine, *J. Chem. Thermodyn.*, **2006a**, 38, 1049.

Dehghani, M. R.; Modarress, H.; Bakhshi, A., Modeling and prediction of Activity Coefficient Ratio of Electrolytes in Aqueous Electrolyte Solution Containing Amino Acids using Artificial Neural Network, *Fluid Phase Equilib.*, **2006b**, 244, 153.

DIPPR 801. Evaluated Process Design Data. Public Release. American Institute of Chemical Engineers. Design Institute for Physical Property Data, (1998). BYU-DIPPR. Thermophysical Properties Laboratory. Provo, Utah.

Drauz, K.; Grayson, I.; Kleemann, A.; Krimmer H.-P.; Leuchtenberger, W.; Weckbecker, C. (2007). Ullmann's Encyclopedia of Industrial Chemistry, John Wiley & Sons, New Jersey.

Duke, M. M.; Hakin, A. W.; McKay, R. M.; Preuss, K. E., The Volumetric and Thermochemical Properties of Aqueous Solutions of L-Valine, L-Leucine, and L-Isoleucine at 288.15, 298.15, 313.15, and 328.15 K, *Can. J. Chem.*, **1994**, 72, 1489.

Dunn, M. S.; Ross, F. J., Qualitative Investigations of Amino Acids and Peptides. IV. The Solubility of the Amino Acids in Water-Ethyl Alcohol Mixtures, *J. Biol. Chem.*, **1938**, 125, 309.

Dunn, M. S.; Ross, F. J.; Read, L. S., The Solubility of the Amino Acids in Water, *J. Biol. Chem.*, **1933**, 103, 579.

Ellerton, H. D.; Reinfelds, G.; Mulcahy, D. E.; Dunlop, P. J., Activity, Density, and Relative Viscosity Data for Several Amino Acids, Lactamide, and Raffinose in Aqueous Solution at 25°, *J. Phys. Chem.*, **1964**, 68, 398.

Fang, G.; Liu, N., Determination of Eight Essential Amino Acids in Mixtures by Chemometrics-Spectrophotometry without Separation, *Anal. Chim. Acta*, **2001**, 445, 245.

Farelo, F.; Lopes, A.; Ferra, M. I. A., Activity Coefficients of Potassium Chloride and Sodium Chloride in the Quaternary System KCl–NaCl–Water–Ethanol, *J. Sol. Chem.*, **2002**, 31, 845.

Fernández-Mérida, L.; Rodríguez-Raposo, R.; García-García, G. E.; Estes, M. A., Modification of the Pitzer Equations for Application to Electrolyte + Polar Non-Electrolyte Mixtures, *J. Electroanal. Chem.*, **1994**, 379, 63.

Foster, P. R.; P. Dunhill, P.; Lilly, M. D., The Precipitation of Enzymes from Cell Extracts of *Saccharomyces Cerevisiae* by Polyethylene Glycol, *Biochem. Biophys. Acta*, **1973**, 317, 505.

Foster, P. R.; P. Dunhill, P.; Lilly, M. D., Kinetics of Protein Salting-Out – Precipitation of Yeast Enzymes by Ammonium Sulfate, *Biotech. Bioeng.*, **1976**, 18, 545.

Friedman, M., Applications of the Ninhydrin Reaction for Analysis of Amino Acids, Peptides, and Proteins to Agricultural and Biomedical Science, *J. Agr. Food Chem.*, **2004**, 52, 385.

Frutos, P.; Torrado, S.; Perez-Lorenzo, M. E.; Frutos, G., A Validated Quantitative Colorimetric Assay for Gentamicin, *J. Pharm. Biomed.*, **2000**, 21, 1149.

Fuchs, D.; Fischer, J.; Tumakaka, F.; Sadowski, G., Solubility of Amino Acids: Influence of the pH Value and the Addition of Alcoholic Cosolvents on the Aqueous Solubility, *Ind. Eng. Chem. Res.*, **2006**, 45, 6578.

- Gao, C.; Vera, J. H., The Activity Coefficients of Glycine, DL-Serine and DL-Valine in Aqueous Solutions Containing Nitrates at 298.15 K, *Can. J. Chem. Eng.*, **2001**, 79, 392.
- Gekko, K., Mechanism of Polyol-Induced Protein Stabilization: Solubility of Amino Acids and Diglycine in Aqueous Polyol Solutions, *J. Biochem.*, **1981**, 90, 1633.
- Gekko, K.; Ohmae, E.; Kameyama, H.; Takagi, T., Acetonitrile-Protein Interactions: Amino Acid Solubility and Preferential Solvation, *Biochim. Biophys. Acta*, **1998**, 1387, 195.
- Ghotbi, C.; Vera, J. H., Extension to Mixtures of Two Robust Hard-Sphere Equations of State Satisfying the Ordered Close-Packed Limit, *Can. J. Chem. Eng.*, **2001**, 79, 678.
- Givand, J. C.; Teja, A. S.; Rousseau, R. W., Effect of Relative Solubility on Amino Acids Crystal Purity, *AIChE J.*, **2001**, 47, 2705.
- Gmehling, J.; Onken, U.; Arlt, W. (1981). Vapor-Liquid Equilibrium Data Collection. Aqueous Organic Systems, Vol. 1, Part 1a, Chemistry Data Series. Dechema, Frankfurt.
- Gross, J.; Sadowski, G., Perturbed-Chain SAFT: An Equation of State Based on a Perturbation Theory for Chain Molecules, *Ind. Eng. Chem. Res.*, **2001**, 40, 1244.
- Gross, J.; Sadowski, G., Application of the Perturbed-Chain SAFT Equation of State to Associating Systems, *Ind. Eng. Chem. Res.*, **2002**, 41, 5510.
- Gude, M. T.; Wielen, L. A. M.; Luyben, K. Ch. A. M., Phase Behaviour of α -Amino Acids in Multicomponent Aqueous Alkanol Solutions, *Fluid Phase Equilib.*, **1996a**, 116, 110.
- Gude, M. T.; Meuwissen, H. H. J.; Wielen, L. A. M.; Luyben, K. Ch. A. M., Partition Coefficients and Solubilities of α -Amino Acids in Aqueous 1-Butanol Solutions, *Ind. Eng. Chem. Res.*, **1996b**, 35, 4700.
- Gupta, R. B.; Heidemann, R. A., Solubility Models for Amino Acids and Antibiotics, *AIChE J.*, **1990**, 36, 333.

- Hakin, A. W.; Copeland, A. K.; Liu, J. L.; Marriott, R. A.; Preuss, K. E., Densities, Apparent Molar Volumes, and Apparent Molar Heat Capacities of L-Arginine, L-Proline and D,L-Methionine in Water at 288.15, 298.15, 313.15, and 328.15 K, *J. Chem. Eng. Data*, **1997**, 42, 84.
- Hakin, A. W.; Duke, M. M.; Klassen, S. A.; McKay, R. M.; Preuss, K. E., Apparent Molar Heat Capacities and Volumes of some Aqueous Solutions of Aliphatic Amino Acids at 288.15, 298.15, 313.15, and 328.15 K, *Can. J. Chem.*, **1994**, 362, 362.
- Hermann, T., Industrial Production of Amino Acids by Coryneform Bacteria, *J. Biotechnol.*, **2003**, 104, 155.
- Huang, S. H.; Radosz, M., Equation of State for Small, Large, Polydisperse, and Associating Molecules, *Ind. Eng. Chem. Res.*, **1990**, 29, 2284.
- Hu Y.-F.; Guo, T.-M., Thermodynamics of Electrolytes in Aqueous Systems Containing both Ionic and Nonionic Solutes. Application of the Pitzer-Simonson-Clegg Equations to Activity Coefficients and Solubilities of 1:1 Electrolytes in four Electrolyte-Non-Electrolyte-H₂O Ternary Systems at 298.15 K., *Phys. Chem. Chem. Phys.*, **1999**, 1, 3303.
- Hurst, P. L.; Sinclair, B. K.; Eason, J. R., Amino Acids Interfere with the Ninhydrin Assay for Asparagine, *Food Chem.*, **1995**, 53, 467.
- Hutchens, J. O. (1976). In *Handbook of Biochemistry and Molecular Biology: Physical and Chemical Data*, vol. 1, 3rd Ed.; Fasman, G. D., Ed.; CRC Press: Cleveland.
- Hutchens, J. O; Figlio, K. M.; Granito, S. M., An Isopiestic Comparison Method for Activities. The Activities of L-Serine and L-Arginine Hydrochloride, *J. Biol. Chem.*, **1963**, 238, 1419.
- Islam, M. N.; Wadi, R. K., Thermodynamics of Transfer of Amino Acids from Water to Aqueous Sodium Sulfate, *Phys. Chem. Liq.*, **2001**, 39, 77.
- Jelińska-Kazimierczuk, M.; Szydłowski, J., Isotope Effect on the Solubility of Amino Acids in Water, *J. Sol. Chem.*, **1996**, 25, 1175.

Jin, X. Z.; Chao, K.-C., Solubility of Four Amino Acids in Water and of Four Pairs of Amino Acids in Their Water Solutions, *J. Chem. Eng. Data*, **1992**, 37, 199.

Jones, D. L.; Owen, A. G.; Farrar, J. F., Simple Method to Enable the High Resolution Determination of Total Free Amino Acids in Soil Solutions and Soil Extracts, *Soil Biol. Biochem.*, **2002**, 34, 1893.

Joullié, M. M.; Trompson, T. R.; Nemeroff, N. H., Ninhydrin and Ninhydrin Analogs. Syntheses and Applications, *Tetrahedron*, **1991**, 47, 8791.

Kabir-Ud-Din; Fatma, W.; Khan, Z., Micelle-Catalyzed Reaction of Ninhydrin with L-Valine in the Absence and Presence of Organic Solvents, *Int. J. Chem. Kinet.*, **2006**, 38, 634.

Kamali-Ardakani, M.; Modarress, H.; Taghikhani, V.; Khoshkbarchi, M. K., Activity Coefficients of Glycine in Aqueous Electrolyte Solutions: Experimental Data for ($\text{H}_2\text{O} + \text{KCl} + \text{Glycine}$) at $T = 298.15 \text{ K}$ and ($\text{H}_2\text{O} + \text{NaCl} + \text{Glycine}$) at $T = 308.15 \text{ K}$, *J. Chem. Thermodyn.*, **2001**, 33, 821.

Karplus, P. A., Hydrophobicity Regained, *Protein Sci.*, **1997**, 6, 1302.

Khavaninzadeh, A.; Modarress, H.; Taghikhani, V.; Koshkbarchi, M. K., Activity Coefficients of Electrolyte and Amino Acid in the Systems (Water + Potassium Chloride + DL-Valine) at $T = 298.15 \text{ K}$ and (Water + Sodium Chloride + L-Valine) at $T = 308.15 \text{ K}$, *J. Chem. Thermodyn.*, **2002**, 34, 1297.

Khavaninzadeh, A.; Modarress, H.; Taghikhani, V.; Koshkbarchi, M. K., Measurements of Activity Coefficients of Amino Acid in Aqueous Electrolyte Solutions: Experimental Data for the Systems ($\text{H}_2\text{O} + \text{NaBr} + \text{Glycine}$) and ($\text{H}_2\text{O} + \text{NaBr} + \text{L-Valine}$) at $T = 298.15 \text{ K}$, *J. Chem. Thermodyn.*, **2003**, 35, 1553.

Khoshkbarchi, M. K.; Vera, J. H., Measurement and Modeling of Activities of Amino Acids in Aqueous Salt Systems, *AIChE J.*, **1996a**, 42, 2354.

Khoshkbarchi, M. K.; Vera, J. H., Measurement of Activity Coefficients of Amino Acids in Aqueous Electrolyte Solutions: Experimental Data for the Systems $\text{H}_2\text{O} + \text{NaCl} + \text{Glycine}$ and $\text{H}_2\text{O} + \text{NaCl} + \text{DL-Alanine}$ at 25°C , *Ind. Eng. Chem. Res.*, **1996b**, 73, 2735.

Khoshkbarchi, M. K.; Vera, J. H., Activity Coefficients of DL-Valine in Aqueous Solutions of KCl at 25 °C. Measurement with Ion Selective Electrodes and Modelling, *J. Sol. Chem.*, **1996c**, 25, 865.

Khoshkbarchi, M. K.; Vera, J. H., A Simplified Perturbed Hard-Sphere Model for the Activity Coefficients of Amino Acids and Peptides in Aqueous Solutions, *Ind. Eng. Chem. Res.*, **1996d**, 35, 4319.

Khoshkbarchi, M. K.; Vera, J. H., Measurement and Correlation of Ion Activity in Aqueous Single Electrolyte Solutions, *AIChE J.*, **1996e**, 42, 249.

Khoshkbarchi, M. K.; Vera, J. H., A Perturbed Hard-Sphere Model with Mean Spherical Approximation for the Activity Coefficients of Amino Acids in Aqueous Electrolyte Solutions, *Ind. Eng. Chem. Res.*, **1996f**, 35, 4755.

Khoshkbarchi, M. K.; Vera, J. H., Effect of NaCl and KCl on the Solubility of Amino Acids in Aqueous Solutions at 298.2 K: Measurements and Modeling, *Ind. Eng. Chem. Res.*, **1997**, 36, 2445.

Khoshkbarchi, M. K.; Soto, A.; Vera, J. H., Interactions of DL-Serine and L-Serine with NaCl and KCl in Aqueous Solutions, *J. Sol. Chem.*, **1997**, 26, 941.

Khoshkbarchi, M. K.; Vera, J. H., A Theoretically Improved Perturbation Model for Activity Coefficients of Amino Acids and Peptides in Aqueous Solutions, *Ind. Eng. Chem. Res.*, **1998**, 37, 3052.

Kikuchi, M.; Sakurai, M.; Nitta, K., Partial Molar Volumes and Adiabatic Compressibilities of Amino Acids in Dilute Aqueous Solutions at 5, 15, 25, 35, and 45 °C, *J. Chem. Eng. Data*, **1995**, 40, 935.

Kirkwood, J. G., Theory of Solutions of Molecules Containing Widely Separated Charges with Special Application to Zwitterions, *J. Chem. Phys.*, **1934**, 2, 351.

Kirkwood, J. G., Theoretical Studies Upon Dipolar Ions, *Chem. Rev.*, **1939**, 24, 233.

- Kleut, G. J.; Sijpkens, A. H., The Solubilities of Five Cyclic Dipeptides in Water at the Temperature 298.15 K, *J. Chem. Thermodyn.*, **1994**, 26, 1115.
- Kleiner, M.; Sadowski, G., Modeling Vapor-Liquid Equilibria of Ethanol + 1,1,1,2,3,3,3-Heptafluoropropane Binary Mixtures using PC-SAFT, *Fluid Phase Equilib.*, **2007**, 260, 190.
- Kuramochi, H.; Noritomi, H.; Hoshino, D.; Nagahama, K., Measurements of Solubilities of Two Amino Acids in Water and Prediction by the UNIFAC Model, *Biotechnol. Prog.*, **1996**, 12, 371.
- Kuramochi, H.; Noritomi, H.; Hoshino, D.; Nagahama, K., Measurements of Vapor Pressures of Aqueous Amino Acid Solutions and Determination of Activity Coefficients of Amino Acids, *J. Chem. Eng. Data*, **1997**, 42, 470.
- Kurosawa, I.; Teja, A. S.; Rousseau, R. W., Solid-Liquid Equilibria in L-Leucine+L-Valine+Water, *Fluid Phase Equilib.*, **2004**, 224, 245.
- Lamothe, P. J.; McCormick, P. G., Influence of Acidity on the Reaction of Ninhydrin with Amino Acids, *Anal. Chem.*, **1972**, 44, 821.
- Lampreia, I. M. S.; Magalhães, S. R. J.; Rodrigues, S. I. M.; Mendonça, A. F. S. S., Solubility of Proline-Leucine Dipetide in Water and in Aqueous Sodium Chloride Solutions from T = (288.15 to 313.15 K), *J. Chem. Thermodyn.*, **2006**, 38, 240.
- Lark, B. S.; Patyar, P.; Banipal, T. S.; Kishore, N., Densities, Partial Molar Volumes, and Heat Capacities of Glycine, L-Alanine, and L-Leucine in Aqueous Magnesium Chloride Solutions at Different Temperatures, *J. Chem. Eng. Data*, **2004**, 49, 553.
- Lark, B. S.; Patyar, P.; Banipal, T. S., Temperature Effect on the Viscosity and Heat Capacity Behaviour of Some Amino Acids in Water and Aqueous Magnesium Chloride Solutions, *J. Chem. Thermodyn.*, **2007**, 39, 344.
- Leuchtenberger, W.; Huthmacher, K.; Drauz, K., Biotechnological Production of Amino Acids and Derivatives: Current Status and Prospects, *Appl. Microbiol. Biotechnol.*, **2005**, 69, 1.

Lin, R.; Hu, X.; Ren, X., Homogeneous Enthalpic Interaction of Amino Acids in DMF-H₂O Mixed Solvents, *Thermochim. Acta*, **2000**, 352-353, 31.

Liu, J.-C.; Lu, J.-F.; Li, Y.-G., Study on the Activity Coefficients and the Solubilities of Amino Acids in Water by the Perturbation Theory, *Fluid Phase Equilib.*, **1998**, 142, 67.

Lopes, A.; Farelo, F.; Ferra, M. I. A., Activity Coefficients of Sodium Chloride in Water-Ethanol Mixtures: A Comparative Study of Pitzer and Pitzer-Simonson Models, *J. Sol. Chem.*, **2001**, 30, 757.

Luk, C. J.; Rousseau, R. W., Solubilities of and Transformations between the Anhydrous and Hydrated Forms of L-Serine in Water-Methanol Solutions, *Cryst. Growth Des.*, **2006**, 6, 1808.

Lye, G. J.; Asenjo, J. A.; Pyle, D. L., Protein Extraction using Reverse Micelles - Kinetics of Protein Partitioning, *Chem. Eng. Sci.*, **1994**, 49, 3195.

Marrero, J.; Gani, R., Group-Contribution Based Estimation of Pure Component Properties, *Fluid Phase Equilib.*, **2001**, 183, 183.

Matsuo, H.; Suzuki, Y.; Sawamura, S., Solubility of α -Amino Acids in Water Under High Pressure: Glycine, L-Alanine, L-Valine, L-Leucine, and L-Isoleucine, *Fluid Phase Equilib.*, **2002**, 200, 227.

McCaldin, D. J., The Chemistry of Ninhydrin, *Chem. Rev.*, **1960**, 60, 39.

McMeekin, T. L.; Cohn, E. J.; Weare, J. H., Studies in the Physical Chemistry of Amino Acids, Peptides and Related Substances. III. The Solubility of Derivatives of the Amino Acids in Alcohol-Water Mixtures, *J. Am. Chem. Soc.*, **1935**, 57, 626.

Merck; O'Neil, M. J.; Smith, A.; Heckelman, P. E.; Budavari, S., "The Merck Index", 11th Edition, Merck Publishing.

Messer, C. E.; Malakoff, G.; Well, J.; Labib, S., Phase Equilibrium behavior of Certain Pairs of Amino Acids in Aqueous Solution, *J. Phys. Chem.*, **1981**, 85, 3533.

Moore, S.; Stein, W., Photometric Ninhydrin Method for use in the Chromatography of Amino Acids, *J. Biol. Chem.*, **1948**, 176, 367.

Moore, S.; Stein, W., A Modified Ninhydrin Reagent for the Photometric Determination of Amino Acids and Related Compounds, *J. Biol. Chem.*, **1954**, 211, 907.

Mortazavi-Manesh, S.; Ghotbi, C.; Taghikhani, V., A New Model for Predicting Activity Coefficients in Aqueous Solutions of Amino Acids and Peptides, *J. Chem. Thermodyn.*, **2003**, 35, 101.

Nass, K. K., Representation of the Solubility Behavior of Amino Acids in Water, *AIChE J.*, **1988**, 34, 1257.

Ninni, L.; Meirelles, A. J. A., Water Activity, pH and Density of Aqueous Amino Acids Solutions, *Biotechnol. Prog.*, **2001**, 17, 703.

Nozaki, Y.; Tanford, C., The Solubility of Amino Acids, Diglycine, and Triglycine in Aqueous Guanidine Hydrochloride Solutions, *J. Biol. Chem.*, **1970**, 245, 1648.

Nozaki, Y.; Tanford, C., The Solubility of Amino Acids in Two Glycine Peptides in Aqueous Ethanol and Dioxane Solutions, *J. Biol. Chem.*, **1971**, 246, 2211.

Orella, C. J.; Kirwan, D. J., The Solubility of Amino Acids in Mixtures of Water and Aliphatic Alcohols, *Biotechnol. Prog.*, **1989**, 5, 89.

Orella, C. J.; Kirwan, D. J., Correlation of Amino Acid Solubilities in Aqueous Aliphatic Alcohol Solutions, *Ind. Eng. Chem Res.*, **1991**, 30, 1040.

Park, B. H.; Yoo, K.-P.; Lee, C. S., Phase Equilibria and Properties of Amino Acids + Water Mixtures by Hydrogen-Bonding Lattice Fluid Equation of State, *Fluid Phase Equilib.*, **2003**, 212, 175.

Pazuki, G. R.; Rohani, A. A.; Dashtizadeh, A., Correlation of the Mean Ionic Activity Coefficients of Electrolytes in Aqueous Amino Acid and Peptide Systems, *Fluid Phase Equilib.*, **2005**, 231, 171.

Pazuki, G. R.; Nikookar, M., A New Local Composition Model for Predicting of Activity Coefficient and Solubility of Amino Acids and Peptides in Water, *Biochem. Eng. J.*, **2006**, 28, 44.

- Pazuki, G. R.; Hosseinbeigi, H. R.; Edalat, M., Correlation of Phase Equilibria of Amino Acids and Peptides in Aqueous Solution Based on the Perturbation Theory of Equation of State, *Fluid Phase Equilib.*, **2006**, 240, 40.
- Pazuki, G. R.; Taghikhani, V.; Vossoughi, M., Correlation and Prediction the Activity Coefficients and Solubility of Amino Acids and Simple Peptides in Aqueous Solution Using the Modified Local Composition Model, *Fluid Phase Equilib.*, **2007**, 255, 160.
- Peres, A. M.; Macedo, E. A., Representation of Solubilities of Amino Acids Using the UNIQUAC Model for Electrolytes, *Chem. Eng. Sci.*, **1994**, 49, 3803.
- Peres, A. M.; Macedo, E. A., Thermodynamic Properties of Sugars in Aqueous Solutions: Correlation and Prediction using a Modified UNIQUAC Model, *Fluid Phase Equilib.*, **1996**, 123, 71.
- Peres, A. M.; Macedo, E. A., Phase Equilibria of D-Glucose and Sucrose in Mixed Solvent Mixtures: Comparison of UNIQUAC – Based Models, *Carbohydr. Res.*, **1997**, 303, 135.
- Perry, R. H.; Green, D. (1984). Perry's Chemical Engineers' Handbook, 6th Ed., McGraw-Hill International Editions, Singapore.
- Phang, S.; Steel, B. J., Activity Coefficients from E.M.F. Measurements Using Cation-Responsive Glass Electrodes. NaCl + Glycine + Water at 273.15, 283.15, 298.15, and 323.15 K, *J. Chem. Thermodyn.*, **1974**, 6, 537.
- Phang, S., Activity Coefficients in the System NaCl - DL-Alanine - H₂O at 298.15 K from E.M.F. Measurements, *Aust. J. Chem.*, **1978**, 31, 419.
- Pinho, S. P. (2000). Phase Equilibria in Electrolyte Systems, Ph.D. Thesis, LSRE, Department of Chemical Engineering, Porto.
- Pinho, S. P., Water Activity in Aqueous Amino Acid Solutions, with and without KCl, at 298.15 K, *J. Chem. Eng. Data*, **2008**, 53, 180.

- Pinho, S. P.; Macedo, E. A., Experimental Measurement and Modelling of KBr Solubility in Water, Methanol, Ethanol, and its Binary Mixed Solvents at Different Temperatures, *J. Chem. Thermodyn.*, **2002**, 34, 337.
- Pinho, S. P.; Silva, C. M.; Macedo, E. A., Solubility of Amino Acids: A Group-Contribution Model Involving Phase and Chemical Equilibria, *Ind. Eng. Chem. Res.*, **1994**, 33, 1341.
- Pitzer, K. S.; Li, Y. G., Thermodynamics of Aqueous Sodium Chloride to 823 K and 1 kilobar (100 MPa), *Proc. Natl. Acad. Sci. U.S. A.*, **1983**, 80, 7689.
- Pradhan, A. A.; Vera, J. H., Effect of Acids and Bases on the Solubility of Amino Acids, *Fluid Phase Equilib.*, **1998**, 152, 121.
- Pradhan, A. A.; Vera, J. H., Effect of the Anions on the Solubility of Zwitterions Amino Acids, *J. Chem. Eng. Data*, **2000**, 45, 140.
- Prausnitz, J. M.; Lichtenthaler, R. N.; Azevedo, E. G. (1999). Molecular Thermodynamics of Fluid-Phase Equilibria, 3rd Ed., Prentice-Hall, New Jersey.
- Prochazkova, S.; Vårum, K. M.; Østgaard, K., Quantitative Determination of Chitosans by Ninhydrin, *Carbohydr. Polym.*, **1999**, 38, 115.
- Profir, V. M.; Matsuoka, M., Processes and Phenomena of Purity Decrease During the Optical Resolution of DL-Threonine by Preferential Crystallization, *Colloid Surf. A-Physicochem. Eng. Asp.*, **2000**, 164, 315.
- Ramasami, P.; Kakkar, R., Partial Molar Volumes and Adiabatic Compressibilities at Infinite Dilution of Aminocarboxylic Acids and Glycylglycine in Water and Aqueous Solutions of Sodium Sulphate at (288.15, 298.15 and 308.15) K, *J. Chem. Thermodyn.*, **2006**, 38, 1395.
- Reid, R. C.; Prausnitz, J. M.; Poling, B. E. (1987). The Properties of Gases and Liquids, 4th Ed., McGraw-Hill Book Co., New York.
- Renon, H.; Prausnitz, J. M., Local Compositions in Thermodynamic Excess Functions for Liquid Mixtures, *AIChE J.*, **1968**, 14, 135.

Richards, M. M., The Effect of Glycine upon the Activity Coefficient of Glycine, Egg Albumin, and Carboxyhemoglobin, *J. Biol. Chem.*, **1938**, 122, 727.

Roberts, R. M.; Kirkwood, J. G., The Activity of Glycine in Aqueous Solutions of Potassium Chloride, from Electromotive Force Measurements, *J. Am. Chem. Soc.*, **1941**, 63, 1373.

Robinson, R. A., The Vapor Pressure of Aqueous Solutions of Alanine, *J. Biol. Chem.*, **1952**, 199, 71.

Rodríguez-Raposo, R.; Fernández-Mérida, L.; Estes, M. A., Activity Coefficients in (Electrolyte + Amino Acid) (aq) The Dependence of the Ion-Zwitterion Interactions on the Ionic Strength and on the Molality of the Amino Acid Analysed in Terms of Pitzer's Equations, *J. Chem. Thermodyn.*, **1994**, 26, 1121.

Romero, C.; González, M. E., Osmotic and Activity Coefficients of Glycine, DL- α -Alanine and DL- α -Aminobutyric Acid in Aqueous Solutions at Temperatures between 288.15 and 303.15 K, *Fluid Phase Equilib.*, **2006**, 250, 99.

Sadeghi, R., A Modified Wilson Model for the Calculation of Vapour + Liquid Equilibrium of Aqueous Polymer + Salt Solutions, *J. Chem. Thermodyn.*, **2005**, 37, 323.

Sadeghi, R., Thermodynamic Representation of Phase Equilibrium Behavior of Aqueous Solutions of Amino Acids by the Modified Wilson Model, *Fluid Phase Equilib.*, **2007**, 260, 266.

Sadovnikova, M. S.; Belikov, V. M., Industrial Applications of Amino-Acids, *Russ. Chem. Rev.*, **1978**, 42, 199.

Sapoundjiev, D.; Lorenz, H.; Seidel-Morgenstern, A., Solubility of Chiral Threonine Species in Water/Ethanol Mixtures, *J. Chem. Eng. Data*, **2006**, 51, 1562.

Schrier, E. E.; Robinson, R. A., A Study of Free Energy Relationships in Some Amino Acid-Sodium Chloride-Water Systems, *J. Sol. Chem.*, **1971**, 246, 2870.

Schrier, E. E.; Robinson, R. A., Free Energy Relationships in Aqueous Amino Acid and Peptide Solutions Containing Sodium Chloride, *J. Sol. Chem.*, **1974**, 3, 493.

Scopes, R. K. (1994). Protein Purification: Principle and Practice, 3rd Ed., Springer-Verlag, New York.

Scott, E.; Peter, F.; Sanders, J., Biomass in the Manufacture of Industrial Products – The use of Proteins and Amino Acids, *Appl. Microbiol. Biotechnol.* **2007**, 75, 751.

Sexton, E. L.; Dunn, M. S., Solubility of Certain Amino Acids in Aqueous Solutions of Amino Acids and Peptides, *J. Phys. Colloid Chem.*, **1947**, 51, 648.

Singh, M.; Pandey, M.; Yadav, R. K.; Verma, H. S., Thermodynamic Studies of Molar Volume, Pair and Triplet Interactions at Increasing Side Chain Length of α -Amino Acids in Aqueous Potassium Chloride Solutions at Different Concentration and 310.15 K, *J. Mol. Liq.*, **2007**, 135, 42.

Smith, E. R. B.; Smith, P. K., The Activity of Glycine in Aqueous Solution at Twenty Five Degrees, *J. Biol. Chem.*, **1937a**, 117, 209.

Smith, P. K.; Smith, E. R. B., Thermodynamic Properties of Solutions of Amino Acids and Related Substances. II. The Activity of Aliphatic Amino Acids in Aqueous Solution at Twenty Five Degrees, *J. Biol. Chem.*, **1937b**, 121, 607.

Smith, P. K.; Smith, E. R. B., Thermodynamic Properties of Solutions of Amino acids and Related Substances. IV. The Effect of Increasing Dipolar Distance on the Activities of Aliphatic Amino Acids in Aqueous Solution at Twenty Five Degrees, *J. Biol. Chem.*, **1939**, 132, 47.

Smith, P. K.; Smith, E. R. B., Thermodynamic Properties of Solutions of Amino acids and Related Substances. V. The Activities of Some Hydroxy- and *n*-Methylamino Acids and Proline in Aqueous Solution at Twenty Five Degrees, *J. Biol. Chem.*, **1940a**, 132, 57.

Smith, P. K.; Smith, E. R. B., Thermodynamic Properties of Solutions of Amino acids and Related Substances. VI. The Activities of Some Peptides in Aqueous Solution at Twenty Five Degrees, *J. Biol. Chem.*, **1940b**, 135, 273.

- Soto, A.; Arce, A.; Khoshkbarchi, M. K.; Vera, J. H., Activity Coefficients of the Electrolyte and the Amino Acid in Water + NaNO₃ + Glycine and Water + NaCl + DL-Methionine Systems at 298.15 K, *Biophys. Chemist.*, **1997a**, 67, 97.
- Soto, A.; Arce, A.; Khoshkbarchi, M. K.; Vera, J. H., Interaction of DL-Threonine with NaCl and NaNO₃ in Aqueous Solutions: E.M.F. Measurements with Ion-Selective Electrodes, *J. Chem. Thermodyn.*, **1997b**, 29, 609.
- Soto, A.; Arce, A.; Khoshkbarchi, M. K.; Vera, J. H., Effect of the Cation and the Anion of an Electrolyte on the Solubility of DL-Aminobutyric Acid in Aqueous Solutions: Measurement and Modelling, *Biophys. Chemist.*, **1998a**, 73, 77.
- Soto, A.; Arce, A.; Khoshkbarchi, M. K.; Vera, J. H., Effect of the Anion and the Cation of an Electrolyte on the Activity of DL-Alanine in Aqueous Solutions, *Fluid Phase Equilib.*, **1998b**, 142, 193.
- Soto, A.; Arce, A.; Khoshkbarchi, M. K.; Vera, J. H., Experimental Data and Modelling of Apparent Molar Volumes, Isentropic Compressibilities and Refractive Indices in Aqueous Solutions of Glycine + NaCl, *Biophys. Chemist.*, **1998c**, 74, 165.
- Soto, A.; Arce, A.; Khoshkbarchi, M. K.; Vera, J. H., Measurements and Modelling of the Solubility of a Mixture of two Amino Acids in Aqueous Solutions, *Fluid Phase Equilib.*, **1999**, 158, 893.
- Su, Ch.-K.; Chiang, B. H., Extraction of Immunoglobulin-G from Colostral Whey by Reverse Micelles, *J. Dairy Sci.*, **2003**, 86, 1639.
- Sun, S.-W.; Lin, Y.-C.; Weng, Y.-M.; Chen, M.-J., Efficiency Improvements on Ninhydrin Method for Amino Acid Quantification, *J. Food Compos. Anal.*, **2006**, 19, 112.
- Teja, A. S.; Givand, J. C.; Rousseau, R. W., Correlation and Prediction of Crystal Solubility and Purity, *AIChE J.*, **2002**, 48, 2629.
- Tumakaka, F.; Gross, J.; Sadowski, G., Thermodynamic Modeling of Complex Systems using PC-SAFT, *Fluid Phase Equilib.*, **2005**, 228, 89.

- Vetere, A., A Simple Modification of the NRTL Equation, *Fluid Phase Equilib.*, **2000**, 173, 57.
- Vetere, A., The NRTL Equation as a Predictive Tool for Vapor-Liquid Equilibria, *Fluid Phase Equilib.*, **2004**, 218, 33.
- Vollhardt, K. P.; Schore, N. E. (2003). Organic Chemistry Structure and Function, 4th Ed., W. H. Freeman and Co., New York.
- Wilce, M. C. J.; Aguilar, M.-I.; Heam, M. T. W., Physicochemical Basis of Amino Acid Hydrophobicity Scales: Evaluation of Four New Scales of Amino Acid Hydrophobicity Coefficients Derived from RP-HPLC of Peptides, *Anal. Chem.*, **1995**, 67, 1210.
- Wolbach, J. P.; Sandler, S. I., Using Molecular Orbital Calculations to Describe the Phase Behavior of Cross-Associating Mixtures, *Ind. Eng. Chem Res.*, **1998**, 37, 2917.
- Xu, X.; Pinho, S. P.; Macedo, E. A., Activity Coefficient and Solubility of Amino Acids in Water by the Modified Wilson Model, *Ind. Eng. Chem. Res.*, **2004**, 43, 3200.
- Yan, Z.; Wang, J.; Liu, W.; Lu, J., Apparent Molar Volumes and Viscosity *B*-Coefficients of some α -Amino Acids in Aqueous Solutions from 278.15 to 308.15 K, *Termochim. Acta*, **1999**, 334, 17.
- Yi, Y.; Hatzivramidis, D.; Myerson, A. S.; Waldo, M.; Beylin, V. G.; Mustakis, J., Development of a Small-Scale Automated Solubility Measurement Apparatus, *Ind. Eng. Chem. Res.*, **2005**, 44, 5427.
- Yin, J.; Tomycz, L.; Bonner, G.; Wang, D. I. C., A Simple and Rapid Assay of Collagen-Like Polymer in Crude Lysate from *Escherichia coli*, *J. Microbiol. Methods*, **2002**, 49, 321.
- Yuan, Q.; Li, Z.-F.; Wang, B.-H., Partial Molar Volumes of L-Alanine, DL-Serine, DL-Threonine, L-Histidine, Glycine, and Glycylglycine in Water, NaCl, and DMSO Aqueous Solutions at T = 298.15 K, *J. Chem. Thermodyn.*, **2006**, 38, 20.

Zhao, H., Viscosity *B*-Coefficients and Standard Partial Molar Volumes of Amino Acids, and their Roles in Interpreting the Protein (Enzyme) Stabilization, *Biophys. Chem.*, **2006**, 122, 157.

Zhao, E.; Yu, M.; Sauvé, R. E.; Khoshkbarchi, M. K., Extension of the Wilson Model to Electrolyte Solutions, *Fluid Phase Equilib.*, **2000**, 173, 161.

Zumstein, R. C.; Rousseau, R. W., Solubility of L-Isoleucine in and Recovery of L-Isoleucine from Neutral and Acidic Aqueous Solutions, *Ind. Eng. Chem. Res.*, **1989**, 28, 1226.

APPENDICES

APPENDIX A.

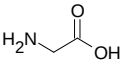
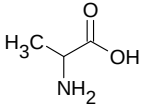
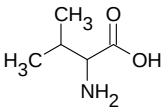
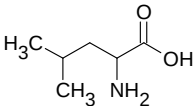
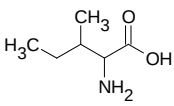
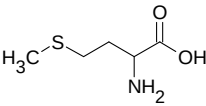
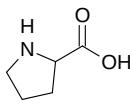
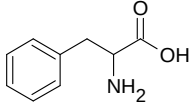
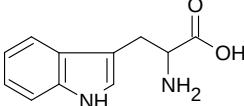
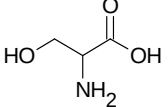
THE CHEMISTRY OF AMINO ACIDS

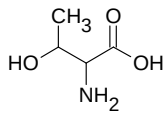
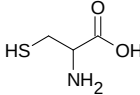
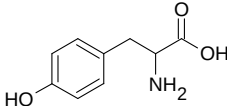
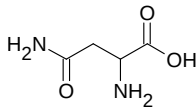
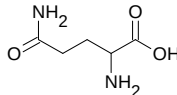
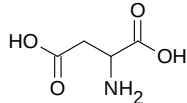
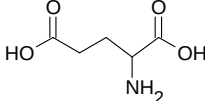
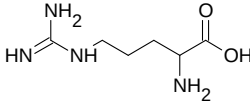
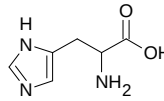
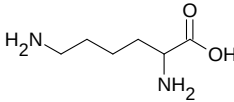
Glycine is the smallest amino acid; it has no side chain. Alanine (R = methyl), valine (R = isopropyl), leucine (R = isobutyl), and isoleucine (R = sec-butyl) have alkyl group (R) as side chains. They have hydrophobic side chains that differ in size: glycine < alanine < valine < leucine, and isoleucine is more spherical than leucine. Methionine (R = -CH₂CH₂SCH₃) due to the presence of sulphur in its side chains, is more polarizable, compared with the alkyl groups mentioned before, leading to stronger dispersion forces. Proline is relatively compact due to the cyclic nature of its side chain and has less conformational flexibility than the other amino acids. Phenylalanine and tryptophan have aromatic rings (large and hydrophobic) incorporated in their side chains. Tryptophan is bicyclic, has a more electron-rich aromatic ring which makes it larger and more polarizable than phenylalanine. Among the amino acids with polar side chain, serine (R = -CH₂OH) is the smallest, and not much larger than alanine. Threonine has a methyl group in place of one of the hydrogens present in the serine molecule, sterically hindering the -OH group and making it less effective to hydrogen bonding. Cysteine (R = CH₂SH) is related to serine in its side chain. Tyrosine, a *p*-hydroxy derivative of phenylalanine, has similar properties to those of phenylalanine and also the facility to engage in hydrogen bonding by its -OH group. Asparagine and glutamine are not amines but amides, their side chain terminal group is -CONH₂ and differ only by a -CH₂ group. They are quite polar, and interact strongly with water molecules by hydrogen bonding. Aspartic and glutamic acid are the most electron-rich units of all the common amino acids. Lysine side chain has four -CH₂ groups and terminates in -NH₂. Arginine has a complex and large side chain that consists of three aliphatic carbons and a complex guanidinium group. The guanidinium group is positively charged in neutral, acidic and even most basic environments, and thus confers basic chemical properties to

arginine. Due to the conjugation between the double bond and the nitrogen lone pairs, the positive charge is de-localized, enabling the formation of multiple hydrogen-bonds. Histidine has an imidazole side chain which has two nitrogens with different properties: one is bound to hydrogen and donates its lone pair to the aromatic ring (slightly acidic) whereas the other donates only one electron pair to the ring so it has a free lone pair (basic). The side chain of histidine is not as basic as that of lysine or arginine and, at biological pH, the concentration of its unprotonated and protonated forms is almost equal (Carey, 2003).

The amino acid backbone determines the primary sequence of a protein, but the nature of the side chains determines the protein's properties. The different amino acids have interesting properties because they have a variety of structural parts which result in different polarities and solubilities. Amino acid side chains can be polar, non-polar. Humans have the capacity to biosynthesize some amino acids, but the others, called essential amino acids and identified as such in the Table A.1 must be obtained from their diet. Histidine and arginine are generally only considered essential in children, because the metabolisms that synthesize these amino acids are not fully developed in children.

Table A.1 α -Amino acids found in proteins.

Name (synonym)	Abbreviation	Polarity	Acidity or Basicity	Structural formula
Glycine (Aminoacetic acid)	Gly (G)	Nonpolar	Neutral	
Alanine (2-amino-propanoic acid)	Ala (A)	Nonpolar	Neutral	
Valine* (2-amino-3-methyl-butanoic acid)	Val (V)	Nonpolar	Neutral	
Leucine* (2-amino-4-methyl-pentanoic acid)	Leu (L)	Nonpolar	Neutral	
Isoleucine* (2-amino-4-methyl-thio-butanoic acid)	Ile(I)	Nonpolar	Neutral	
Methionine* (2-amino-3-hydroxy-propanoic acid)	Met (M)	Nonpolar	Neutral	
Proline (2-pyrrolidine-carboxylic acid)	Pro (P)	Nonpolar	Neutral	
Phenylalanine* 2-amino-3-phenyl-propanoic acid	Phe (F)	Nonpolar	Neutral	
Tryptophan* (2-amino-3-indoyl-propanoic acid)	Trp(W)	Nonpolar	Neutral	
Serine (2-amino-3-hydroxy-propanoic acid)	Ser (S)	Polar	Neutral	

Threonine* (2-amino-3-hydroxy- butanoic acid)	Thr (T)	Polar	Neutral	
Cysteine (2-amino-3-mercapto- propanoic acid)	Cys (C)	Polar	Acidic	
Tyrosine (2-amino-3-(4-hydroxy- phenyl)-propanoic acid)	Tyr (Y)	Polar	Neutral	
Asparagine (2-amino-3-carbamoyl- propanoic acid)	Asn (N)	Polar	Neutral	
Glutamine (2-amino-4-carbamoyl- butanoic acid)	Gln (Q)	Polar	Neutral	
Aspartic Acid (2-amino-butane-dioic acid)	Asp (D)	Polar	Acidic	
Glutamic Acid (2-amino-pentane-dioic acid)	Glu (E)	Polar	Acidic	
Arginine* (2-amino-5-guani- dopentanoic acid)	Arg (R)	Polar	Strongly basic	
Histidine* (2-amino-3-imidazole- propanoic acid)	His (H)	Polar	Weakly basic	
Lysine* (2,6-diamino-hexanoic acid)	Lys (K)	Polar	Basic	

*essential amino acids

APPENDIX B.

MECHANISM OF THE REACTION OF NINHYDRIN

The mechanism of the reaction of ninhydrin with a primary amino group can be explained in terms of polar and steric effects associated with the reactants. The ninhydrin interacting with α -amino acids, produce carbon dioxide, aldehyde, ammonia, hydrindantin, and Ruhemann's purple (McCaldin, 1960) (Figure B.1). The reaction proceeds through the formation of a schiff base which is unstable and undergoes decarboxylation, and hydrolysis to yield 2-amino-indanedione (A) as a stable intermediate. 2-Amino indanedione acts as a reactant in the formation of ammonia and Ruhemann's purple (RP). Two reactions, (i) hydrolysis and (ii) condensation occur simultaneously. These two reactions depend strongly upon the pH, atmospheric oxygen, and temperature. 2-Amino-indanedione is extremely sensitive to molecular oxygen; in the presence of atmospheric oxygen instead of the Ruhemann's purple (RP), a yellowish colored product is formed. At low pH, the reaction proceeds mainly by route (i) where there is no Ruhemann's purple (RP) formation. In solutions of pH at or near 5.0, route (ii) predominates however the route (i) cannot be ruled out completely (Kabir-Ud-Din, 2006). The rate-determining step in the ninhydrin reaction appears to involve a nucleophilic displacement of a hydroxy group of ninhydrin by a nonprotonated amino group. The rate of the reaction pass through a maximum as function of pH where the products of the concentrations of reactive species, protonated ninhydrin and non-protonated amino acid, is a maximum (Lamothe and McCormick, 1972). The kinetic and mechanistic studies on the behaviour of structurally different amino acids in ninhydrin reactions indicated that the reaction of α -amino acids with ninhydrin entails two molecules of ninhydrin for each molecule of amino acid to form Ruhemann's purple (RP) (Friedman, 2004).

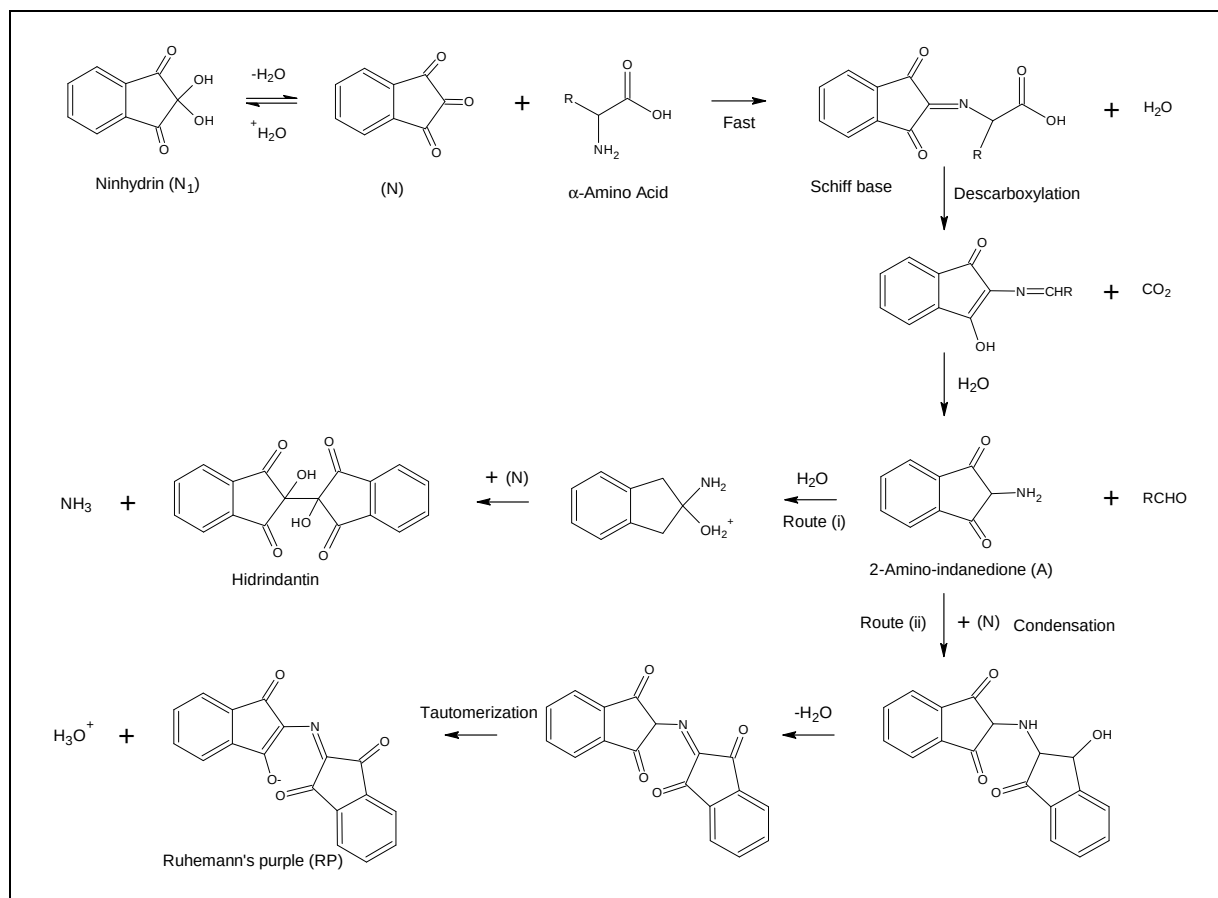


Figure B.1 Mechanism of the reaction of ninhydrin.

APPENDIX C.

CALIBRATION CURVES

In this appendix the calibration curves prepared by assaying standard amino acid solutions at 8 different concentrations, ranging from 0.00005 to 0.0005 g amino acid/100g of water, obtained by dilution of an initial solution of known concentration (0.01 g amino acid/100g of water) are presented. Since calibration curves are made for each amino acid studied on each analysis day, using freshly prepared calibration standards, Figures C.1 to C.3 are just examples of one working day.

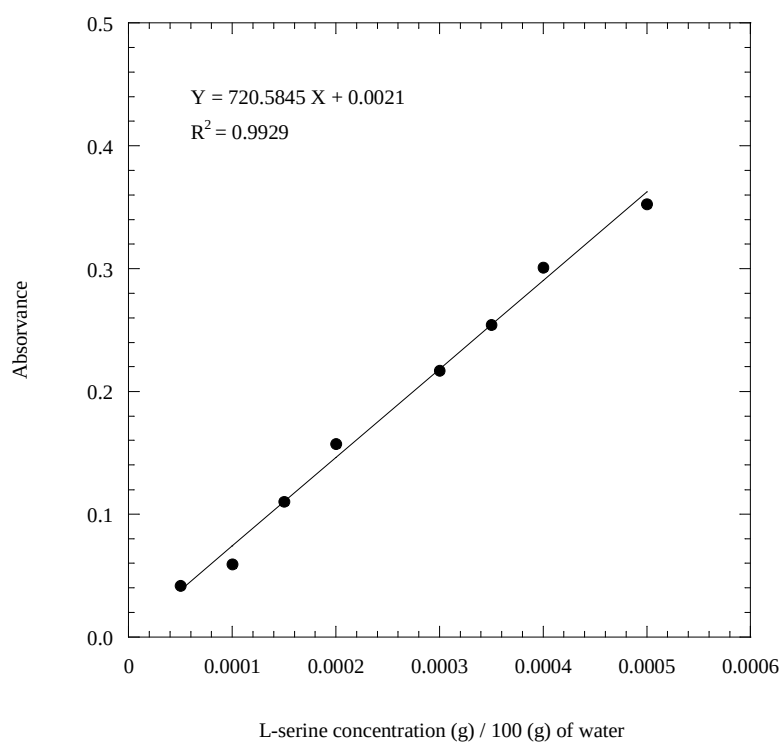


Figure C.1 Calibration curve for L-serine.

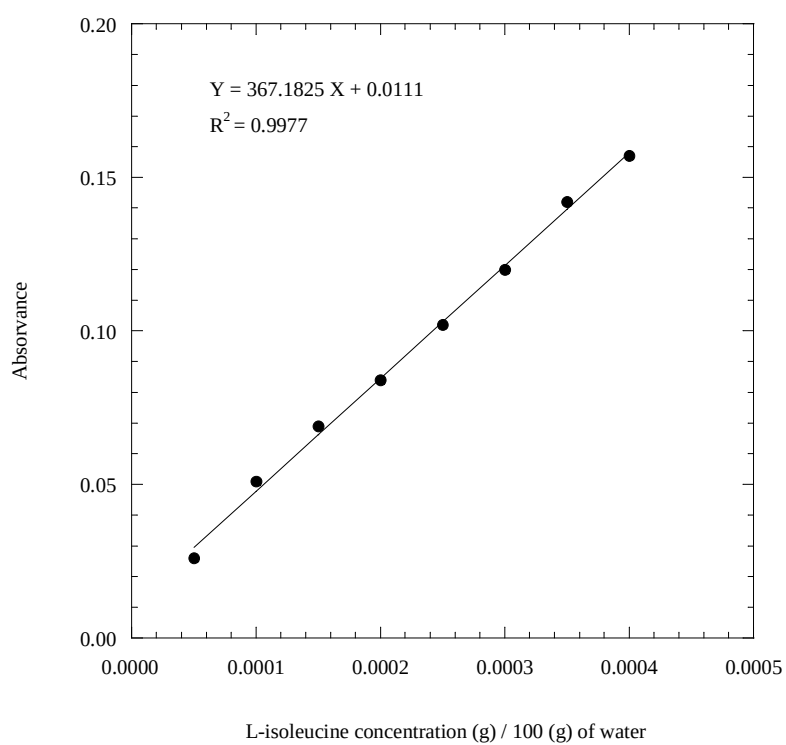


Figure C.2 Calibration curve for L-isoleucine.

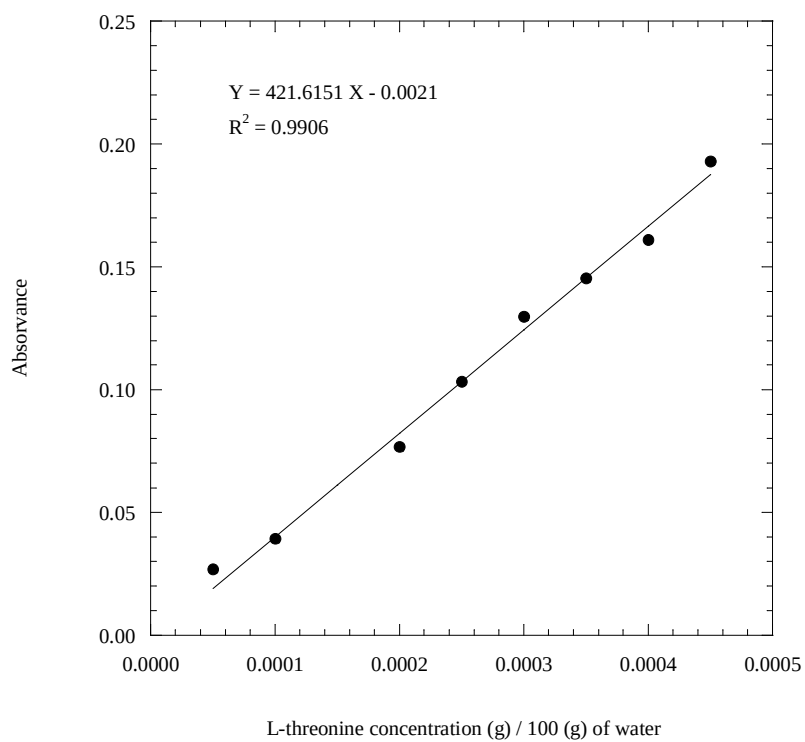


Figure C.3 Calibration curve for L-threonine.

APPENDIX D.

SUMMARY OF EQUATIONS (PERTURBED-CHAIN SAFT EoS)

A summary of equations for calculating thermophysical properties using the PC-SAFT EoS is given in this appendix. From the residual Helmholtz free energy A^{res} all the other properties can be obtained. In the following, a tilde ($\sim$) corresponds to reduced quantities.

The total reduced residual Helmholtz free energy ($\tilde{a}^{res}$) is given by:

$$\tilde{a}^{res} = \frac{A^{res}}{RT} \quad (D.1)$$

and is considered as a sum of different contributions:

$$\tilde{a}^{res} = \tilde{a}^{hc} + \tilde{a}^{disp} + \tilde{a}^{assoc} \quad (D.2)$$

Hard-Chain Reference Contribution (Gross and Sadowski, 2001)

The contribution to the hard chain term ($\tilde{a}^{hc}$) is made up of two contributions; the hard-sphere and the chain terms:

$$\tilde{a}^{hc} = \bar{m} \tilde{a}^{hs} - \sum_i x_i (m_i - 1) \ln g_{ii}^{hs}(\sigma_{ii}) \quad (D.3)$$

here $\bar{m}$ is a mean segment length defined as $\bar{m} = \sum_i x_i m_i$, and the hard-sphere term is given

on a per-segment basis:

$$\tilde{a}^{hs} = \frac{1}{\zeta_0} \left[\frac{3\zeta_1\zeta_2}{(1-\zeta_3)} + \frac{\zeta_2^3}{\zeta_3(1-\zeta_3)^2} + \left(\frac{\zeta_2^3}{\zeta_3^2} - \zeta_0 \right) \ln(1-\zeta_3) \right] \quad (D.4)$$

The radial distribution function of the hard-sphere is given by:

$$g_{ij}^{hs}(\sigma_{ij}) = \frac{1}{(1-\zeta_3)} + \left(\frac{d_i d_j}{d_i + d_j} \right) \frac{3\zeta_2}{(1-\zeta_3)^2} + \left(\frac{d_i d_j}{d_i + d_j} \right)^2 \frac{2\zeta_2^2}{(1-\zeta_3)^3} \quad (D.5)$$

The ζ_n values are defined by:

$$\zeta_n = \frac{\pi}{6} \rho \sum_i x_i m_i d_i^n \quad n \in \{0,1,2,3\} \quad (D.6)$$

where ρ is the total number density of molecules, x_i is the mole fraction of component i , m_i is the number of segments per chain of component i , and d_i is the temperature-dependent segment diameter of component i :

$$d_i = \sigma_i \left[1 - 0.12 \exp\left(-\frac{3\varepsilon_i}{kT}\right) \right] \quad (D.7)$$

here σ_i is the temperature-independent segment diameter and ε_i is the segment energy parameter, k is the Boltzman constant and T the absolute temperature.

Dispersion Contribution (Gross and Sadowski, 2001)

The dispersion contribution ($\tilde{a}^{disp}$) is given by the following expression:

$$\tilde{a}^{disp} = -2\pi\rho I_1(\eta, \bar{m}) \overline{m^2 \varepsilon \sigma^3} - \pi\rho \bar{m} C_1 I_2(\eta, \bar{m}) \overline{m^2 \varepsilon^2 \sigma^3} \quad (D.8)$$

Here, η is the packing fraction ($\eta = \zeta_3$) and C_1 is an abbreviation for the compressibility expression:

$$\begin{aligned} C_1 &= \left(1 + Z^{hc} + \rho \frac{\partial Z^{hc}}{\partial \rho} \right)^{-1} \\ &= \left(1 + \bar{m} \frac{8\eta - 2\eta^2}{(1-\eta)^4} + (1-\bar{m}) \frac{20\eta - 27\eta^2 + 12\eta^3 - 2\eta^4}{[(1-\eta)(2-\eta)]^2} \right) \end{aligned} \quad (D.9)$$

where Z is the compressibility factor, with $Z = Pv/(RT)$; P is the pressure, v is the molar volume and R the ideal gas constant.

The other abbreviations are defined as:

$$\overline{m^2 \epsilon \sigma^3} = \sum_i \sum_j x_i x_j m_i m_j \left(\frac{\epsilon_{ij}}{kT} \right) \sigma_{ij}^3 \quad (\text{D.10})$$

$$\overline{m^2 \epsilon^2 \sigma^3} = \sum_i \sum_j x_i x_j m_i m_j \left(\frac{\epsilon_{ij}}{kT} \right)^2 \sigma_{ij}^3 \quad (\text{D.11})$$

Conventional combining rules are employed to determine the parameters for a pair of unlike segments:

$$\sigma_{ij} = \frac{1}{2} (\sigma_i + \sigma_j) \quad (\text{D.12})$$

$$\epsilon_{ij} = (1 - k_{ij}) \sqrt{\epsilon_i \epsilon_j} \quad (\text{D.13})$$

being k_{ij} the binary interaction parameter.

The integrals of the perturbation theory are substituted by simple power series in density written as:

$$I_1(\eta, \bar{m}) = \sum_{i=0}^6 a_i(\bar{m}) \eta^i \quad (\text{D.14})$$

$$I_2(\eta, \bar{m}) = \sum_{i=0}^6 b_i(\bar{m}) \eta^i \quad (\text{D.15})$$

the coefficients a_i and b_i depend on the chain length according to:

$$a_i(\bar{m}) = a_{0i} + \frac{\bar{m}-1}{\bar{m}} a_{1i} + \frac{\bar{m}-1}{\bar{m}} \frac{\bar{m}-2}{\bar{m}} a_{2i} \quad (\text{D.16})$$

$$b_i(\bar{m}) = b_{0i} + \frac{\bar{m}-1}{\bar{m}} b_{1i} + \frac{\bar{m}-1}{\bar{m}} \frac{\bar{m}-2}{\bar{m}} b_{2i} \quad (\text{D.17})$$

where, a_{0i} , a_{1i} , a_{2i} , b_{0i} , b_{1i} ; b_{2i} are universal model constants (Gross and Sadowski, 2001).

Association Contribution (Kleiner and Sadowski, 2007)

The expression for the association contribution to the Helmholtz energy ($\tilde{a}^{assoc}$) is:

$$\tilde{a}^{assoc} = \sum_i x_i \sum_{A_i} \left(\ln X^{A_i} - \frac{X^{A_i}}{2} + \frac{1}{2} \right) \quad (D.18)$$

where x_i is the mole fraction of the component i and X^{A_i} the mole fraction of molecules i not bonded at site A described by:

$$X^{A_i} = \left[1 + \rho \sum_j x_j \sum_{B_j} X^{B_j} \Delta^{A_i B_j} \right]^{-1} \quad (D.19)$$

$\sum_{B_j}$ corresponds to the summation over all sites on molecule j : A_j , B_j , ..., and $\sum_i$ means summation over all components, and the association strength $\Delta^{A_i B_j}$ can be approximated as:

$$\Delta^{A_i B_j} = \sigma_{ij}^3 g_{ij}^{hs}(d_{ij}) \kappa^{A_i B_j} \left[\exp\left(\frac{\epsilon^{A_i B_j}}{kT}\right) - 1 \right] \quad (D.20)$$

To describe mixtures of two associating substances, the strength of the cross-associating interactions $\epsilon^{A_i B_j}$ and $\kappa^{A_i B_j}$ between unlike molecules is estimated applying combining rules as suggested by Wolbach and Sandler (1998):

$$\epsilon^{A_i B_j} = \frac{1}{2} (\epsilon^{A_i B_i} + \epsilon^{A_j B_j}) \quad (D.21)$$

$$\kappa^{A_i B_j} = \sqrt{\kappa^{A_i B_i} \kappa^{A_j B_j}} \left[\frac{\sqrt{\sigma_{ii} \sigma_{jj}}}{1/2(\sigma_{ii} + \sigma_{jj})} \right]^3 \quad (D.22)$$