

**THERMODYNAMICS AND HYDROPHOBIC HYDRATION  
OF SOME BIOCHEMICAL MODEL COMPOUNDS IN  
AQUEOUS AND MIXED AQUEOUS SOLUTIONS**

**A Thesis Submitted  
In Fulfilment of the Requirement for the Degree of  
DOCTOR OF PHILOSOPHY**

**By  
AWADHESH KUMAR MISHRA**

to the

**DEPARTMENT OF CHEMISTRY  
INDIAN INSTITUTE OF TECHNOLOGY, DELHI  
JANUARY, 1981**

TH  
542.6  
MIS - T

I. I. T. DELHI.  
LIBRARY  
Acc. No. TH 825 R<sub>2</sub>

187/1/87

I. I. T. DELHI  
LIBRARY  
RECORDED

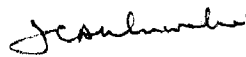
समर्पित -  
पूज्य माँ और पिताजी के  
चरणकमलों पर

CERTIFICATE

This is to certify that the thesis entitled "THERMODYNAMICS AND HYDROPHOBIC HYDRATION OF SOME BIOCHEMICAL MODEL COMPOUNDS IN AQUEOUS AND MIXED AQUEOUS SOLUTIONS" being submitted by Mr. A.K. Mishra to the Indian Institute of Technology, Delhi, for the award of the degree of Doctor of Philosophy in Chemistry, is a record of bonafide research work carried out by him. Mr. Mishra has worked with my guidance and supervision and has fulfilled the requirements for the submission of this thesis, which to my knowledge, has reached the requisite standard.

The results contained in this dissertation have not been submitted, in part or in full, to any other University or Institute for the award of any degree or diploma.

I.I.T., Delhi  
29.12.1980

  
( J. C. Ahluwalia )  
Professor  
Department of Chemistry

## ACKNOWLEDGEMENT

It is with great pleasure I express my deep sense of gratitude -

To Professor J.C. Ahluwalia for suggesting the research problems and for his inspiring guidance throughout the course of the present investigations without which this work would not have been possible.

To Dr. S.K. Suri and Dr. B. Chawla and to my colleagues Dr. K.P. Prasad, Mr. N. Roy Choudhury, Mr. P.K. Singh, and Mr. R.V. Jasra who have helped me formulate my ideas in stimulating discussions and for their unfailing co-operation,

To Mr. Shiv Singh for his splendid expertise to fabricate and repair various electronic and mechanical systems whenever it was needed and to Mr. Anil K. Tangry for his dedicated assistance.

To Mr. N.L. Arora for listening with patience and good humor and then drawing the graphs and illustrations.

To those typists who have transcribed one unreadable draft after another Mr. S. Anand, Mr. T.R. Sharma, Mr. Ashok Kumar and Miss Rama and to the entire valient staff of the Department of Chemistry, for having helped me at various stages of this work.

To the Indian Institute of Technology, Kanpur and Indian Institute of Technology, Delhi which provided part of my support.

To the Department of Science and Technology, New Delhi for the financial assistance at a time when it was needed most.

To my teachers and professors whose earnest interest and good wishes have always encouraged me and especially to Professors D. Balasubramanian and G. Mehta for providing HPLC facility at the University of Hyderabad, and last but not the least,

To the Shivalik, which housed, the many restaurants that nourished; and friends and acquaintances who comforted, prodded and cajoled.

*A.K. Mishra*

## ABSTRACT

Investigation of small model compounds is known to be a very useful method for elucidating various solute-solvent and solute-solute interactions prevalent in the aqueous solutions of biological macromolecules. Thermodynamic investigations on some model compounds of proteins and polypeptides such as amino acids, N-acetyl amino acids, and peptides in water, aqueous alcohol, and aqueous urea solutions are described in this thesis. The nature of interactions of these solutes with water and mixed aqueous solvents has been deduced from the thermodynamic quantities such as enthalpy and heat capacity of dissolution and apparent molal volume of the solute. Enthalpies and heat capacities of dissolution are obtained by calorimetry and apparent molal volumes from density measurements.

Chapter 1 of the thesis describes briefly the 'water structure' -peculiar nature of which is supposed to be the origin of most of the typical solute-solvent and solute-solute interactions in aqueous solutions. A brief account of typical features of aqueous solutions and the techniques through which the thermodynamic data reveal the nature of molecular interactions in aqueous solutions are also incorporated in this Chapter.

Chapter 2 deals with the experimental set up of the isoperibol submarine calorimeter and the oscillating tube digital density meter and their operational procedure.

In Chapter 3, enthalpy, heat capacity, and apparent molal volume changes due to transfer of some amino acids from water to aqueous tert-butanol solutions are reported. Enthalpy of transfer of diglycine from water to aqueous tert-butanol and to aqueous ethanol solutions are also given in this Chapter. Entropies of transfer of a peptide backbone unit ( $\text{CH}_2\text{CONH}$ ) and peptide group ( $\text{CONH}$ ) from water to aqueous ethanol solutions were evaluated from the enthalpy of transfer data and the free energies of transfer of glycine, diglycine and  $\alpha$ -alanine available in literature.

The thermodynamic transfer functions are discussed in terms of solute-solute interactions. Observed trends in the thermodynamic transfer functions of these model compounds have been also utilized to rationalize the effect of alcohols on the conformational stability of proteins and polypeptides.

Chapter 4 consists of the results of integral enthalpies of solution of sodium salts of some N-acetyl amino acids. Limiting partial molal heat capacity contributions of the peptide backbone unit ( $\text{CH}_2\text{CONH}$ ) and the peptide group ( $\text{CONH}$ ) are evaluated from the enthalpy of solution data and discussed in terms of the solute-solvent interactions.

The results of the apparent molal volume measurements of some amino acids, N-acetyl amino acids and peptides are described in Chapter 5. Group additivity relationships for the molal volumes and the effect of electrostriction in these series of compounds have been examined. Concentration dependence of the apparent molal volumes have been explained in terms of hydrophobic and hydrophilic interactions.

Limiting apparent molal volumes of these compounds are estimated from the van der Waals atomic volume increments and appropriate volume decrements due to hydrophilic hydration of polar (CONH and COOH) and zwitterionic ( $\text{H}_3\text{N}^+ \dots \dots \text{COO}^-$ ) groups.

Chapter 6 embodies the results of the limiting apparent molal volume changes due to transfer of some amino acids and peptides from water to aqueous urea solutions. The results have been interpreted in the framework of Friedman's cosphere overlap model.

Care has been taken to give proper credit for the investigations done by other workers. The author would like to apologize for any omission which might have occurred by oversight or error in judgement.

# C O N T E N T S

				<i>Page</i>
CERTIFICATE	...	...		iii
ACKNOWLEDGEMENT	...	...		iv
ABSTRACT	...	...		ix
 <u>CHAPTER 1</u>				
<u>INTRODUCTION</u>				
1.1	STRUCTURE OF WATER	...	...	2
	1.1.1	Continuum Model	...	3
	1.1.2	Simple Two State and Cluster Models	...	4
	1.1.3	Interstitial Models	...	7
	1.1.4	Computer Simulations	...	7
	1.1.5	Present Status of Water Structure	...	11
1.2	AQUEOUS SOLUTIONS	...	...	12
	1.2.1	Concept of Structure-making and structure-breaking Effects of Solutes	...	12
	1.2.2	Ion Induced Solvent Structural Changes - Ion Hydration	...	13
	1.2.3	Structural Changes by Apolar Solutes - Hydrophobic Hydration	...	16
	1.2.4	Hydrophobic Interaction	...	24
1.3	INFORMATION ON MOLECULAR INTERACTION FROM THERMODYNAMIC DATA	...	...	28
	1.3.1	Limiting Thermodynamic Properties	...	28
	1.3.2	Concentration Dependence of the Thermodynamic Properties	...	30

1.4	OUTLINE OF PRESENT RESEARCH PROBLEMS	...	...	32
	Figures	...	...	35
	References	...	...	43

## CHAPTER 2

### *INSTRUMENTS AND EXPERIMENTAL PROCEDURE*

2.1	CALORIMETER	...	...	53
2.2	DENSITY METER	...	...	60
	Table	...	...	66
	Figures	...	...	67
	References	...	...	69

## CHAPTER 3

### *THERMODYNAMIC STUDY OF THE INTERACTIONS IN THE TERNARY SYSTEM OF WATER, ALCOHOL, AND AMINO ACID OR PEPTIDES*

3.1	INTRODUCTION	...	...	70
3.2	EXPERIMENTAL	...	...	75
3.3	THERMODYNAMICS OF TRANSFER OF AMINO ACIDS FROM WATER TO t-BUTANOL + WATER MIXTURES	...	...	77
	3.3.1 Results	...	...	77
	3.3.2 Discussion	...	...	81
	3.3.3 Conclusion	...	...	92
3.4	THERMODYNAMICS OF TRANSFER OF DIGLYCINE FROM WATER TO AQUEOUS ALCOHOL MIXTURES	...	...	93
	3.4.1 Results	...	...	93
	3.4.2 Discussion	...	...	95
3.5	ALCOHOL INDUCED CONFORMATIONAL TRANSITIONS OF PROTEINS AND POLYPEPTIDES IN AQUEOUS SOLUTIONS	...	...	97
	Tables	...	...	102
	Figures	...	...	115
	References	...	...	125

CHAPTER 4*HEAT CAPACITY OF SOLUTION OF SOME**PEPTIDE DERIVATIVES IN WATER*

4.1	INTRODUCTION	...	...	131
4.2	EXPERIMENTAL	...	...	137
	4.2.1	Preparation	...	137
	4.2.2	Measurement of Enthalpy of Solution	...	138
4.3	RESULTS	...	...	138
4.4	DISCUSSION	...	...	139
	Tables	...	...	145
	References	...	...	151

CHAPTER 5*APPARENT MOLAL VOLUMES OF AMINO ACIDS,**N-ACETYL AMINO ACIDS, AND OLIGO-PEPTIDES**IN AQUEOUS SOLUTIONS*

5.1	INTRODUCTION	...	...	153
5.2	EXPERIMENTAL	...	...	157
5.3	RESULTS	...	...	158
5.4	DISCUSSION	...	...	161
	5.4.1	Limiting Apparent Molal	...	161
	5.4.2	Group Contributions	...	169
	5.4.3	Estimates of $\phi_v^\circ$ from van der Waals Volumes	...	172
	5.4.4	Concentration Dependence of $\phi_v$	...	178
	Tables	...	...	181
	Figures	...	...	194
	References	...	...	201

CHAPTER 6*APPARENT MOLAL VOLUMES OF SOME AMINO ACIDS AND**PEPTIDES IN AQUEOUS UREA SOLUTIONS*

6.1	INTRODUCTION	...	...	207
6.2	EXPERIMENTAL	...	...	209
6.3	RESULTS	...	...	209

6.4	DISCUSSION	...	...	...	210
	Tables	...	...	...	215
	Figures	...	...	...	217
	References	...	...	...	222
	SUMMARY	...	...	...	225
	<u>APPENDIX - A</u> Tables of Raw Data	...	...	...	232
Table A-1	Enthalpy of Solution of some Amino Acids in Aqueous tert-Butanol Mixtures	...	...	...	233
Table A-2	Densities and Apparent Molal Volumes of Glycine in Aqueous tert-Butanol at 298.15 K	...	...	...	241
Table A-3	Densities and Apparent Molal Volume of ~0.1 m Amino Acids in Aqueous tert-butanol Mixtures at 298.15 K	...	...	...	244
Table A-4	Densities and Apparent Molal Volumes of some Amino Acids and Peptides in Aqueous Solutions at 298.15 K.	...	...	...	250
Table A-5	Densities, Apparent Molal Volumes and Degree of Dissociation of some N-Acetyl Amino Acids in Aqueous Solutions at 298.15 K	...	...	...	253
Table A-6	Densities and Apparent Molal Volumes of some Amino Acids and Peptides in Concentrated Aqueous urea solutions at 298.15 K	...	...	...	255
	<u>APPENDIX - B</u> Glossary of Symbols	...	...	...	260

\* \* \*