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REVIEW

Complexation Behaviour of Glutathione with Metal Ions

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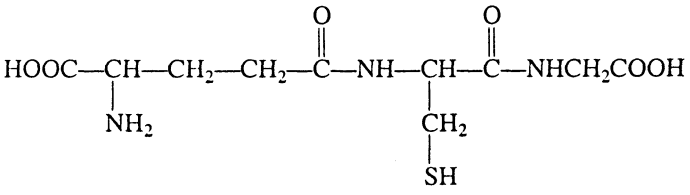
Metal complexes of glutathione (GSH) serve important functions in our biological system and play a dominant role in protein metabolism. They are important constituents of enzymes, proteins and present in many parts of the biological system. Glutathione (GSH, γ-glutamate-cystein-glycine) is the most common cellular non-protein thiol in the cell; it exists predominantly in the reduced form (GSH) at concentration 0.1–10 mm and is readily oxidized to the disulphide (GSSG). Because of the presence of potential binding sites in glutathione (reduced), its coordination chemistry is complicated. The present review discusses the interaction of glutathione with various transition and non-transition elements at the molecular level and their biological significance.

Key Words: Group metals, glutathione (GSH), Metal complexes, Biological importance.

Most of the recent research in coordination chemistry is based on the complexation reaction of the metal ions with various biologically active molecules. In this context, study of metal-peptide interaction is of biological interest since it provides a model to elucidate the binding sites and the nature of linkages involved in metal protein binding. The coordination chemistry of glutathione is of vital importance as it serves as a model system for the binding of metal ions by larger peptide and protein molecules. Metal glutathione complexes are involved in the toxicology of several metals[1, 2]. Glutathione is present in cellular systems at a relatively high concentration and, generally, is the most abundant non-protein thiol. Because of the high affinity of sulfur for many metals, glutathione may be involved in their uptake and excretion[1, 2] and almost certainly will be involved in their intracellular coordination chemistry.

Glutathione (**I**) is a polydentate ligand, offering potential binding sites, *i.e.*, two carboxylate oxygens, an amino nitrogen, thiol group and two amide groups[3].



I

The structure of glutathione is such that all its potential binding sites cannot be simultaneously coordinated to the same metal ion and consequently its coordination chemistry is characterized by the formation of protonated and polynuclear

complexes. Also the thiol group of glutathione (GSH) is readily oxidized and its oxidation by O_2 is catalyzed by traces of metal ions such as Cu(II), Fe(III), Co(II), Mn(II) and Cr(VI). The concomitant reduction of the metal ion alters its reactivity with cellular components[3]. Glutathione has antioxidant properties[4,5] and plays an important role in tissue protection[4].

Glutathione (reduced), a biological reducing agent in thiol dependent enzyme reactions, has recently been reported to have anticancer activity[6,7]. Novi[8] administered reduced glutathione to rats bearing aflatoxin B_1 induced liver tumors and observed regression of tumor growth that resulted in the survival of the animals. Since glutathione is a harmless natural product, it merits further investigation as a potential antitumor drug for human [9]. Glutathione reductase was chosen as target molecule for the design of parasiticidal drugs to combat parasitic infectious diseases[10].

In view of the dominant role of glutathione and its metal complexes in various biochemical systems, this review emphasises on the nature of the complexes at the molecular level and their biological significance. The chemical and biological aspects of the coordination chemistry of glutathione with various metal ions are discussed.

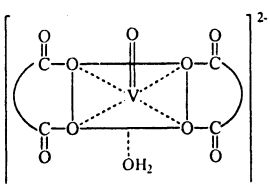
Complexes of Glutathione

The interactions of glutathione with various transition and non-transition elements (toxic, biologically essential and present in various drugs) have been described.

(a) Group 5 metal complexes

Vanadium Complexes: Vanadium is an essential trace element in both plants and animals[11]. Vanadate ion is taken up by cells by the same system that transports phosphate[12] and is an inhibitor of $(Na^+ + K^+)$-ATPase[13]. However, the extent of inhibition of $(Na^+ + K^+)$-ATPase activity in human erythrocytes by vanadate is considerably less than expected[12] because of reduction of vanadate to V(IV) by cytoplasmic glutathione[14]. The V(IV) then binds to haemoglobin. The reduction is not enzymatic since the time courses for reduction when GSH is added to a solution of haemoglobin and vanadate and for reduction in human erythrocytes are similar[14].

Delfini and coworkers[15] identified the binding sites in the V(IV)-$(GSH)_2$ complex **(II)** and measured the kinetics of dissociation of GSH from the complex. Extensive broadening of the gly-COO^- and glu-COO^- ^{13}C NMR resonance together with a sizeable broadening of those for the gly-Cα, glu-Cα and glu-Cβ carbons, indicates binding to four carboxylate groups in the 1 : 2 complex. By comparison of the EPR parameters for the 1 : 2 complex with those for model complexes, it was concluded that the four carboxylate groups lie in the equitorial plane, with an oxoligand and a water molecule in the axial positions[15].



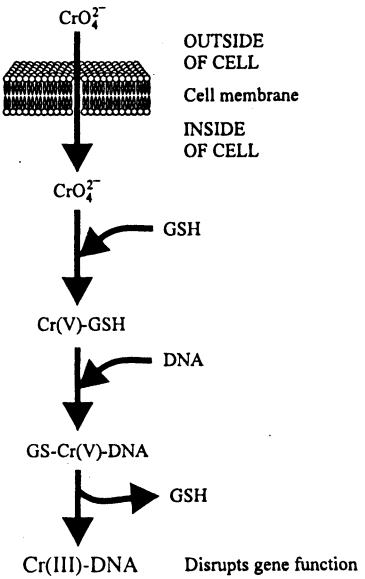
II

Complex formation[16, 17] between GSH and oxovanadium(IV) occurred between pH 4 to 10, but at pH > 5 complexes were found to be unstable. The binding ratio of GSH to VO^{2+} in complexes exhibited eight lines EPR spectra at room temperature and liquid N_2 temperature. The ^{13}C NMR and EPR parameters correlating the g_0 and A_0 values suggest three characteristic types of coordination environment for the GSH-VO^{2+} complex depending on pH values of the solution. Vanadate ion was reduced by excess of GSH and subsequently gave a complex with GSH[16, 17]. This suggests that vanadate is reduced to oxovanadium ion by GSH in cells or organs and may be the cause for protein synthesis inhibition in reticulocyte lysates[16, 18]. The effect of solubilization and effect of vanadium glutathione complex on inhibitor against eosinophil cyclic AMP-specific phosphodiesterase has been studied[19, 20].

Vanadium, a dietary micronutrient, has recently been found to possess a potent antitumor activity during chemical induced rat liver carcinogenesis[16]. The basic mechanism of the antitumor response of vanadium by monitoring liver cells during the early preneoplastic steps of diethylnitrosamine (DENA)-induced hepatocarcinogenesis[21]. It provides evidence that vanadium dependent induction of GSH-mediated glutathione S-transferase catalyzed detoxificational capacity of the host is presumably related to its suppressive effect against chromosomal aberrations. This may explain, in part, the antitumor efficacy of this trace element [21].

(b) Group 6 Metal Complexes

(i) Chromium Complexes: Chromium(VI) is known to be toxic and carcinogenic to organisms[22]. Although full details of how this element causes cancer are unknown, its mechanism of entering cells and becoming immobilized has recently been worked out as indicated in model for cellular uptake and genotoxic action of chromate ion[22] **(Scheme-I)**.

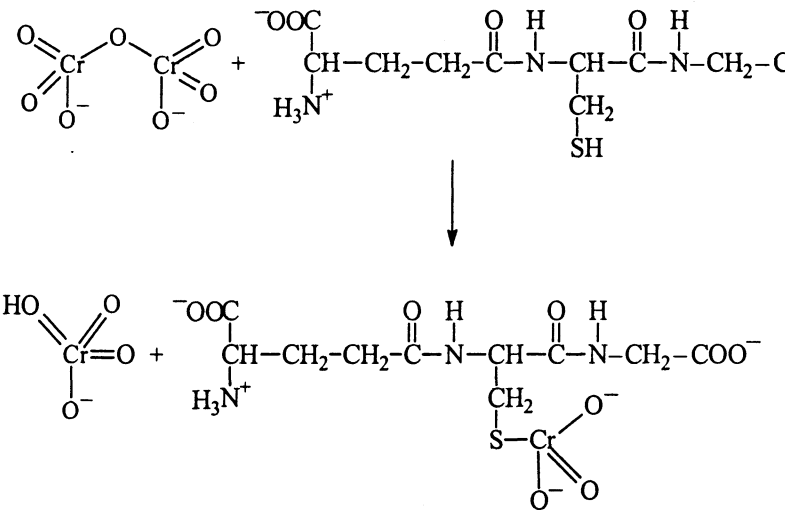


Scheme-I

The CrO_4^{2-} ion that enters the cell through specific channels is reduced by glutathione and binds covalently to DNA[22]. Cr(VI) is carried into cell by the anion transport system[22]. In the cytoplasm, it reacts with glutathione, an intracellular tripeptide present in *ca.* 5 mM concentration that contains a cysteinyl sulfhydryl group. In its reaction with GSH, Cr(VI) is reduced to Cr(V) and Cr(IV), and a Cr-S bond is formed[22–24]. The metal ion is thus trapped in the cell and cannot diffuse back out into plasma. Binding to DNA and subsequent reduction ultimately to Cr(III) are thought to constitute additional steps in the carcinogenic action of this ion[22]. Glutathione reacts with chromium(VI) *in vitro* under physiological conditions[25, 26] and is considered to be a detoxifying agent against chromate poisoning at or near conditions similar to those of the cellular environment[25]. The ability of carcinogenic chromium(VI) compounds to damage DNA in cells increases glutathione concentration *in vitro* as well as in cultured cells[27–29]. Glutathione has been postulated to reduce the chromium(VI), via a Cr(VI) glutathione thioester intermediate[25, 30–32]

$$GSH + Cr(VI) \longrightarrow GSCr(VI) \qquad (1)$$

Based on kinetic studies on the reaction of GSH with chromium(VI) under acidic conditions, the existence of a GS-Cr(VI) thioester intermediate has been proposed[31, 33]. The spectral studies provides the first structural evidence for a Cr(VI) glutathione thioester, which has the cysteinyl thiolate of glutathione bound to chromium(VI)[32] as shown in **Scheme-II**.



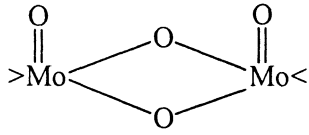
Scheme-II

Chromium-glutathione complexes and its mixed ligand complexes of amino acid have been synthesized[34–36] and characterized by spectroscopic and magnetic studies. The results indicate that glutathione coordinates through glycinyl oxygen, thiol sulfur and amide oxygen. Results from electron paramagnetic resonance studies show that the reaction of GSH-Cr(VI) in aqueous solution at pH 6–8

produces relatively long-lived Cr(V) species as intermediates in the reduction to Cr(III)[37]. The dependence of their EPR signals on the GSH : Cr ratio suggests that they may be 2 : 1 and 1 : 1 GSH complexes of Cr(V) coordinated *via* deprotonated thiol groups[37].

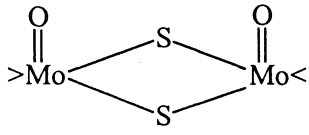
The kinetics of oxidation of DL-penicillamine and glutathione by potassium chromate has been studied at pH ≥ 7 under pseudo-first order conditions of an excess of thiol at ionic strength 0.50 mol dm^{-3} ($NaClO_4$)[38]. A common mechanism which is compatible with the kinetics of both reactions is proposed[38]. Chromium(VI) is also taken up by liver, whereas the uptake of Cr(III) is much less. After exposure to Cr(VI), chromium present in the liver of rats is Cr(III) and only Cr(III) is excreted in the bile. A GSH-dependent carrier mechanism that facilitates the transfer of Cr(III) across the canalicular membrane into bile has been suggested[39].

(ii) Molybdenum Complexes: Glutathione is oxidized by Mo(VI) with the formation of Mo(V) and GSSG[40]. A complex between Mo(V) and GSH has been isolated[41] in which Mo(V) is diamagnetic and dioxobridged **(III)**.



III

In phosphate buffer (at pH 8–10) the dinuclear complex dissociates to give a paramagnetic dinuclear complex which has been studied by EPR. Mitchell and Scarle[42] studied thiolato complexes of Mo(V) and Mo(III) by EPR studies. A μ-disulfidodioxodimolybdenum(V)-GSH complex has been studied by Raman spectroscopy as a model for a molybdenum enzyme[43] **(IV)**.



IV

Glutathione reacts with Mo(VI) in acid buffer (pH 2.5–5.3) to give a blue 1 : 1 complex, soluble in water, with maximum absorbance at 825 nm. The conditional stability constants of the complex were determined. The value of log K was 3.75 (pH = 2.90; μ = 0.1 M; T = 25 ± 0.5°C, here T is absolute temperature)[44]. The reaction between glutathione and molybdenum(VI) is suitable for the spectrophotometric determination of glutathione in micro quantities[44, 45]. A Mo(V)-GSH complex shows catalytic activity in the conversion of acetylene to ethylene and hydrazine to ammonia by borohydride[46].

(c) Group 7 Metal Complexes

(i) Manganese Complexes: Manganese(II) complexes of glutathione have been studied by infrared spectra, magnetic susceptibility and electronic spectra. The electronic spectral bands at 465, 315 and 280 nm of the Mn(II)-GSH complexes correspond to transition, $^6A_{1g} \rightarrow {}^4E_g(G)$, $^6A_{1g} \rightarrow {}^4A_{1g}(G)$ and $^6A_{1g} \rightarrow {}^4T_{2g}(D)$, respectively. All these bands are due to *d-d* transition and its magnetic moment data showed that the complexes are normal and in spin-free octahedral. The complexes were tested for antifungal activity against some plant pathogenic fungi using slide germination technique[47]. The potentiometric determination of stability constant for Mn(II)-GSH[48] and its mixed ligand complexes with anthranilic acid, ascorbic acid, nicotinic acid and sulphanilic acid have been investigated potentiometrically[49].

The effect of chronic exposure of manganese on antioxidant enzymes particularly glutathione in different regions of rat brain have also been studied[50, 51]. Adult male Sprague-Dawley rats were dosed with different amounts of $MnCl_2$. Results indicate that the glutathione content was significantly reduced in cerebellum, whereas no change was observed in other brain regions[50]. The electrocatalytic reactions of glutathione by water soluble manganese porphyrin have been studied. Mn(III) is reduced to Mn(II) by oxidizing reduced glutathione[52].

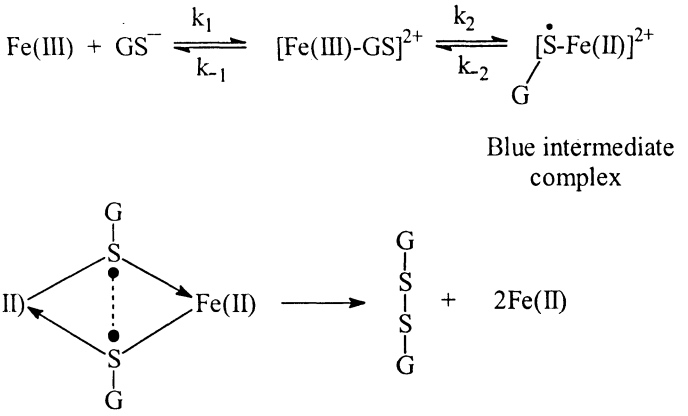
(ii) Technetium Complexes: The organ distribution of ^{99m}Tc-glutathione complexes has been studied by the use of ^{99m}Tc as an imaging agent[53, 54]. Complexes were prepared by mixing ^{99m}Tc-pertechnetate solution with GSH solution[54] and with $SnCl_2$-GSH solution[55]. In the first preparation, the thiol group of GSH serves as the reducing agent for reduction of pertechnetate, while in the second, reduction is by the stannous ion. Different biodistribution of ^{99m}Tc-glutathione complexes arises as a result of differences in the chemical composition of the complexes. The accumulation of ^{99m}Tc glutathione in the pancreas was found to be insufficient for visualization of the pancreas[55]. Johannsen and coworkers[56] studied pharmacokinetic properties of technetium-labeled thiolate complexes and labeling of human immunoglobulin was done with ^{99m}Tc by a direct method[57].

(d) Group 8 Metal Complexes

(i) Iron Complexes: Mössbauer studies of the binding reaction of iron(III) to glutathione have clearly demonstrated the production of iron(II)[58, 59]. Anaerobic reactions of ferric salts with glutathione and related thiols were studied by Mössbauer spectroscopy and fast reaction kinetic techniques at low pH. In all cases the final product contained iron(II) under anaerobic conditions, GSH reduces Fe(III) to Fe(II)[59].

$$2Fe(III) + 2GSH \longrightarrow 2Fe(II) + GSSG \quad (2)$$

Complex formation and electron transfer are both thought to be involved in the overall mechanism[59] **(Scheme-III)**.

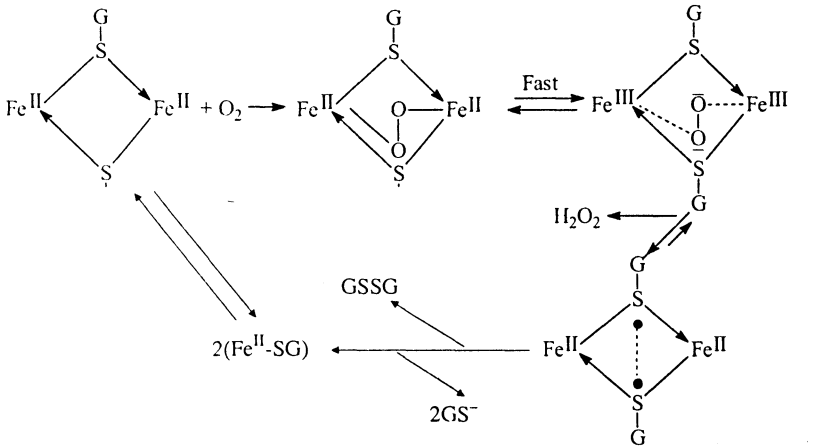


Scheme-III

Stopped-flow kinetic experiments show that initial binding and electron transfer leading to the blue intermediate complex are very rapid and its formation is rate limited by a second-order process. In Step II, the blue intermediate complex decays to the final product, in which the iron was determined by Mössbauer spectroscopy on frozen solutions to be Fe(II). Since GSH is the reducing species, the other product was assumed to be GSSG. The Mössbauer results indicate binding of Fe(II) only to oxygen and chloride ligands, with no evidence for sulfur binding Mössbauer measurements and pH titration experiments have confirmed that in the final product Fe(II) binds to reduced glutathione in the pH range 3–7 *via* the carboxylate groups and the amide groups may also bind but the sulfurs do not[60].

Spectral studies of the 1 : 1 and 1 : 2 iron(II)-glutathione complexes have been carried out and studies show that the 1 : 1 compound containing high spin iron(II) is in distorted five and six-coordinated environment, whereas the 1 : 2 complexes contain only distorted six-coordinated high spin iron(II). The nature of the coordinating ligands are discussed[61]

The iron(II) and iron(III) catalyzed oxidation of GSH by molecular O_2[62] is shown in **Scheme-IV**.



Scheme-IV

The first step (not shown) in the Fe(III) catalyzed oxidation is the reaction of Fe(III) with GSH to produce Fe(II), which then forms a complex with another molecule of GSH. This Fe(II)-GSH complex reacts with oxygen to form a red complex that undergoes an autocatalytic oxidation of the thiol[62]. The overall reaction catalyzed by this cycle is

$$2GS + O_2 + 2H^+ \longrightarrow H_2O_2 + GSSG \quad (3)$$

The protective mechanism operating in the gastrointestinal tract to counteract the potential oxidizing effects of excess free iron was tested in rat fed with excess iron[63]. The activities of some antioxidant enzymes, the levels of GSH, and the extent of lipid peroxidation at the site of iron absorption were measured. It has been suggested that antioxidative enzymes play a key role in rendering the intestinal mucosal cells resistant to iron induced oxidative damage in rats[63]. The conversion of oxidized form of glutathione to the reduced form of glutathione by iron porphyrin was studied in aqueous solution by electrochemical method[52]. Reduced glutathione reduces Fe(III) porphyrin to Fe(II)-porphyrin in the basic medium and in turn itself gets oxidized while in acidic medium the reverse process takes place[52]. An EPR study[64] of rapid and frozen solution of iron-glutathione showed the progressive reduction of iron(III) with time and the transient presence of a g = 2 radical signal.

This signal is discussed in terms of an intermediate in the reduction pathway containing a high spin iron(II) centre weakly coupled to a sulfur radical. Similar experiments were carried out at pH 9 in the presence of oxygen. The stabilities of mixed ligand complexes of Fe(III) involving oxalic acid as primary ligand and glutathione as secondary ligand in aqueous medium have been studied[65].

(ii) Ruthenium Complexes: Only very few literature data on ruthenium glutathione complexes is available. The kinetics of oxidation of glutathione by ruthenium has been discussed[66]. The antitumor active complex *trans*-tetrachlorobis (imidazole) ruthenate(III) completely changes its ligand configuration within 1 h in H_2O in the presence of glutathione and L-histidine[67]. It has been observed that the release of the *trans*-standing imidazole ligands at 37° that occurs in addition to chloride substitution reactions has to be taken into consideration for further studies into the mode of action of this new antitumor drug[67].

(e) Group 9 Metal Complexes

(i) Cobalt Complexes: It was first pointed out by Martin and Edsall [48] that glutathione may coordinate to cobalt(II) both as an amino acid *via* the γ-glutamyl side chain, and *via* the sulfur atom. Complexation of GSH by the Co(III) in cobalamines corrinoids and related model compounds has been the subject of several studies[68–73]. Four of the Co(III) coordination positions are occupied by the more or less planar corrin ring. The two remaining coordination positions are on opposite sides of the ring, and thus chelation of other ligands to the Co(III) is not possible. Binuclear complexes involving Co(III) coordination to the amino and thiol groups have been found in studies with model compounds[72]. Under anaerobic conditions both aqua and hydroxo forms of cobala-

mine react with GSH to form a single complex of 1 : 1 stoichiometry[68, 69, 71]. Cyanide easily displaces GSH to form dicyanocobalamine[70, 71].

Potentiometric and spectrophotometric studies[48, 74, 75] of the Co(II)-GSH system over the pH range 5–12 indicate that at low pH, the predominant complex is Co(HL). The Co(II) is chelated by the $^{-}O_2CCH_2NH_2$ group of the glutamyl residue and the thiol group is protonated. As the pH is increased from 6–10, bands characteristic of tetrahedrally coordinated Co(II) increase in intensity in the UV-Vis spectrum. The tetrahedral complex is of stoichiometry CoL_2^{4-} and is postulated on the basis of spectrophotometric results to involve sulfur and oxygen coordination. In strongly basic solution (pH *ca.* 12), Co(II) also binds to deprotonated amide nitrogen. Synthesis and characterization of cobalt(II) glutathione complexes has been studied[76–79]. On the basis of electronic and IR spectral data, glutathione is pentadentate in the polymeric octahedral complexes[79]. The binding of the metal occurs through S and O of the cysteine fragment, O atom of the glycine fragment and the N and O of the glutamine fragment[79]. Potentiometric studies of some mixed ligand complexes cobalt with glutathione have also been performed[49, 65].

(f) Group 10 Metal Complexes

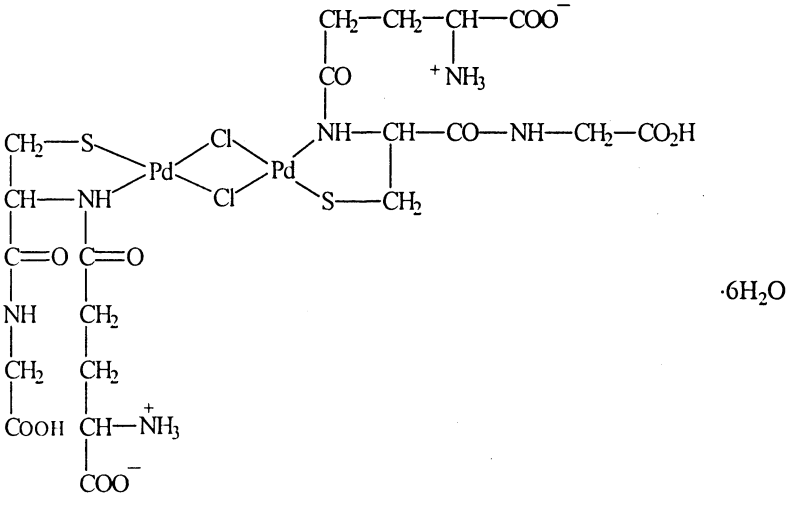
(i) Nickel Complexes: From circular dichroism(CD)[80], ^{1}H NMR and UV-Vis spectra[81] and IR, ^{13}C NMR UV-Vis, pH-metric studies[82] on the Ni(II)-glutathione system in aqueous solution, it has been established that both octahedral and square-planar complexes exist in an equilibrium. This equilibrium is strongly dependent upon pH[80–82]. At least two octahedral and two square-planar complexes occur in the region between pH 4 to 12. From these investigations, it was concluded that the octahedral species formed at the lowest pH values involve coordination by the carboxyl and amine groups of the glutamic acid residue[83]. Stability constant and thermodynamic parameters (ΔG, ΔH and ΔS) of complexes of glutathione with Ni(II) have been determined in aqueous medium pH-metrically at different ionic strength and temperature[84]. The formation constant ($\log \beta_n$) has been calculated using the weighted least squares method. Species distribution curve has been plotted as a function of pH, which shows that complex formation starts at pH *ca.* 3.30 and becomes maximum at pH range 3.40–3.50 and after that it decreases to zero concentration in the pH range 11.0–12.0[84].

Various kinetic studies and solid state structural studies[47, 85] of Ni(II)-glutathione complexes have been performed and showed antifungal activity against some plant pathogenic fungi using slide germination technique[47, 86]. Catalytic polarographic currents in the nickel-glutathione system[87, 88] have also been studied. A few studies on rats of Ni(II) toxicology have considered the interaction of Ni(II) with GSH[89, 90]. The concentration of GSH level decreases in rat liver due to bilary excretion of Ni-GSH complexes, diffusion of GSH out of the liver, and/or depletion of the hepatic GSH pool due to induction of hepatic metallothionin formation[90].

(ii) Palladium Complexes: Very little is reported in literature on palladium-glutathione complexes. Sovago and Martin[91] studied the 1 : 2 Pd(II) : GSH complex by potentiometric titration. It reveals that only two equivalents of base are needed to reach pH 8. This indicates that an especially stable thiol bridged polynuclear complex is formed. In this bridged 1 : 2 complex the ammonium group deprotonates upon addition of the third equivalent of base at a higher pKa *ca.* 10.3 than in the free ligand. In all 1 : 2 solutions, the NMR peaks from the cysteine

residue of GSH are especially broadened. These findings indicate that in these solutions Pd(II) binds only to the thiol group and the $—NH_3^+$ group titrates freely on addition of the third equivalent of base. In an equimolar solution of Pd(II) and GSH, addition of 3 equivalents of base raised the pH to 7. Broadening recurs in the NMR spectra, but in this case the $—CH_2CH$ part of the γ-glutamate residue is most broadened, indicating chelation at the α-amino terminus in addition to thiol coordination. A single Pd(II) is probably coordinated to the glutamate amino terminus of one GSH molecule and to the thiol group of another. Addition of a fourth equivalent of base from pH 7 to 11 results in a considerable change in the NMR spectrum. Most notable is an upfield shift and shifting of 0.1 ppm of the glycyl CH_2 proton[91].

The equilibrium constants for complex formation by the $[PdCl_4]^{2-}$ ion with glutathionate ions of different degree of deprotonation have been determined by the pH-metric method. Isolation and characterization of $[Pd(NH_3)_2(gluH_2)]_2[PdCl_4]$[92] indicates Pd—S bond formation. Chow and coworkers[76] reported that glutathione coordinates as a monoanionic ligand in $[Pd(GluH_2)Cl]\cdot 3H_2O$. The absorption in the IR spectrum at 1725 cm^{-1} indicates that an unionized carboxylic acid group is present[93, 94]. The only absorption in the 400–200 cm^{-1} region occurs at 275 cm^{-1} which is assignable to the ν(Pd—Cl) stretch of the Pd—Cl—Pd bridging linkage[95]. As in all the other complexes there is an absence of the ν(SH) suggesting sulfide coordination. The most likely structure of this complex is **(V).**



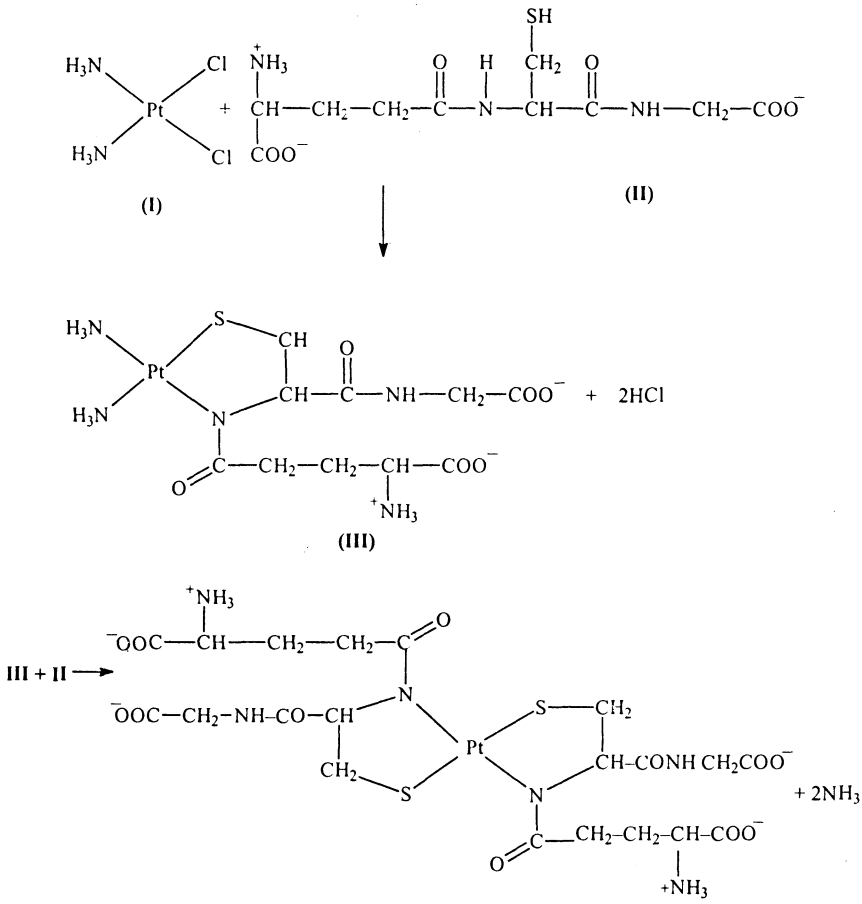
V

A similar coordination has been found in the dimeric $[Pd(pen)Cl]_2\cdot 2H_2O$ complex[96]. The stability constants and ionization constants have been determined from solution studies[97, 98] which indicated the nature of binding of palladium-glutathione complexes. A spectroscopic method for thiol analysis, based on the complexation reaction with Pd(II), is studied[99]. This proposed method is simple and sensitive and can be used for a rapid analysis of thiols in human lymphocytes[99]. The kinetics of the complex formation between $[Pd(H_2O)_4]^{2+}$ and glutathione was investigated [100] in the presence of sodium dodecyl sulfate in the acidity range from 2 M $HClO_4$ to

pH 5. Acceleration (from 2 M $HClO_4$ to pH 3.5) and retardation (3.5 < pH < 5) of the complex formation in the presence of anionic micelles was observed and compared with the kinetic data in aqueous solution[100]. The effect of the nature of the modified electrode (Pd + Pt) (90% + 10%) was selected for peroxide oxidation[101]. The anodic oxidation of the interfering substrates ascorbic acid, uric acid and glutathione was studied on this electrode. They are oxidized in the same potential range as H_2O_2 but at lower rates[101].

(iii) Platinum Complexes: *Cis*-dichlorodiammineplatinum(II) (Cisplatin) is a widely used chemotherapeutic agent towards numerous human tissues[102, 103]. The two chloro ligands of cisplatin are relatively labile and can be replaced by water molecules or hydroxide ions. Platinum(II) is a 'soft' Lewis acid and consequently is expected to have a relatively high affinity for sulfur ligands[81, 102–110].

The reaction scheme proposed for the complexation of GSH by cisplatin[104] are shown in **Scheme-V**.



Scheme-V

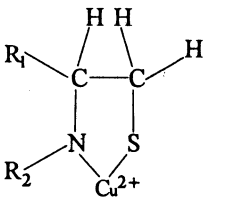
Each GSH coordinates as a bidentate chelating ligand to Pt(II) *via* its sulfur and the amide nitrogen of the glutamyl residue. It is postulated that coordination of Pt(II) to sulfur at a site vacated by a chloro ligand labilizes the *trans*-ammine ligand, making possible the binding of a second GSH ligand[104].

Binding of cisplatin by GSH *in vivo* and its possible involvement in the toxicity of cisplatin has been studied using rats[105–108, 111]. Shanjin and coworkers[112] studied glutathione-*cis*-diamminediaquaplatinum(II) complexation using potentiometric and spectrophotometric technique (Job's method). They proposed sulfur atom as the preferential ligating atom in complex formation. Kinetics and mechanism for reduction of *trans*-dichlorotetracyanoplatinate(IV) by glutathione in aqueous solution was studied by stopped-flow spectrophotometry[113]. The spectra showed that reduction takes place directly without initial substitution at Pt(IV). NMR studies[103, 114] of platinum(II) and reduced glutathione have also been reported. Platinum(II) complexes of glutathione are used as anticancer agents[111, 115–119].

(g) Group 11 Metal Complexes

(i) Copper Complexes: Cu(II) is the most important oxidation state of copper in many physiological systems. Complexation of Cu(II) by glutathione may be involved in the metabolism of both Cu(II) and GSH[1, 120]. The hemolytic anemia that typically accompanies copper toxicity is usually accounted for by Cu(II)-catalyzed oxidation of GSH and by inhibition of glutathione reductase[1, 121, 122]. Both interactions reduce the ability of GSH to protect cells from damage by hydroperoxide generated in cellular processes. Incubation of leukocytes with Cu(II) also results in a decrease in both GSH content and glutathione reductase activity[120].

NMR and EPR studies of the Cu(II)-glutathione interaction indicate the coordination sites and character of metal-peptide bonding[123–125]. In acid medium[123], before the carboxyl group undergoes dissociation, the stepwise addition of copper ions to the glutathione solution causes a slight broadening of all ^1H NMR lines simultaneously. This may suggest very slight coordination of metal by the totally protonated ligand. At higher pH both the thiol group of the cysteine residue and the amino group glutamic acid undergo dissociation and the interaction of these groups with copper ions in solution becomes possible[123]. The ^1H NMR spectrum of glutathione in basic solution by the addition of small amounts of Cu(II) ions cause at first broadening of the quartet derived from the —CH cysteine group and next the multiplet of the cysteine —CH_2 group. With larger amounts of copper ions the α-CH group triplet of glutamic acid first broadens and then decays. No considerable change in the CH_2 singlet of glycine was observed even at high Cu(II) ion concentration. The results suggest that the peptide linkage between cysteine and the glutamic acid occur first. Since the Cu(II) promotes the deprotonation of the peptide linkage in basic medium, it would be expected that the deprotonated nitrogen atom will be bonded to the metal ion. The sulfur atom combines with the same metal ion, forming the five membered ring[123] **(VI)**.



$R_1 = CONHCH_2COO^-$
$R_2 = COCH_2CH_2CHNH_2COO^-$

VI

From ^1H NMR spectra it has been concluded that the glutamic acid copper terminal contributes to the coordination by copper ion[123]. The EPR spectra of aqueous solutions with peptide : metal molar ratio 1 : 1 and 1 : 2 and of pure salt $CuCl_2 \cdot 2H_2O$ solution, at pH 3, show the symmetric line characteristic of the copper aqua ion. Slight differences in the g parameter value, 2.153, 2.164 and 2.186 for molar ratio of a solution 1 : 1, 1 : 2 and for the $CuCl_2$ solution at T = 120 K could be caused by the weak interaction of copper ions with carboxyl groups. In basic medium (pH 11.8) with different molar ratios, the results indicate the existence of two non-equivalent copper ions coordinated by glutathione[123] at copper : glutathione ratio > 1 : 1.

TABLE-1
EPR SPETRA OF AQUEOUS SOLUTIONS WITH PEPTIDE : METAL MOLAR RATIO[123]

Molar ratio	$A_{\|\|} \times 10^4$ (cm^{-1})	$g_{\|\|}$	$A_\perp \times 10^4$ (cm^{-1})	$g_\perp$	$A_{\|\|} \times 10^4$ (cm^{-1})	$g_{\|\|}$	$A_\perp \times 10^4$ (cm^{-1})	$g_\perp$
1 : 1	189.7	2.221	16.2[1]	2.062[2]	—	—	—	—
1 : 1.5	189.8	2.222	16.2[1]	2.062[2]	194.9	2.274	29.6	2.044
1 : 2	190.8	2.221	16.2[1]	2.062[2]	195.1	2.272	29.5	2.044

[1]A estimated from A_{av} obtained from 1 : 1 molar ratio solution.
[2]Estimated with g_{av} from 1 : 1 ratio liquid solution using equation $g_{av} = 1/3(g_{||} + 2g)$.

The EPR spectra have been employed to study the rates and mechanism of reactions of copper/peroxide/thiol systems relevant to biological conditions, with special emphasis on the oxidation by Cu(II) of the antioxidant GSH to GSSG [and Cu(I)] and the subsequent generation of hydroxyl radicals from reactions between Cu(I) and H_2O_2[125]. Evidence is presented that the reaction between Cu(II) and GSH in water proceeds considerable more rapidly than with cysteine or penicillamine, but that the reoxidation of the copper proceeds relatively slowly for glutathione *via* the reaction of the complex Cu(II)-SG with H_2O_2[125].

Transition metals are crucial for thiol autoxidation[126]. The role of copper and ceruloplasmin in thiol-dependent mutagenesis was studied in *Salmonella typhimurium* strain TA102. The effect of copper and ceruloplasmin on thiol-dependent mutagenesis were similar to their effects on thiol-driven lipid peroxidation. The results indicate that the role of copper and ceruloplasmin in the

enhancement of thiol mutagenesis is the facilitation of the transfer of electrons from a thiol to iron (of added Fe), rather than in catalysis of the Fenton reaction[126].

GSH interacts with Cu(II) in the vicinity of DNA (pH *ca.* 7) to form the DNA-Cu(I) complex, which can be quantified by characteristic absorption changes under initial conditions of Cu(II)/GSH $\gg$ 1 and DNA (base)/Cu(II) $\gg$ 5, the stoichiometry is 1 DNA-Cu(I) per SH group[127]. Stopped-flow kinetic studies[127] show that the complex is formed with half lives of 1–30 s, depending on the environment, but independent of O_2. DNA-Cu(I) generation is much slower, less efficient, and O_2 dependent at Cu(II)/GSH < 1, or when GSH interacts with Cu(II) before the addition of DNA. Interaction of GSH with Cu(II) in the presence of DNA (at Cu(II)/GSH > 1) leads to DNA-associated transients, probably DNA-GS^--Cu(I); DNA-Cu(I) formation under these conditions is proposed to occur by ligand exchange: DNA-GS^--Cu(I) + Cu(II) $\rightleftharpoons$ DNA-Cu(I) + GS^--Cu(II). There is no evidence for generation of free thioyl radicals ($GS^\bullet$) on reaction of Cu(II) with GSH[127]. Formation of DNA-Cu(I) is involved in DNA-strand cleavage by GSH in the presence of Cu(II)[128]. In this context the question of the pro-oxidative and/or antioxidative activity of GSH, when combined with copper, is discussed. GSH also generates Cu(I) complexes with other nucleic acids. An updated order of affinities of various nucleic acids for Cu(I) is presented. Cu(I) exhibits a high preference for alternating dG-dC sequences and might even be a Z-DNA inducer. The poly (C)-Cu(I) complex seems to form a base-paired structure at pH *ca.* 7, as demonstrated by intercalation of ethidium bromide[127].

Oxygen radicals are known to interact with a variety of macromolecules leading to lipid peroxidation, DNA strand breakage and a variety of changes in proteins, including thiol oxidation[129]. The protein binding of homocysteine, cysteine and glutathione in a human cell line culture exposed to homocysteine and copper ions in order to elucidate the possible role of homocysteine in cell injury and artherogenesis[129]. It is shown that homocysteine has the highest tendency of the thiols investigated to create disulfide bonds with proteins. The interaction with protein cysteine thiol groups, which are involved in the function of many enzymes, structural proteins and receptors might disturb many metabolic functions in the cell. This finding might therefore be one reason for the cell-damaging effects of homocysteine[129]. To explain the cytotoxicity of excessive copper accumulation in the liver of sheep, Cu-Zn superoxide dismutase activity, GSH levels and lipid peroxidation in homogenates and subcellular fractions of hepatocytes has been measured[130, 131].

Cu(II)-GSH complexes were studied using IR, electronic spectra and coordination sites were ascertained[132, 133]. The ternary complexes of Cu(II)-GSH have been studied by absorption and ESR spectrometries. Measurements were done by stopped-flow techniques. The results showed that glutathione functioned as a monodentate thiol ligand and were stabilized significantly by ligation of an imidazole nitrogen to the metal atom[134]. But the electronic spectrum of the Cu(II) complexes of glutathione showed a strong band at 640 nm characteristic of octahedral geometry and at room temperature the magnetic moment was found to be 2.07 B.M., a normal and spin free octahedral range[47]. Cu(II)-GSH

complexes were tested for their antifungal activity against some plant pathogenic fungi using slide germination technique[47].

The protective effects of GSH, vitamin E and selenium on the lipid peroxidation in the liver and kidney of copper treated rats has been studied[135]. Simultaneous effects on copper accumulation and glutathione cycle are also described. Copper toxicity can be prevented by supplementing diet with one of these nutrients. A combined effect of all the three nutrients is being studied, which might be helpful in elucidating the biochemical basis of copper-GSH, copper-vitamin E and copper-selenium interactions[135].

Cu(I) is another important oxidation state of copper in physiological systems. Cu(I)-thioamino complex formation serves not only to improve the chelation therapy for treating copper intoxication but may also provide a better understanding of many facets of normal copper metabolism[136-138]. The blue crab (*Callinectes sapidus*) has a very dynamic copper metabolism associated with the biosynthesis and degradation of its respiratory pigment hemocyanin[138]. It has been reported as the cellular defence mechanism is used by the crab to protect itself from copper toxicity[138]. Formation constants for the ternary mixed ligand complexes of Cu(I) with cysteine, glutathione and penicillamine by potentiometric titrations[136]. The formation constants of Cu(I)-thioamino acids determined were used in an improved computer simulation of copper speciation in blood plasma, which incorporates redox equilibrium[136]. The possible structure of polymeric Cu(I)-glutathione complexes were discussed by NMR and X-ray absorption spectroscopy[139]. The high thermodynamic stability of Cu(I)-S bonds in complexes may provide efficient and specific pathways for the transport of copper in cells[139].

(ii) Silver Complexes: Silver(I) has high affinity for sulfur. Polarographic and potentiometric studies of 1 : 1 and 1 : 2 (Ag : GSH) complexes have been reported[140]. In tris buffer at pH 7–8, a 1 : 2 Ag : GSH complex forms in which Ag(I) coordinates to deprotonated thiol-groups (GS-Ag-SG) while the amino group is largely protonated[141]. But 1 : 1 Ag : GSH complexes are polymeric involving both amino and thiol groups, with the two groups of a single molecule binding to different Ag(I) ions. Zegzhda *et al.*[142, 143] studied stability and ionization constants of Ag(I) complexes of glutathione. The Ag(I) with thiol group of GSH has been widely used for detecting and determining GSH in blood and tissue[144, 145].

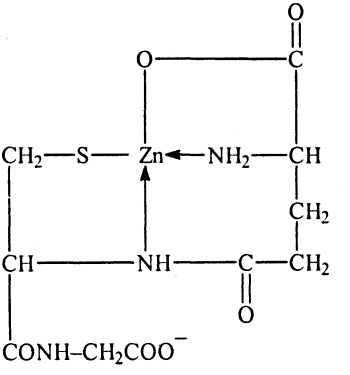
(iii) Gold Complexes: The biological activity of Au(I) containing drugs used as antitumor agent has been discussed[146]. Gold(I) has a strong affinity for thiolate sulfur. The ^{1}H and ^{13}C NMR of GSH readily displaces thiomalate from Au(I)-thiomalate and thioglucose from Au(I)-thioglucose, the two most widely used gold drugs for rheumatoid arthritis[147, 148]. The metallodrug auranofin [(1-thio-β-D-glucopyranose-2,3,4,6-tetraacetato-S (triethylphosphine) gold] with biological ligands was studied by radioisotope methodology also used for the treatment of rheumatoid arthritis[149, 150].

The GSH exchange reactions on Au(I) are facile and probably involve a polynuclear mixed cluster of the type Au_4S_6 with GSH bound through its thiol groups[147]. Bio-analytical work[151, 152] on plasma protein adsorption on to glutathione immobilized on gold has also been carried out.

(h) Group 12 Metal Complexes

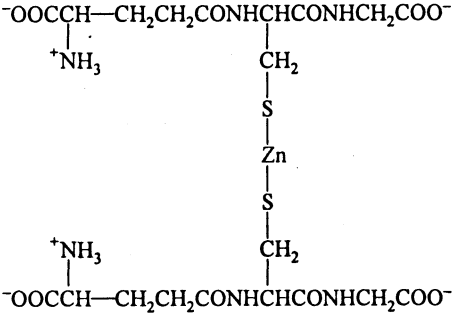
(i) Zinc Complexes: The abundance of GSH in biological systems and its

high affinity for Zn(II) is important in biological chemistry. It has been determined by ^{1}H NMR that GSH is coordinated by Zn(II) in intact human erythrocytes[153]. Perrin and Watt[154] studied complex formation of zinc with glutathione. They proposed several possible structures for the complexes ML^- and ML_2^{4-} involving coordination to one or more of the following sites in the glutathione anion: an oxygen atom of the glutamyl residue (A), the amino nitrogen (B), the peptide oxygen (C) or nitrogen (D), the sulfur atom (E), the second peptide oxygen (F) or nitrogen (H). They suggested a model for zinc complexes in which the metal is bonded through the sites A, B, D and E. ZnL is postulated to have the structure **VII**, showing the bonding of the zinc to the glutamyl portion.



VII

NMR studies[155] of the solution chemistry of Zn(II)-GSH complexes in D_2O indicate ^{13}C chemical shifts to be sensitive to metal binding. The chemical shift data indicate no binding to the peptide linkage between the glutamyl and cysteinyl residues at pD6 < 10.5, and a small amount of binding to the glutamyl residue up to pD6 presumably involving only the carboxylic acid group. At pD > 6, zinc is binding simultaneously to both the amino and carboxyl groups, by analogy with the coordination of zinc by glycine. Thus, in the 1 : 2 complex, the major species in solution for these conditions, with the thiol group as the coordination site[155], is shown as **VIII**.



VIII

Synthesis and characterization[78, 156] of zinc-glutathione ternary complexes has been carried out by IR and thermal analyses. Formation constants have been determined by the pH titration method for Zn(II)-GSH complexes[3, 48, 84, 154, 155, 157–160]. The results indicate a lack of agreement as to which complexes are formed and the magnitude of their formation constants. Formation constants which have been reported from these studies[3, 154, 157] are listed in Table-2.

TABLE-2
FORMATION CONSTANT OF Zn(II)-GSH COMPLEXES[a 3, 154, 157]

Metal	$\log K^{M}_{M(HL)}$	$\log \beta^{M}_{M(HL)_2}$	$\log K^{M}_{ML}$	$-\log K^{M}_{M(HL)_2}$	$-\log K^{H}_{M(HL)(L)}$	$-\log K^{H}_{ML}$	$-\log K^{H}_{ML_2}$
Zn(II)[b]	4.74	9.76	7.84	7.04	9.15	8.82	9.86
Zn(II)[c]	4.88	10.86	8.57	7.35	9.68	8.64	9.96
Zn(II)[d]	5.00	10.17	—	—	7.15	8.96	—

[a]Formation constant = $[M(HL)]/[M][HL]$, $K^{M}_{M(HL)_2} = [M(HL)(L)][H]/[M(HL)_2]$, $\beta^{M}_{M(HL)_2} = [M(HL)_2]/[M][HL]^2$,

[b]I = 0.15 (37°C) $\beta^{Zn}_{Zn(HL)_3} = 10.62$[154], [c]I = 3.00 (25°C)[157],

[d]I = 0.30 (25°C); $\beta^{Zn}_{Zn(HL)_3} = 14.01$; $-\log K^{M}_{M(HL)_3} = 8.60$[3].

The kinetics of formation and dissociation of mono and bis complexes of Zn(II) with reduced glutathione by the reactions represented by eqns. (4) and (5) have been studied by temperature-jump relation method[158]. The important reaction are, therefore:

$$H_2L^- \underset{}{\overset{K_{H_2L}}{\rightleftharpoons}} HL^{2-} + H^+ \qquad \text{Rapid equilibrium}$$

$$\left.\begin{aligned} Zn^{2+} + HL^{2-} &\overset{K_1}{\rightleftharpoons} ZnHL \\ Zn^{2+} + H_2L^- &\overset{K_1'}{\rightleftharpoons} ZnHL + H^+ \end{aligned}\right] \qquad (4)$$

$$\left.\begin{aligned} ZnHL+ + HL^{2-} &\overset{K_2}{\rightleftharpoons} Zn(HL)_2^{2-} \\ ZnHL+ + H_2L^- &\overset{K_2'}{\rightleftharpoons} Zn(HL)_2^{2-} + H^+ \end{aligned}\right] \qquad (5)$$

Results of the kinetic experiments are reported in Table-3.

TABLE-3
RELAXATION TIMES AND RATE CONSTANTS FROM TEMPERATURE-JUMP EXPERIMENT AT 25°C[158]

Total Zn(M)[a]	Relaxation times (msec)			
	4.58	4.78	4.88	4.98
0.010	—	—	1.31	—
0.012	—	—	1.22	—
0.014	—	1.35	0.926	0.933
0.016	1.52	1.01	0.800	0.622
0.018	1.35	0.777	0.730	0.640
0.020	1.17	0.695	0.700	0.557
0.022	0.975	0.668	0.629	0.570
0.024	0.870	0.589	0.529	0.501

COMPLEX FORMATION (k_n M^{-1} sec^{-1})[a] AND DISSOCIATION (k_{-n} (sec^{-1})[b] RATE CONSTANTS

K_1 $9.3 \times 10^7 \pm 24\%$	K_{-1} 9.3×10^2
K_1' $3.9 \times 10^3 \pm 43\%$	K_{-1}' 1.7×10^7
K_2 $5.1 \times 10^7 \pm 25\%$	K_{-2} 3.4×10^2
K_2' $1.9 \times 10^3 \pm 43\%$	K_{-2}' 5.7×10^7

[a]All experiments were performed with total GSH concentration equal to twice total Zn concentration.
[b]Calculated from equilibrium constants and measured forward rate constants.

Considering the H_2L^- and HL^{2-} as the only reactive forms of ligand, the species H_2L^- has both the amine and thiol group protonated. If the species HL^{2-} has only the amine proton left, then it will have a zwitterionic amino acid moiety in one region, and deprotonated thiol and carboxylato groups in the remaining portion of the molecule. In addition, HL^{2-} can be partitioned into another form with the proton on the sulfur instead of the amine. However, if the sulfur is about twice as acidic as the amine[3], the majority of HL^{2-} would be the sulfur deprotonated form.

The stability constant of mixed ligand complexes of Zn(II) has been studied[49, 65, 161]. The relative stabilities of ternary complexes were compared with those of the corresponding binary complexes in terms of Δ log K values. The concentration distribution diagrams of the complexes were evaluated[161]. Spectral characteristics of GSH-capped Zn-S nanocrystallites were significantly influenced by pH and by the stoichiometry of zinc sulfide and glutathione in the complex[162]. Samples containing least glutathione and highest sulfide showed maximal luminescence at pH 6, whereas those with higher glutathione and lower sulfide content showed maximal luminescence[162] at pH 11. Biliary secretion is the main route for elimination of zinc(II)[163, 164]. GSH does not play a regulatory

role in biliary secretion of Zn(II)[163]. GSH has little or no effect on Zn(II) transport across the brush border surface of intestinal cells[165].

(ii) Cadmium complexes: The complexation of Cd(II) by GSH is involved in cadmium toxicology. Although blood is not the target site in cadmium poisoning, it seems to transport Cd(II) to other tissues. Cadmium(II) in blood is located mainly in the erythrocytes[166]. It has been demonstrated directly by ^{1}H NMR studies that Cd(II) in intact human erythrocytes is complexed predominantly by GSH and hemoglobin[167]. Lipid peroxidative damage on cadmium exposure and alterations in antioxidant system in rat erythrocytes has been studied[166, 168].

^{13}C NMR studies of the cadmium-glutathione system indicate that the chemical shifts of the three cysteinyl carbons are a function of pD[155]. For a cadmium to glutathione ratio of 1 : 2, the chemical shifts of selected carbon atoms of the glycyl and glutamyl residues showed the same conditions. At pD < 2, there is some binding to the two carboxylic acid groups but no detectable binding to the amino group, the thiol group, or the peptide linkages. Between pD 2 and 13.2, cadmium is bound to some extent to the thiol group and possibly to the peptide linkage between the cysteinyl and glycyl residues. The chemical shift of the Glu-CONH carbon resonance is not changed by the presence of cadmium over the entire pD range, indicating no detectable binding to the peptide linkage joining the glutamyl and cysteinyl residues. At pH 6.59 the result indicates that thiol is the principal donor group up to a ratio of 0.5. Coordination to the glutamyl end becomes important only when the cadmium to glutathione ratio is greater than 0.5[155]. The complexation of glutathione by Cd(II) was investigated as a model for the coordination chemistry of Cd(II) by thiol containing peptides. Experimental data obtained by differential pulsed polarography (DPP) for different Cd(II) to GSH concentration ratios at fixed pH have been globally analyzed by the multi-variate curve resolution (MCR) method[169, 170]. Polarograms from experiments at fixed Cd(II) to GSH concentration ratios but changing pH values have been univariably deconvoluted at each pH value. In both cases, the obtained single DPP peaks can be associated to specific electrode processes[169]. It has been proposed as a model for the complexation of Cd(II) by GSH involving the formation of $Cd(GSH)_2$ and $Cd_2(GSH)_2$ complexes[169]. The formation constants of Cd(II)-glutathione complexes have been determined[154, 157] and the structures of the complexes were proposed[154].

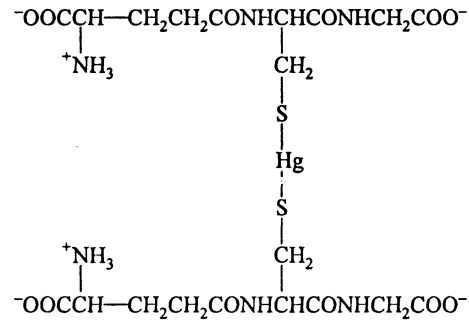
Binary and ternary complexes of Cd(II) with N-(2-acetamido) iminodiacetic acid (ADA) and glutathione have been studied. The contribution of the complex species has been evaluated[171]. Recent studies have shown that cadmium deplete glutathione and protein-bound sulfhydryl groups, resulting in the production of reactive oxygen species[172]. As a consequence enhanced lipid peroxidation, DNA damage and altered calcium and sulfhydryl homeostasis occur[172]. Lipid peroxidation is often discussed as a cause of metal-induced toxicity, although many others suggest a pivotal role of interaction with sensitive SH groups in determining cadmium toxicity[172]. The possible protective role of selenium against Cd toxicity in rat kidney has been studied[173, 174]. Evidence has been presented for the involvement of Cd(II)-GSH complexes in the elimination of Cd(II) by biliary

secretion[165, 175, 176]. Cadmium(II) is reported to form complexes with GSH in rat bile[175] and GSH is thought to be a carrier for its efflux across the canalicular membrane[165, 175].

(iii) Mercury complexes: Mercury(II) has a strong affinity for thiol ligands[177] and GSH plays a significant role in the transport and tissue deposition of mercury compounds[2, 178–180]. Tissue GSH levels are a determinant of tissue mercury deposition and mercury-GSH complexes have been isolated from various tissue. Glutathione is the most abundant intracellular non-protein thiol[181], and GSH has been implicated in numerous other studies of the toxicology of Hg(II)[2, 178–180]. Direct evidence has been presented for the complexation of Hg(II) by GSH in intact human erythrocytes[182] and biliary secretion of Hg(II) has been shown to depend in large part on the biliary transport of GSH[2, 178–180]. The interactions of inorganic mercury and GSH have been discussed[183, 184]. It is explained how these pertain to the translocation of mercury between tissues, to the renal and hepatic processing of mercury, and to the nephrotoxicity. It underlies the biological activity of Hg, deposition of Hg and thiols in erythrocytes and plasma, in liver, and finally in the primary organ kidneys. The nephrotoxic heavy metals such as $HgCl_2$ and $NaAsO_2$ react with endogenous sulfhydryls (GSH) delivered to the kidney. Arsenic and mercury accumulation has been determined by proton-induced X-ray emission. Exogenous GSH decreased $HgCl_2$ cytotoxicity and was correlated to a decrease in Hg accumulation in rabbit renal cortical slice[183, 184].

The formation constants of mercury(II)-glutathione complexes with another biologically active ligand has been studied[185, 186]. The solid 1 : 1 Hg(II)-GSH complexes have been isolated and characterized by IR, electronic and thermal analyses [78]. The results indicate that coordination occurred between the two acid groups through the deprotonation of COOH group of the non-amino acid part, the $—COO^-$ group of the amino acid part remaining unbound. The ν(SH) band at 2500 cm^{-1} in glutathione disappeared on complexation. This indicates the linking of the thiol sulfur to the metal in the complexes[78].

^{13}C NMR study of the binding of mercury with glutathione indicates, for 1 : 2 mercury:glutathione ratio, that the chemical shifts for all the other carbon atoms of glutathione were identical with those obtained for solutions containing no complexing metal ion. The nearly constant chemical shift values for the three cysteinyl carbons over the entire accessible pD range, indicate that binding occurs exclusively to the thiol group at mercury to glutathione ratios up to 0.5 at pH 5. Precipitation prevented studies at higher ratios[156]. Hg(II) does not bind to the glutamyl end at pD > 3 for metal to glutathione molar ratios of 0.5. Hg(II) binds to thiol groups of the two glutathione molecules over the whole pD range accessible, resulting in complexes of structure similar to **IX** except that the state of protonation of the carboxyl and amino groups may be different depending on the pH of the solution[156].



IX

The complexation of Hg(II) by glutathione has been studied by polarimeter[187] under conditions of excess ligand with the objective of characterizing formation of the 1 : 3 complex, Hg(glutathione)$_3$. The value obtained for the formation constant $K_f = 1.5 \times 10^3$ indicates that binding of the third ligand to form Hg(glutathione)$_3$ is much weaker than binding of the first two glutathione ligands. However, calculations indicate that binding is sufficiently strong that a significant fraction of Hg(II) is present as Hg(glutathione)$_3$ under physiological conditions. Equilibrium constants were determined by polarimetry and by ^{13}C NMR for the displacement of one thiolate ligand by another[187]. The mechanism of oxidation of elemental mercury in the presence of —SH compounds has also been studied[188–190] in aqueous environment. A mixture of HgSH compound and phosphate buffer (pH 6.5) was incubated in the dark at 25°C. Hg(II) concentration increased with increasing incubation time. Hg(II) formation by SH compound decreased in the order of glutathione > L-cysteine > D-penicillamine[190].

(iv) Methylmercury complexes: The significance of $CH_3Hg(II)$–GSH complexes in the toxicology of methylmercury is well established. A $CH_3Hg(II)$ has been considered to be secreted from liver to bile as a complex with glutathione[191]. The efflux of $CH_3Hg(II)$ from liver is closely coupled to the carrier mediated transport system by which GSH is secreted into bile[178, 192–194]. Methyl mercury(II) complexes of GSH have been identified in the brains of rats[195] and squirrel monkeys[196] exposed to methylmercury. The elimination of accumulated methylmercury from nervous and non-nervous tissues of fish has been studied[197]. The results showed that the significant detoxification either by glutathione or vitamin B complex or their combination. The best results were obtained in combination therapy[197].

In the equimolar 1 : 1 $CH_3Hg(II)$-GSH complex, $CH_3Hg(II)$ binds exclusively to the deprotonated thiol group with no detectable dissociation over the pH range 0–4[198, 199]. ^1H NMR chemical shift data indicates that the acid-base chemistry of the two carboxylic acid groups and the ammonium group of the $CH_3Hg(II)$ complexed GSH are essentially identical to those of free GSH, consistent with no binding to the thiol group. ^1H NMR chemical shift data suggests, however,

that there is some protonation of the $CH_3Hg(II)$ complexed thiol group[199] at pH < 2.

The formation constant of methylmercury(II) and thiol group of glutathione was studied potentiometrically[200]. Kinetic studies[201] indicate that $CH_3Hg(II)$-GSH complexes are extremely labile, in spite of their high thermodynamic stability and can account for the mobility of $CH_3Hg(II)$ in biological systems[202]. At physiological pH, exchange of $CH_3Hg(II)$ among glutathione and other thiol ligands is predominantly displacement by free ligand[202] [equation (6)].

$$CH_3HgSG + G^*S^- \longrightarrow CH_3HgSG^* + GS^- \quad (6)$$

The tissue levels, half-life and synthetic rates of GSH were investigated in methylmercury treated mice[203]. Methylmercury increased the levels of GSH in the plasma and kidney and decreased in liver and blood. GSH half-life was shortened in the liver and lengthened in the kidney. Thus methylmercury changed GSH metabolism in kidney and liver[203]. Bile is the main route for elimination of methylmercury and several studies have shown a close coupling of efflux of $CH_3Hg(II)$ and secretion of GSH *via* its biliary transport system[2, 165, 178, 192, 193, 204–206]. A somewhat smaller fraction of the methylmercury in mouse, rabbit and guinea pig bile is complexed by GSH[205]. Also $CH_3Hg(II)$ uptake by the kidney is reduced when the GSH level is depressed[204] and it has been suggested that the CH_3Hg-GSH excreted into the bile can be reabsorbed into the kidney[206].

(i) Group 14 Metal Complexes

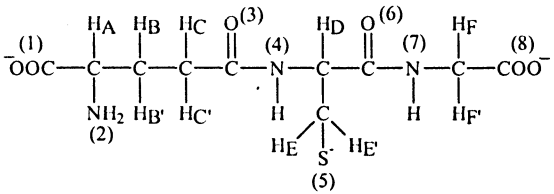
(i) Tin complexes: Organotin(IV) compounds are selective biocides and pesticides and have high mammalian toxicity[207, 208]. They inhibit mitochondrial oxidative phosphorylation[209] and functions of the nervous system[210]. Complex formation equilibrium of tin(IV) with glutathione have been investigated[211–213]. Stoichiometry and stability constants for the complexes formed were determined at temperature 25°C and ionic strength 0.1 M $NaNO_3$. The binding of tin(IV) occurs through the terminal amino group, carboxylate oxygen and the amide nitrogen atoms[211–213]. A solid tri-*n*-butyl tin(IV) complex of GSH has been synthesized and studied by Mössbauer spectroscopy and ^{13}C NMR spectroscopy. The results indicate one Sn(IV) to be four coordinate and the other five coordinate[214, 215].

The comparison of hepatotoxicity caused by mono-, di- and tributyl tin compounds in mice has been studied[216]. The results suggest that dibutyltin chloride (DBTC) is more hepatotoxic than tributyltin chloride (TBTC) and that dibutyltin inside the cells may be the main form of tin which is responsible for induction of hepatotoxicity following *in vivo* TBTC and DBTC. The generation of free radical species, as evaluated by lipid peroxidation and GSH levels, may not be associated with the hepatotoxicity caused by butyltin compounds[216].

(ii) Lead complexes: The binding of lead(II) by glutathione is the subject of interest for toxicity of metal ions in biological systems. The accumulation of thiol groups (SH) in tissues decreases lead toxicity[217]. Lead was administered to rats and total glutathione (GSH) and SH contents and metabolic turnover in their

livers and kidneys were determined. The increased total content of GSH and SH in the lead-administered rat tissues increased the biological availability in rats of binding sites for the complexing of ionic lead. Therefore, GSH and SH block the lead toxicity in rat tissues. The increased total GSH content in bile also was expected to protect the liver against lead toxicity[217]. In a thermodynamic study of lead(II) interaction with several amino acids and peptides, Corries and William[218] suggested the formation of a lead glutathione complex (PbL), with binding to the lead through the glutamyl carboxylate and amine groups as well as the cysteinyl sulfur and the glycyl carboxylate groups. For 2 : 1 glutathione : lead(II) complex they suggested that the species PbL_2H is formed. Binding in this complex was thought to be through the four donor groups used in the 1 : 1 complex for one ligand and only through either the S or the NH_2 for the other ligand. In contrast, from ^{13}C NMR studies, Fuhr and Rabenstein[155] suggested that in a 2 : 1 glutathione : lead(II) complex, binding occurs only through the cysteinyl sulfur (between pD 5.4–12), and to a lesser extent to the glycyl carboxylic acid group (significant at pD < 9). At pD > 9 lead-hydroxyglutathione mixed complexes were formed. Binding to glutathione in these mixed complexes was thought to be through the cysteinyl sulfur only. They also postulated binding through either terminal carboxylic acid group at pD < 2.

A high field NMR study of the binding of lead(II) and glutathione by Kane-Maguire and Riley[219] in acid solution indicated no interaction in the low pH region, but in alkaline solution at pD 12.9 glutathione has eight possible binding sites as shown in **X.**



X

Data obtained in alkaline solution indicate the formation of a 2 : 1 glutathione : lead(II) complex in the presence of excess glutathione. However, when there is a molar excess of lead the 1 : 1 glutathione : lead(II) complex is favoured[219].

Complexation equilibria and evalution of thermodynamic parameters of lead(II)-glutathione complexes were determined pH-metrically[84]. Computer modelling[220] of metal speciation in human blood serum has led to the design of new therapeutic chelating agents[220, 221]. Computer simulation and speciation details are especially important in predicting whether a metal ion or its complex will be toxic. Complexation of Pb(II) by GSH has also been studied in relation to the decreased activity of 5-aminolevulinic acid dehydratase in lead induced anemia[222]. Recent studies have shown that lead depletes glutathione and protein bound sulfhydryl groups, resulting in the production of reactive oxygen species. As a consequence enhanced lipid peroxidation, DNA damage and altered calcium and sulfhydryl homeostasis occur[223]. It has been sugested that the lipid peroxida-

tion induced by lead is connected with the decrease in antioxidative enzyme activity[223]. The role of glutathione in excretion of lead in rat bile is also studied[224].

(j) Group 15 Metal Complexes

(i) Arsenic complexes: There are only few studies on interaction between GSH and arsenic[225, 226]. The influence of arsenic on GSH content, the level of GSH in blood and tissues of mice were detected in acute and subchronic experiment[226]. The results showed that in acute experiment, GSH content in blood and kidney increased gradually in a dose-effect manner and the GSH level in liver and heart increased significantly during the low-dose and decreased significantly during the high dose. In subchronic experiment, the level of blood GSH increased in high arsenic group and decreased in ultra arsenic group significantly[226].

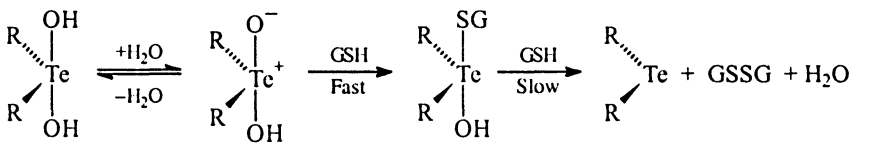
(ii) Bismuth complexes: The glutathione is thought to play an important role in the pharmacology of bismuth drugs, but no chemical studies of bismuth glutathione complexes have been reported[227]. The interactions of the antiulcer compound ranitidine bismuth citrate and $[Bi(EDTA)]^-$ with glutathione in aqueous solution in intact red blood cells have been studied by NMR spectroscopy[227]. The deprotonated thiol group is shown to be the strongest binding site for Bi(III), and a complex with the stoichiometry $[Bi(GS)_3]$ is formed as determined by ^{13}C NMR titrations[227]. A remarkably large low-field shift of *ca.* 1.37 ppm for the β-CH_2 ^1H NMR resonance of GSH was observed on binding to Bi(III). The complex $Bi(GS)_3$ is stable over the pH range 2–10. A formation constant log K = 29.6 (I = 0.1 M, 298 K) for $[Bi(GS)_3]$ was determined by displacement of EDTA by GSH. The rate of exchange of GSH between free and bound forms is pH dependent, ranging from slow exchange (on the ^1H NMR timescale) at low pH (*ca.* 3 s^{-1} at pH 4.0) to intermediate exchange at biological pH (*ca.* 1500 s^{-1}). Such facile exchange may be important in the transport and delivery of Bi(III) *in vivo*[227]. Spin-echo ^1H NMR showed that bismuth citrate reacts with GSH in red cells both *in vivo* and *in vitro*. A first order reaction of bismuth citrate with red blood cells was observed *in vitro* ($k = 0.20\ h^{-1}$, $t_{1/2} = 3$ h, at 310 K), and the rate determining step appeared to involve the passage of Bi(III) through the cell membrane[227].

(k) Group 16 metal complexes

(i) Selenium Complexes: Selenium is an essential trace element to human kind primarily as the element source for the selenium containing enzymes glutathione peroxidase[228]. These enzymes reduce hydrogen peroxide, fatty acid peroxides and phospholipid and cholesterol hydroperoxides in the body[229]. All aerobic cells, including aerobic transformed cells, rely on a variety of antioxidative systems, which include the glutathione peroxidases, to protect against the potentially lethal effects of lipid peroxidation and other oxidative damage[230–233]. These processes work against the therapeutic oxidative stress treated by the generation of singlet oxygen in photodynamic therapy (PDT)[234]. PDT is recently used in cancer treatment in which light, oxygen and a photosensitizer combine at a tumor site to produce singlet oxygen, which is cytotoxic.

In human, murine leukemia cell lines, cells fed a selenium-deficient diet were more susceptible to photoperoxidation and photo killing than selenium-sufficient control populations[235, 236]. In the human leukemia cell lines, 5 to 10-fold lower glutathione peroxidase activity was observed in the selenium deficient cells[236]. Important conclusion from these studies is that impairment of the glutathione-glutathione peroxidase repair cycle in transformed cells might lead to more effective treatment with singlet-oxygen-generating photosentizers[236]. Selenite reacts with excess of glutathione to form a yellow coloured solution of Se(O)-GSH type of complex in alkaline medium[237]. Cyclic voltammetry study indicates a cathodic reduction peak with peak potential –0.89 V. No anodic peak is found. The maximum complex formation in 1 : 10 molar ratio of Se(IV): GSH occurs at pH 12. The electrochemical reduction is irreversible. The reaction mechanism for the reduction process is discussed[237]. To explore the mechanism by which Se exerts its cancer-chemopreventive activity[238], the chemopreventive effects of selenite may be related in part to the generation of reactive oxygen species resulting from the reaction between selenite and GSH.

(ii) Tellurium complexes: The oxidized telluropyrylium dyes react with glutathione and become a potential means for depleting treated cells and tumors of glutathione[236]. Telluroxide is hygroscopic and picks up water upon standing[236]. This suggests that the hydration of the telluroxide is reversible[236] as shown in **Scheme-VI.**



Scheme-VI

Glutathiones are excellent nucleophiles and should enter into the ligand sphere of tellurium(IV) to form a tellurium(IV) hydroxide thiolate as proposed by Engman *et al.*[239]. This process would not involve a change in oxidation state at tellurium and is probably the fast reaction observed by stopped flow spectroscopy[236].

(l) Lanthanide complexes

Only few research papers on lanthanide glutathione complexes are available. NMR study of the conformation of free and lanthanide-complexed glutathione in aqueous solution has been discussed[240]. The stability constants of lanthanide(III) complexes of glutathione (GSH) have been determined by potentiometric estimation of $[H^+]$ in aqueous medium at different ionic strengths and at various temperatures[241]. The effect of dielectric constant on stability has been studied at different percentages of solvent variation and at different solvent systems. The $\bar{n}$, pL and S_{min} values have been calculated[241]. The order of stability constants is found to be La(III) < Pr(III) < Nd(III) < Gd(III) < Ce(III) < Sm(III) < Tb(III) < Dy(III) < Ho(III) < Yb(III). The thermodynamic parameters ΔG, ΔH and ΔS have also been calculated[241].

(m) Actinide complexes

(i) Thorium complexes: Potentiometric and calorimetric studies of glutathione-thorium complexes have been used for detrmination of the stability constant for formation of 1 : 1 Th(IV)-GSH complex and the associated enthalpy and entropy changes[242].

(ii) Uranium Complexes: Marzotto[243] prepared dioxouranium(VI) glutathione complexes of different molar ratios and studied them by IR, ^{1}H NMR, electronic absorption and circular dichroism spectroscopies. The results indicate that coordination occurs at the carboxylato groups acting as monodentate ligands, whereas no significant interaction with the amino and thiol group takes place, and the complexes have polymeric structures. Stability constants and entropy and enthalpy changes have been obtained for uranyl(VI)-glutathione systems studied by potentiometric and colorimetric investigations[244].

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