Chapter - 2

LITERATURE SURVEY

2.1 Literature on amino acids

Lot of work on amino acids had been done during the past four decades. Even though all the twenty three amino acids known to us are not studied in detail, literature is available on glycine, methionine, L-cysteine, arginine, alanine, phenylalanine, aspartic acid, valine, DL-tryptophane, L-threonine, lysine, leucine, isoleucine and glutamine and also their complexes with metal ions and other protein chains.

Measurement of densities and heat capacities of aqueous solutions of glycine, L-alanine, L-serine and L-threonine at 288.15, 298.15, 313.15 and 328.15K using a Picker flow micro calorimeter and calculation of apparent molar volumes and heat capacities and associated standard state partial molar properties were reported by Andrew and Preuss.\(^{19}\)
Thermodynamics of amino acids is a key to understand protein chemistry. Many studies on measurements of heat capacities of aqueous solutions of amino acids were made at a standard temperature of 298.15K.\textsuperscript{19} The reason for this is that only at this temperature many living systems which contain proteins, polypeptides and hence amino acids operate. This work proves that there are classes of organisms which exist in submarine hot springs at temperatures greater than 383.15K.

The volumetric and thermochemical properties of aqueous solutions of L-valine, L-leucine and L-isoleucine at 288.15, 298.15, 313.15 and 328.15K were reported and volumes and heat capacities of some amino acids in water at 25°C were calculated by Jolicoeur and Riedl.\textsuperscript{20} A report on aqueous solutions containing amino acids and peptides was made by Cabani and others.\textsuperscript{21}

In addition to the study of thermodynamic properties of aqueous solutions, structure of pure amino acids and their various complexes were also studied by many researchers. C. Bakke and A. Mostad reported the structure of DL-tryptophan.\textsuperscript{22} Similarly, Varughese and Srinivasan
reported the structure of isoleucine.\textsuperscript{23} A three dimensional structure of glycine had been reported by Kvick and others\textsuperscript{24}, using neutron diffraction methods. L-Arginine dihydrate used in pituitary function determination was studied by Karle and others and the properties were reported.\textsuperscript{25} A three dimensional structure of \(\beta\)-Alanine was reported by Willart and others in the year 1981.\textsuperscript{26} Sequeria, Rajagopal and Ramanatham applied the neutron study method to get the three dimensional structure of DL-Aspartic acid.\textsuperscript{27} Torii and Litaka reported the structure of L-Valine in the year 1970.\textsuperscript{28} A refined structure of L-threonine at a temperature of 12 K was reported by Janctak, Zobel and Lugar.\textsuperscript{29}

Amino acids, because of their dipolar nature were able to form complexes with other metallic ions and metallic compounds. Their affinity to these substances is so strong that the complexes were found to be more stable. Few metallic complexes of some amino acids reported in literature are quoted just for reference and their significance in complex chemistry.

Kirkwood and Willett report about the complex of \(\beta\)-alanine tetrochloro copper.\textsuperscript{30} Gorbitz and Etter gave the
structure of L-phenylalanine formate. Srinivasan and Rajaraman reported details of the complex L-phenylalanine perchlorate. Dawson reported the characterisation of complexes of aspartic acid, such as DL-aspartic acid hydrochloride.

As amino acids complexes are very essential for metabolic activities and applied in preparing medicines to cure some diseases, growth and characterization of some sulfur containing amino acids such as methionine, L-cysteine and L-cystine may help in the preparation of such medicines.

Metallic complexes of L-methionine play a very important role in complex chemistry. L-methionine, a less occurring amino acid has a sulphur atom in addition to the amino group (NH₃⁺) and carboxylic group (CHCOO⁻). The sulphur atom is attached to CH₂CH₂ molecules forming the longer chain CH₃SCH₂CH₂. This chain helps methionine to have a strong affinity with metallic ions and other amino groups. Large number of complexes with methionine have been prepared and reported in literature.

Nambley and Webster report tripyridyl copper nitrate - L - methionie Oxime, L-methionine cobalt - tetrochloric -
iron\textsuperscript{35} L-methionine Platinum\textsuperscript{36}, L-methionine - dichloro - palladium\textsuperscript{37} L-methionine palladium Nitrate\textsuperscript{38}, L-Methionine dicalcium\textsuperscript{39}, L-methionine chloride hydrochloride\textsuperscript{40}, L-methionine perchlorate - mercury dihydrate\textsuperscript{41}, L-methionine copper sesquihydrate\textsuperscript{42}, DL-Methionine nitrate, N-Acetyl - DL - Methionine, Phenylalanyl - Methionine, L-glutamyl - L - Methionine, are some of the other complexes reported so far.\textsuperscript{43} L-methionine in its present form is used as an anti-ulcer agent\textsuperscript{44} and also as an antidote in the medical field.\textsuperscript{45}

In addition to the structural aspects and complex formation of amino acids some of their physical and chemical properties and also their thermodynamical parameters were also measured and reported. Lark and others\textsuperscript{46} had reported the viscosities and viscosity B-coefficients of aqueous solutions of some amino acids at various temperatures ranging from $-5^\circ C$ to $40^\circ C$. They have chosen glycine, B- Alanine, Sarcosine and DL - Valine and concluded that the Viscosities are more temperature – dependent and that temperature has a structure breaking effect on DL – alanine and structure reinforcement on glycine due to the presence of CH$_2$ groups.
Deuterium NMR study of Amino acids' coordination to chromium III was reported by Ivan Lagg and others.\textsuperscript{47} Complex formation of Alanine, Glycine, Leucine, methionine, Pheynylalanine, serine and L - threonine with chromium III was studied and the reports of these experimental results bring out the following two major conclusions.

1. The instability of all the amino acid chromium complexes seen from NMR spectral studies accounts for the difficulties in isolation and characterization.
2. Isotropic shifts are observed in the spectral studies and they show semi hyperfine contact interactions.

Similarly volumetric and thermochemical properties of aqueous solutions of L - valine, L - Leucuie and L-Isoleucine at 288.15, 298.15, 131.15 and 328.15K were studied by Duke.\textsuperscript{48} He has calculated thermochemical and volumetric properties and found that these properties are temperature dependent.

A survey of the literature on amino acids for the past four decades show the complex behaviour of the amino acids and their complexes. The literature also reveals that most of
the amino acid complexes find wide applications in the medical field.

2.2. Literature on L – Cystine

The single HSCH$_2$ molecule of L – Cysteine and the twin CH$_2$S – SCH$_2$ molecules of L – Cystine which contain sulphur atoms show significant variation in their behaviour as complexes with other organic and inorganic molecules.

Literature available on L-cystine is mostly related to the medical field and few of the available do not throw light on the physical aspect of the system; especially, the reports on structure, growth and complex formation of L-cystine are meager.

L-cystine is a form that two molecules of L-cysteine bind together by disulfide bonding. It is contained in many proteins and makes an important contribution to protein structure. It is Glycogenic. L-cystine undergoes a reciprocal conversion with L-cysteine in the oxidation reduction mechanism. L-cystine is necessary for the formation of skin and accelerates healing. $^{49}$
Obata and Tanaka with their studies on photolysis of L-cysteine and L-cystine obtained the formation of the flavour of cooked rice.  

Yuji Baba, Tetsuhiro Sekignchi and Iwao Shimoyama studied the fragment-ion desorption from sulfur-containing amino acids (such as methionine, L-cysteine and L-cystine), by localized core-level excitation. They studied desorption of fragment ions from sulfur-containing amino acids following the sulfur K-edge photo excitation using monochromatised synchrotron radiation. The samples investigated are pellets of neutral L-cystine. It was found that only atomic S ions are desorbed following the $\sigma^*$ resonant excitations. Various kinds of fragment ions containing carbon, nitrogen and oxygen atoms are desorbed by the low energy electron irradiation. It was concluded that the core-to-valence resonant photo excitation and succeeding specific, S-S and S-C bond scissions play a major role in ionic fragmentation in the present amino acids.

L-cystine, with the molecular formula $C_6H_{12}N_2O_4S_2$ is a white crystal or crystalline powder, tasteless, practically insoluble in water, ethanol and ether, and very soluble in
acids and alkali. It dissolves in dilute hydrochloric acid. Its solubility in 100 gm of water is 0.009 g at 20°C, 0.018 g at 40°C and 0.033 g at 60°C. In solution form it is clear and colorless. It has a specific rotation in the range – 215 to – 225 and has pH in the range 5.0 to 6.5.

It is widely used in pharmaceutical, food and cosmetic industry. Cystine promotes oxidation and reduction ability in the human cell. It increases white blood cell, cures the infectious diseases such as asthma, nerve ache and eczema. It is a good drug for prevention of skin infections and revitalizes old and feeble skin. It cures a disease called cirrhosis of liver commonly found among people who consume excess alcohol.

Mcbean and Flynn,52 reported on the molecular mechanisms of cystine transport. When L-cystine is given to rats, rat brain synoptosome occurs. Almost 90% of L-cystine transport is by a low-affinity, sodium-dependent mechanism mediated by the family of glutamate transporters. These results suggest that L-cystine binds to a site which is different from the glutamate transporters. In the sodium
dependent transport in rat brain, L-glutamate and L-cystine are necessary for maintaining glutathione levels.

Ruiz, Siow, Bartlett, Jenner, Sato, Bannai and Mann\textsuperscript{53} found that vitamin C inhibits diethylmaleate-induced L-cystine transport in human vascular smooth muscle cells. According to them, adaptive increase in intracellular glutathione (GSH) to oxidative stress are mediated by induction of L-cystine uptake via the anionic amino acid transport system. This property of antioxidant behaviour helps to protect smooth muscle cells from injury.

Gukasyan, Kannan, Lee and Kim\textsuperscript{54} studied the regulation of L-cystine transport and intracellular GSH level by a nitric oxide donor in primary cultured rabbit conjunctival epithelial cell layers. The purpose of their investigation was to find the metabolism and transport of cysteine which are critical for the maintenance of the intracellular glutathione (GSH) level. In their study, transport mechanisms of L-cystine and regulation of GSH biosynthesis in the presence or absence of NO-induced oxident stress were investigated in primary cultured rabbit conjunctival epithelial cells. The conclusion drawn from this study was that understanding
sulfur amino acid precursor – dependent cellular mechanisms of GSH homeostasis would be of value in devising GSH – based treatment of conjunctival or other ocular disorders.

Knert,\textsuperscript{55} found the utilization of L-cystine as a source of carbon and nitrogen by various fungi. He studied thirty fungal species from various taxonomic and ecologic groups. They were cultivated on two glucose – peptone media enriched with cystine. Nineteen species showed ability to utilize cystine not only as a source of sulfur but also that of carbon and nitrogen. Six species utilized cystine completely. Excess sulfur was excreted after oxidation in the form of inorganic sulfate or sulfite back into the medium. Cystine metabolism was on an average more rapid and complete on medium with higher peptone content and better growth. These fungi showed a lower tendency to cystine utilization and belong to the species which do not utilize this amino acid at all.

Perez, Martinez, Tomas and Carpena,\textsuperscript{56} worked on the feasibility of the spectro fluorimetric flow injection method for the individual and successive determination of L-cysteine and L-cystine in pharmaceutical and urine samples. The
actual analysis involved the rapid oxidation of L-cysteine by thallium (III) with concomitant formation of fluorescent thallium (I). This method was successfully applied to the determination of L-cysteine and L-cystine in urine samples.

Thoene, et.al. 57 studied the effect of intracellular cystine depletion leading to the chronic renal failure and a disease called cystinosis.

Certain aminothiols rapidly deplete cultured cystinotic skin fibroblasts of their abnormally high free (nonprotein) cystine pool. The free cystine contents of these cells if reduced over 90% in 1 hour, this disulfide—cystamine, is also able to deplete cystinotic cells of free cystine. A patient with nephropathic cystinosis and end-stage renal disease was treated with cysteamine, both intravenously and orally. They found that determination of the proper place of aminothiol therapy in this disease will depend upon further clinical trial with patients whose kidney function has not deteriorated to the point of irreversible change, accompanied by careful monitoring of plasma aminothiol levels.
As much of the literature available on L-cystine converge to give biological, agricultural and medical informations, the need to give attention to the physical aspects of L-cystine and its complexes, such as growth, characterization and analysis becomes relevant to the present study.

The most widely accepted structure of L-cystine is:

![L-cystine structure](image)

Thus the literature scan showed the importance of L-cystine and its complexes in the medical field for several diseases. The study of complexes of L-cystine with metallic ions such as Cu and Cd is lacking and the present report
aims at it. In order to see the feasibility of complex formation and to predict, if possible, some structural parameters, an initial attempt was made to simulate the structure of the two said complexes using the SIRIUS package. An account of the methodology followed by the discussion of the results so obtained are given in the next chapter.