The Biological and Medicinal Chemistry of Bismuth

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Bismuth compounds have been widely used in medicine for more than 200 years, and new bismuth-containing drugs are now being developed. However the biological chemistry of bismuth is poorly understood. We review here methods for the study of bismuth compounds, and use of Bi(III) in antiulcer, antibacterial, anti-HIV and radiotherapeutic agents is described. The chemistry of Bi(III) carboxylates and aminocarboxylates is dominated by intermolecular interactions which lead to polymeric structures. Bi(III) exhibits a highly variable coordination number and coordination geometry, and alkoxide ligands can induce a strong stereochemical "lone-pair effect". Bi(III) can bind to both Zn(II) sites (e.g. metallothionein) and Fe(III) sites (e.g. transferrin) in proteins. Further work is needed to understand the relationship between the structures and dynamics of bismuth compounds and their bioactivity.

Abbreviations: [15]-aneN$_4$, 1,4,8,12-tetraazacyclopentadecane; BSS, bismuth subsalicylate; CBS, colloidal bismuth subcitrate; dien, diethylenetriamine; dota, 1,4,7,10-tetra-azacyclododecane *N,N'',N'',N'''*-tetraacetate; dtpa, diethylenetriamine pentaacetate; edta, ethylenediamine tetraacetate; egta, ethyleneglycol-*O,O'*-bis(2-aminoethyl)-*N,N,N',N'*-tetraethanoate; gsh, glutathione (γ-L-Glu-L-Cys-Gly); hbdtta, *N,N'*-bis(2-hydroxybenzyl)diethylenetriamine-*N,N',N''*-triacetate; himda, *N*-(2-hydroxycthyl)iminodithanoate; ida, iminodiethanoate; IL2, interleukin; nac, *N*-acetyl-L-cysteine; nta, nitrilotriacetate; RBC, ranitidine bismuth citrate; trien, triethylenetetramine.

1 The Chemistry of Bismuth

1.1 Properties of the Element

Bismuth was first discovered as early as the 15th century, and was established as an element in 1739 by Potts and Bergmann[1]. It is most commonly found as the oxide (Bi_2O_3), bicarbonate [$(BiO)_2CO_3$] and sulfide (Bi_2S_3), and is also obtained as a by-product of lead, zinc and copper mining. It is the heaviest stable element in the periodic table, in group 15 together with nitrogen, phosphorus, arsenic and antimony. The main features of bismuth are shown in Table 1.

1.2 Oxidation States and Redox Chemistry

There are two major oxidation states of bismuth (III and V), with the 3+ state being the most common and most stable form, in contrast to arsenic and antimony. The electronic configurations of bismuth(III) and bismuth(V) are:

Bi(III): $(Xe)4f^{14}5d^{10}6s^2$
Bi(V): $(Xe)4f^{14}5d^{10}$

Peter Sadler (right) studied for his first and second degrees at the University of Oxford. His doctorate was on bioinorganic chemistry under the direction of Professors H. O. A. Hill and R. J. P. Williams. After two years as a Medical Research Council Research Fellow at the University of Cambridge and National Institute for Medical Research, he joined the Department of Chemistry at Birkbeck College, University of London, where he was successively Lecturer, Reader in Biological Inorganic Chemistry, and Professor of Chemistry. On the 1st of October 1996 he took up the Crum Brown Chair of Chemistry at the University of Edinburgh. In 1993 he was recipient of the Royal Society of Chemistry Award for Inorganic Biochemistry and he is currently Chairman of the EC COST Action D8 [The Chemistry of Metals in Medicine]. His research interests lie in this area.

Hongzhe Sun (left) received his M. Sc in 1990 from The Chinese Academy of Sciences and then he moved to Nanjing University (China) as a research associate and later a lecturer. He received his Ph. D. from the University of London in 1996 under the supervision of Professor Peter Sadler and is currently a Glaxo Wellcome Research Fellow in Professor Sadler's laboratory at the University of Edinburgh.
Hongyan Li (center) is doing her Ph. D. work under the supervision of Peter Sadler.

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Table 1. Main features of bismuth

Atomic number	83
Electronic structure	(Xe)$4f^{14}5d^{10}6s^26p^3$
Occurrence in earth's crust	0.2 ppm
Natural abundance	100%
Nuclear spin quantum number	9/2
Radio isotope	212 (alpha emission with half-life 1 h and max. energy 6.090 and 8.78 MeV)[39]
Atomic weight	208.9804 (relative to ^{12}C = 12.000)
Radii: Bi(III) (CN: 6)	1.03 Å
Bi(V) (CN: 6)	0.76 Å

Bi(V) is a powerful oxidant in aqueous solution, with a Bi(V)/Bi(III) potential E^0 = 2.03 V, although recently two Bi(V) complexes with benzenoid and non-benzenoid arenes as ligands were reported to be stable even in aqueous media[2]. Little redox data for either Bi(III) or Bi(V) complexes appears to have been reported.

1.3 Bismuth(III) Compounds

Bismuth is widely used in medicine[3] but is also of current interest in high T_c superconducting materials and sol-gel and vapor deposition precursor compounds[4]. However very little is known about its solution and solid-state chemistry.

According to Pearson's HSAB (Hard–Soft Acid–Base) theory[5], Bi(III) should be a broadline or soft metal ion[6]. Recently Bi(III) was found to have a high affinity for both oxygen and nitrogen ligands in aqueous solution. It binds to nitrogen donor macrocycles even in strongly acidic solutions (pH ≈ 0) Figure 1a[7]. The stability constants of Bi(III) with a series of nitrogen donor ligands have been determined by differential pulse polarography. Separate peaks can be observedin the differential pulse polarograms for free Bi(III) and complexed Bi(III), which greatly simplifies the calculations. Bi(III)-binding to ligands is usually fast with equilibration within a few minutes. An exception is the macrocycle 1,4,8,12-tetraazacyclopentadecane ([15]aneN$_4$), which required two to three weeks. Some Bi(III) binding constants can be predicted from the following empirical equation:

$$\log K_1 \text{ (polyamine)} = 1.152 \log\beta_n(NH_3) + (n-1)\log 55.5^{[7a]}$$

where β_n refers to the stability of the complex containing n NH_3 analogues of the polymaine. This equation has been extended to polyaminocarboxylates such as nta and edta[8]. Stability constants for the binding of Bi(III) to some common ligands are listed in Table 3[9]. It has been found that there is a good linear free energy relationship between $\log K_1$ values for Bi(III) and Pb(II) complexes, and between $\log K_1$ values for Bi(III) and Fe(III) complexes (Figure 2).

The structures of Bi(III) compounds are similar to those of As and Sb compounds, but more complicated. The coordination number of Bi varies from 3 to 9[10,11]. Compared to As and Sb, more structures of Bi complexes are known. Table 2 lists the range of stereochemistries and coordination numbers of Bi(III) compounds.

Figure 1. Structures of (a) a Bi(III) tetraaza macrocycle complex, where the ligand is 1,4,7,10-tetrakis[(S)-2-hydroxypropyl]-1,4,7,10-tetraazacyclo-dodecane:[7c] this complex was crystallized at pH 0; (b) $[Bi_6O_4(OH)_4]^{6+}$[13]

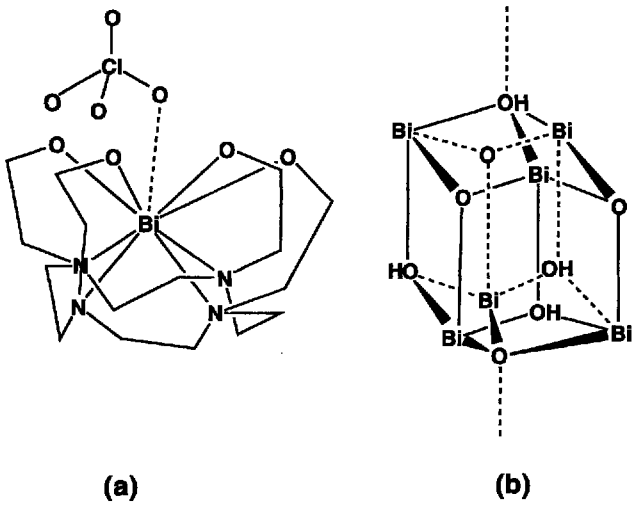
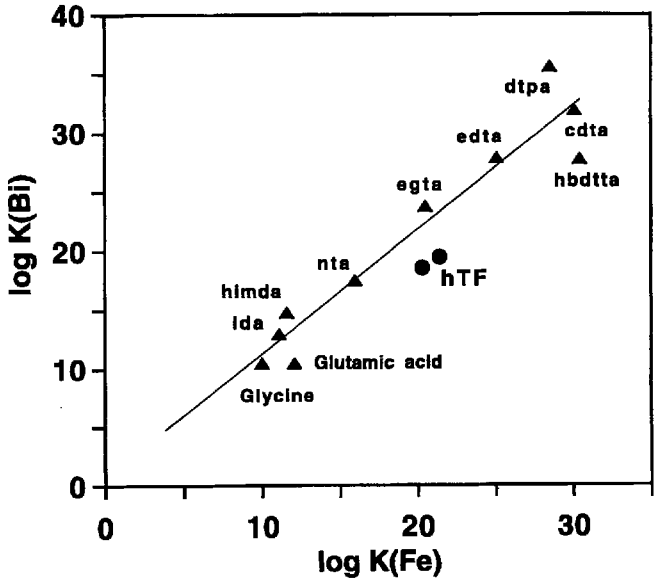


Figure 2. Linear free energy relationship for the complexation of Bi(III) and Fe(III) with oxygen and nitrogen donor ligands. The data are taken from ref.[9]. The points for the two binding constants of transferrin are shown as solid circles



Bi_2O_3 is strictly basic, whereas As_2O_3 and Sb_2O_3 are amphoteric. Bi_2O_3 dissolves in mineral acids to give salts. The hydroxide product, $Bi(OH)_3$, can be precipitated by the addition of base. The first pK_a for a Bi(III) aqua ligand is 1.51. At high pH $[Bi(O)]^+$ forms, and polymeric cations such as $[Bi_6O_4(OH)_4]^{6+}$ always form in solution before precipitation of the hydroxide complex. The X-ray crystal structure of this cation shows that six Bi atoms are at the apices of an octahedron at non-bonding separations. The octahedron is face-capped by O and OH, forming Bi–O–Bi bridges (Figure 1b)[12]. The lone pair of electrons on Bi is directed away from the cage[13]. Bi(III) also shows a

Table 2. Structural data for Bi(III) compounds[10]

Coord. number	Geometry	Examples	Ref.
3	pyramidal	$[Bi(SAr)_3]$ (for structure see Scheme 1)	[17]
4	trigonal bipyramidal	$[Bi\{OP(NMe_2)_3\}_2][Fe(CO)_2(\eta\text{-}C_5H_5)_5F_2][PF_6]$	[18]
5	square-based pyramidal	$Na_2[Bi(SC_6F_5)_5](THF)_4$	[19]
5	trigonal antiprism	{Bi(NO$_3$)bis[1-azepanyl-4-(2-thienyl)-2,3-diazapenta-1,3-diene-1-thiolato-N^3,S]}	[55]
6	octahedral	$[(R'S)_3Bi(SR)_3]$, $[Bi_6O_4(OH)_4]^{6+}$	[12]
7	trigonal dodecahedron	{Bi(NO$_3$)bis[1-azepanyl-4-(2-pyridyl)-2,3-diazapenta-1,3-diene-1-thiolato-N',N^3,S]}	[55]
8	bicapped trigonal prism	[Bi(nta) $(H_2O)_2$] and [Bi(Hedta)] • 2 H_2O	[47]
9	tricapped trigonal prism	$[Bi(H_2O)_9](CF_3SO_3)_3$	[10]
9	monocapped square antiprism	(guanidinium)$_2$[Bi(dtpa)] • 4 H_2O	[47]

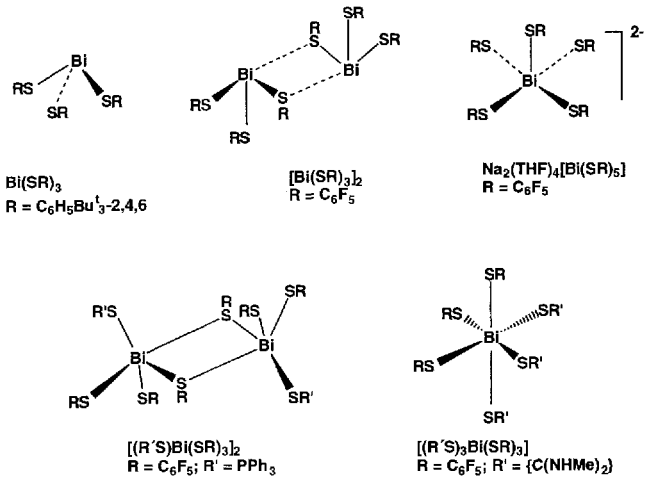
Table 3. Binding constants of Bi(III) with common ligands. Data from[9]

Ligand	log K_1	Ligand	log K_1
hydroxide (OH^-)	12.49	2-mercaptoethanol	13.6
ammonia	5.1	glycine	10.0
dien	17.4	aspartate	10.5
trien	21.9	glutamate	10.5
[15]-aneN$_4$	23.5	nta	17.5
oxalate	7.7.	ida	12.9
L-malate	9.9	hda	12.5
citrate	13.5	edta	27.8
ascorbate (vitamin C)	25.3	egta	23.8
succinate	8.8	cdta	31.9
fumarate	6.9	dtpa	35.6

high affinity for other oxygen donor ligands. Bi(III) complexation with polyethylene glycols and crown ethers has been reported recently[14]. Most of the Bi(III) polyethylene glycol complexes are dimeric, with bridging by two alkoxide oxygen atoms. Coordination of each Bi(III) is completed by a nitrate anion. The Bi–alkoxide bonds are short (2.24 Å), indicative of strong covalent character; this strong bond appears to give rise to a stereochemical role for the lone pair of electrons. The crown ether complexes do not exhibit such strong covalent interactions, and do not display lone-pair effects. Bismuth alkoxide complexes {e.g. $[Bi(OC_2H_5)_3]$ and $[Bi(O\text{-}iC_3H_7)_3]$} show potential as precursors for new superconductor formulations[15].

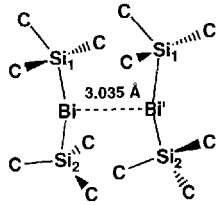
Another class of complexes which is relevant to the biological chemistry of bismuth is that with thiolate-containing ligands as described by Atwood et al., and Norman et al.[16–19]. In these complexes, bismuth exhibits coordination numbers of three, four, five and six with thiolate ligands. Overall the structures are either monomers, or loose dimers as shown in Scheme 1. The Bi–S bond distance is ca. 2.5–2.6 Å for intramolecular interactions and ca. 3.1–3.3 Å for intermolecular (long-range) interactions.

Scheme 1. Structures of bismuth thiolate complexes



Bi–Bi bonds are also observed in some other complexes. The most significant one is tetrakis(trimethylsilyl)dibismuthane, which shows a trans-staggered conformation. The intramolecular d(Bi–Bi) is 3.035 Å, and the intermolecular d(Bi–Bi) is 3.804 Å (Scheme 2). The molecules are linked into chains in the crystal[20]. Other bismuth organometallic complexes have been extensively reviewed in *Comprehensive Coordination Chemistry*[21].

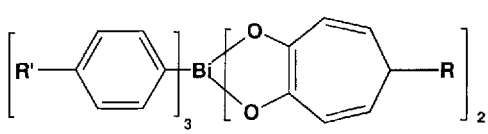
Scheme 2. [Bi(III)$_2$(SiCH$_3$)$_4$] showing the short Bi–Bi bond



1.4 Bismuth(V) Compounds

Most of the Bi(V) complexes prepared to date are five-coordinate, although Bi(V) in triarylbismuthate complexes such as $Ar_3Bi(OCHO)_2$ (where Ar = Ph, p-Tol)[22], is six-coordinate. Almost all of these Bi(V) complexes are unstable. However seven-coordinate complexes tri(aryl)bis(tropolonato)bismuth(V) (where R = H, NO_2; R' = H, CH_3, Scheme 3) have recently been reported to be unusually stable, probably due to the steric shielding of the bismuth ion[2].

Scheme 3. Tri(aryl)tropolonato)bismuth(V)



2 Physical Methods for the Study of Bismuth Compounds

Table 4 summarizes the various physical methods used for the study of bismuth compounds. There are few informative methods for the direct probing of Bi in bismuth

Table 4. Physical methods for the study of bismuth complexes

Technique	Information	Comments
NMR spectroscopy	types and number of coordinated ligands, conformation, dynamics (ligand exchange)	only two reports of ^{209}Bi NMR, probing ligand rather than Bi usually ^{1}H, ^{13}C, ^{15}N and ^{31}P, generally in solution, but also possible in solid state
Infrared and Raman spectroscopy	ligand and Bi–ligand vibration frequencies	difficult to assign Bi–S or Bi–O vibration frequencies. Usually compare free ligand and complex
UV-visible and circular dichroism spectroscopy	electronic energy levels and symmetry	absorptions are usually weak in the visible region charge transfer absorptions can be observed in the UV region
Mass spectrometry	molecular mass of parent and fragments	compound must be introduced into the ion source, fragmentation pattern can be obtained
Atomic absorption spectroscopy	total Bi, detection limit 0.09 ppb (μg/l)	need to digest the sample, desalt biological samples
ICP-MS	total Bi, detection limit 0.01 ppb (μg/l), direct observation of ^{209}Bi isotope	samples only need dilution by 10% HNO_3, less interference (than AAS)
X-ray crystallography	3D structure (bond lengths, angles)	need single crystals
X-ray spectroscopy: XAS, XANES and EXAFS	number and type of bonded atoms, interatomic distances	requires synchrotron radiation, can be used for both solution and amorphous solid, difficult to distinguish, N and O, and P, S and Cl
Differential pulse polarography	half-wave potentials and concentrations, detection limit, ca. 10^{-7} M	distinguishes between Bi(III) and Bi(V); speciation, Bi binding at very low concentration (μM), wide pH range

complexes. ^{209}Bi NMR has not been widely used because Bi has a large nuclear quadrupole moment (-0.4×10^{-24} cm^{-2}), thus leading to broad resonances. Resonances for ^{209}Bi can be detected only in highly symmetrical environments, and only two ^{209}Bi-NMR spectra have been reported: those of $Bi(NO_3)_3$ (dissolved in nitric acid) with a linewidth of 3.2 kHz ($\delta = -24$) and $[BiF_6]^5$, with a linewidth of 44 Hz, $\delta = 0.0$ and $J_{Bi\text{-}F}$ 3823 ± 3 Hz[23]; no useful chemical studies have been carried out. NMR studies of bismuth complexes have therefore relied on observations of ligand nuclei (e.g. ^{1}H, ^{13}C). Nuclear quadrupole resonance has also not been useful for studies of bismuth. Single crystal X-ray diffraction gives useful structural information if suitable crystals can be obtained, and since bismuth is a very heavy element, very small crystals are used to avoid absorption effects. X-ray absorption near edge structure (XANES), and extended X-ray absorption fine structure (EXAFS, e.g. Bi L_{III} edge) are likely to be useful in the study of bismuth coordination spheres but there appear to be no previous reports of this. Using EXAFS we have recently been able to determine the coordination spheres of Bi in glutathione and transferrin complexes[24].

The most common method of determining the total bismuth content of a sample is by atomic absorption spectroscopy (AAS), and more recently by inductively-coupled plasma mass spectrometry (ICP-MS). The former requires digestion of the sample, and large interferences from high concentrations of salts retard the application to biological samples. ICP-MS seems to be a suitable method for determining bismuth in all kinds of materials. Since bismuth is a very heavy element (atomic mass, 208.98), there is little interference from other elements, and the detection limit can be as low as 0.01 ppb (μg/l, 4.8×10^{-9} M[25]. Using Direct-Injection Nebulization (DIN) instead of pneumatic nebulization (PN), memory effects can be dramatically suppressed and less sample volume is required[25]. By combining HPLC (FPLC) and ICP-MS, it is possible to study the speciation of Bi in biological systems. Recently the Bi content of blood plasma was determined by ICP-MS before, during and after the intake of the bismuth anti-ucler drug CBS[26].

3 The Chemistry of Bismuth in Medicine

3.1 History

The earliest use of bismuth in medicine appears to have been in the Middle Ages as bismuth subnitrate, a white pigment, also used in beauty care and painting. The first full account of the internal administration of a bismuth compound was in 1786 by Louis Odier[27].

In the UK, bismuth seems to have entered firmly into medicinal use as early as the 19th century via Alexander Marcet at Guy's Hospital, London (1805)[28]. He used bismuth subnitrate to treat spasms of the stomach. In the later 19th century, several new bismuth compounds were introduced into medicine specifically for the treatment of intestinal and gastric disorders. These included derivatives of phenol, bromophenol, pyrogallol, naphthol and salicylate.

In 1889, Felix Balzer first discovered that bismuth might be useful as an antisyphilitic agent[29]. Even after the introduction of penicillin as a safe and rapid treatment for treponemal infections, bismuth was still found to be useful. Its gradual action was thought to be particularly valuable in the tertiary and quaternary stages of syphilis. In addition, bismuth nitrate in combination with morphine was a constituent of Ferrier's snuff, an inhalation for nasal catarrh. Bismuth and iodoform were even widely advocated as a surgical would dressing due to their antimicrobial and antibacterial effects[30].

During this century, various bismuth complexes (subnitrate, subgallate, subcitrate, tartrate, subcarbonate and subsalicylate) have been used to treat syphilis, hypertension, infections, skin conditions and gastrointestinal disorders[31]. Since the 1970s, two bismuth compounds have been most commonly used worldwide: bismuth subsalicylate (BSS, Pepto-Bismol; the Procter & Gamble Company, Cincinnati, Ohio, USA) for the prevention and treatment of diarrhoea and dyspepsia, and colloidal bismuth subcitrate (CBS, De-Nol; Gist Brocades, Delft, The Netherlands, launched in 1976) for the treatment of peptic ulcers. The latter (CBS)

Table 5. X-ray crystal structure determinations of bismuth citrate complexes

Complexes	Bicit	CN	Comments	Ref.
$K_{4.75}(NH_4)_{0.25}[Bi_2(cit)_2(Hcit)] \cdot 13\ H_2O$ (**1**)	2:3	9	two citrate tetraionized (cit^{4-}), one is triionized ($Hcit^{3-}$); shortest Bi–OC 2.12 Å; Bi–Bi 5.849 Å	[32b]
$K[Bi(cit)] \cdot 3\ H_2O$ (**2**)	1:1	9	citrate tetraionized (cit^{4-}), shortest Bi–OC ca. 2.16 Å; distorted pentagonal bipyramid; dimeric characterized by a planar Bi_2O_2 four-membered ring	
$Na_2[Bi_2(cit)_2] \cdot 7\ H_2O$ (**3**)	1:1	6	citrate tetraionized (cit^{4-}); shortest Bi–OC 2.21 Å; bi–Bi 6.188 Å; dimeric unit, aggregation via double carboxylate bridge	[51]
$(NH_4)_2[Bi_2(cit)_2] \cdot 4\ H_2O$ (**4**)	1:1	6	similar structure to **3**; shortest Bi–OC 2.13 Å	[33b]
$K(NH_4)[Bi_2(cit)_2] \cdot 4\ H_2O$ (**5**)	1:1	6	similar structure to **3**; shortest Bi–OC 2.13 Å	[33a]
$K(NH_4)[Bi_2(cit)_2(H_2O)] \cdot 5\ H_2O$ (**6**)	1:1	6	similar dimeric unit structure to **3**; dimeric unit aggregation via single carboxylate bridge	[33a]
$K_2[Bi_2(cit)_2(H_2O)] \cdot 5\ H_2O$ (**7**)	1:1	6	similar structure to **6**	[33b]
$(NH_4)_4[Bi(cit)(Hcit)(H_2O)_2] \cdot H_2O$ (**8**)	1:2	9	one citrate is tetraionized (cit^{4-}), one is triionized ($Hcit^{3-}$); Bi–OC 2.14 Å (the shortest); Bi–Bi 5.972 Å	[32b]
$(NH_4)_6[Bi_6O_4(OH)_4(Hcit)_4] \cdot 2\ H_2O$ (**9**)	3:2	7	hexanuclear cluster	[49b]
$(NH_4)_{12}[Bi_{12}O_8(cit)_8] \cdot 10\ H_2O$ (**10**)	3:2	7	dodecanuclear Bi(III)-oxo citrate cluster	[49a]

has been successfully used in the treatment of both gastric and duodenal ulcer diesease. It has proved as effective as the histamine H_2-antagonists such as cimetidine. CBS differs from previous bismuth compounds in that it is highly water-soluble, giving a colloidal solution in contrast to bismuth subnitrate and subsalicyalte, and its solid-state structure has been investigated[32,33]. Recently the effectiveness of bismuth has been attributed to its bactericidal action against *Helicobacter pylori* (an organism which was first discovered in 1983 named as *Campylobacter pyloridis*, later amended to *C. pylori*). Several bismuth compounds have been found to be effective against *H. pylori* in vitro, and the longer remission times achieved with bismuth therapy are probably due to clearance of the organism by bismuth[34]. Clinical studies with CBS and BSS show that patients treated with bismuth alone experience a slower relapse than patients treated with other ulcer-healing agents[35], due to bactericidal action of these two complexes against *H. pylori*.

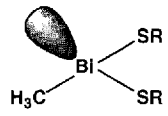
There was an outbreak of bismuth-induced encephalopathy in France and Australia in the 1970s which led to the withdrawal of bismuth drugs in these countries[36]. However, there have been few such side-effects reported in the UK and USA where there is a widespread use of bismuth drugs (200 and 507 tons per annum in UK and USA, respectively). The mechanism of action of bismuth drugs needs to be investigated, as toxic side effects may be related to bismuth interactions with certain molecules in the body. As with other metallodrugs, such as gold antiarthritic and platinum anticancer agents, new methods (i.e. different from those used routinely for organic drugs) need to be developed in clinical chemistry laboratories to determine both the total contents of biological samples and to investigate speciation. This will lead to their safer use.

New bismuth-containing drugs are currently being developed. Recently, a new ranitidine bismuth citrate compound (made by GlaxoWellcome plc.) has been approved for marketing and is on sale in a number of countries around the world. It combines the antisecretory action of ranitidine with the mucosal protectant and the bacteriocidal properties of bismuth[37,38].

Another use of bismuth in medicine is in radiotherapy. ^{212}Bi, is a strong alpha-particle emitter, has a short half-life (1 h)[39], and can be obtained in large quanties from a ^{224}Ra generator. This isotope has been used as a targeted radiotherapeutic agent for cancer therapy when attached via complexing ligands such as dtpa (diethylene-triaminepentaacetate) and dota (1,4,7,10-tetra-azacyclododecane N,N″,N″,N‴-tetraacetate) to monoclonal antibodies[40]. Using the method developed by Krejcarek and Tucker[41], ^{212}Bi has been successfully conjugated to anti-Tac, a monoclonal antibody directed towards the human interleukin-2 (IL-2) receptor. Recently ^{212}Bi-anti-Tac has shown potential for eliminating alloreactive T-cell in graft-versus-host disease, and in organ allograft settings, where inflamed tissues are more rapidly targeted because of favourable circulatory distribution and vascular leakage[42].

Bismuth complexes such as $\{Na_2[BiO(mp)_3] \cdot 3\ H_2O\}$ and $\{Bi(tgn)_3(H_2O)] \cdot 3.5\ H_2O\}$ (where mp = 6-mercaptopurine; tgn = thioguanine) have been shown to exhibit anticancer activity[43]. Skinner, Swatzell and Lewis found that $Na_2[BiO(mp)_3] \cdot 3\ H_2O$ complex was signficantly effective in treating Dunning ascitic leukemia in rats[44]. Recently it was found that some organometallic bismuth(III) thiolates complexes (Scheme 4) exhibit an optimum cure rate of 100% against fluid Ehrlich ascites tumor with a therapeutic index of 3.2–5.0[45]. Some bismuth complexes have recently been shown to inhibit HIV-1 virus production from chronically infected H9 cells with a selective index of ca. 5[46].

Scheme 4. Organometallic bismuth(III)thiolate complexes which exhibit antitumor activity[45]. R = CH_3, *p*-Ph-NH_2 and *p*-Ph-$N^+H_2CH_3$



3.2 Structures of Anti-Bacterial and Anti-Ulcer Bismuth Complexes

Although some bismuth complexes have been known for a long time to exhibit anti-bacterial or anti-ulcer activity, the structures of several of them have only recently been

solved, and some of them are still unknown. [Bi(nta) $(H_2O)_2$], [Bi(Hedta)] • 2 H_2O and (guanidinium)$_2$[Bi(dtpa) • 4 H_2O all exhibit antibacterial activities, and their structures were determined in 1994 by Palenik et al.[47]. The least soluble complex, [Bi(nta) $(H_2O)_2$] (solubility in aqueous solution ca. 2 mM), has been found to be the most active. In the neutral complex [Bi(nta) • 2 H_2O], bismuth is eight-coordinate, bound to four oxygens and one nitrogen from nta, and two carboxylate oxygen atoms from a neighboring [Bi(nta)] $(H_2O)_2$] molecule, with the coordination sphere being completed by two water molecules, forming a bicapped trigonal prism (Figure 3a). In [Bi(Hedta)] • 2 H_2O (solubility ca. 30 mM pH 7.0), the bismuth atom is also eight-coordinate with a bicapped trigonal prism arrangement (Figure 3b), but there is no water coordinated to bismuth. The edta ligand coordinates in hexadentate mode via the two nitrogen atoms and four oxygen atoms of the carboxylates; two carboxylate oxygen atoms from another edta ligand complete the coordination sphere. In (guanidinum)$_2$[Bi(dtpa)] • 4 H_2O, bismuth is coordinated in a monocapped-square antiprism arrangement (Figure 3c).

The empirical formula of colloidal bismuth subcitrate (CBS) is often given as $K_3(NH_4)_2[Bi_6O_3(OH)_5(Hcit)_4]$[48]. By variation of the pH and the ratios of bismuth and citrate, nine different bismuth citrate adducts have been isolated and characterized by X-ray crystallography, as summarized in Table 5. Most of them contain the stable dinuclear unit [Bi(cit)$_2$Bi]$^{2-}$ with additional O^{2-}, OH- and H_2O ligands as shown in Figure 4a. This dinuclear unit can aggregate further by citrate bridging to form channels and can also form sheets by H-bonding; the high solubility of CBS is probably due to the formation of such channels and sheets. The coordination number of bismuth in these complexes is usually high, from 6 to 9, and there are short Bi–alkoxide (C–O of citrate) bonds of ca. 2.13 Å. The stereochemical role played by the lone pair of electrons of Bi(III) in these compounds is particularly notable. All of the bound atoms lie on the same side of the Bi coordination sphere, and the vacant axial site is occupied by the lone pair of electrons. However none of these complexes has exactly the same composition as CBS; therefore the solid-state and solution structures of CBS are still unknown, although it seems likely that it consists of dinuclear units [Bi(cit)$_2$Bi]$^{2-}$ aggregated through citrate bridges and H-bonding. It is clear that bismuth citrate complexes can have complicated structures, being dependent on pH, concentration, [Bi:cit] ratio and the counter cations. At neutral pH, multinuclear clusters such as $[Bi_6O_4(cit^{4-})_4]^{6-}$ (Figure 4c) and $[Bi_{12}O_8(cit^{4-})_8]^{12-}$ are also formed by citrate bridging[49]. The latter cluster can further aggregate to form Bi_{24} in concentrated solutions, and dissociates into smaller clusters with some release of citrate when diluted[49].

Ranitidine bismuth citrate (RBC) is highly soluble in water (ca. 1.0 g/ml), giving a pH of 4.6. Possible solution and solid-state structures of RBC have been discussed[50,51]. In aqueous solution, different bismuth citrate species are in fast exchange on an NMR time-scale; at pH* >6.2 the exchange rate decreases and different species can be observed[50,52]. Ranitidine is not coordinated to Bi and appears to be involved in specific second-coordination sphere interactions with bismuth citrate via its $HNMe^+$ group. Complexation of Bi(III) to both citrate and ranitidine in acidic solutions (pH 2.5–3.0) was detected by differential pulse polarography. There is a rapid deprotonation equilibrium between at least two species in solutions containing Bi(III)/citrate in 1 : 10 mol ratio. At pH >5.8 a second peak appeared suggesting that at least two types of Bi(III) citrate complex exist under these conditions, in agreement with NMR data. The solid-state structure of ranitidine bismuth citrate may be closely related to that of $Na_2[Bi_2(citrate)_2]$ • 7 H_2O in view of the similarity in their chemical compositions (Bi:cit = 1:1), crystallization conditions (ca. pH 4), and solid-state ^{13}C-NMR spectra[51,52]. $Na_2[Bi_2(citrate)_2]$ • 7 H_2O contains the highly stable dimeric unit [Bi(cit)$_2$Bi]$^{2-}$ in

Figure 3. X-ray structures of (a) [Bi(nta) $(H_2O)_2$], (b) [Bi(Hedta)] • 2 H_2O, and (c) (guanidinum)$_2$[Bi(dtpa)] • 4 H_2O[47]. In all three cases, singly and doubly bridged dimers are further cross-linked by carboxylate oxygens to give a polymeric lattice. In (a) one of the bonds to H_2O is long and not shown

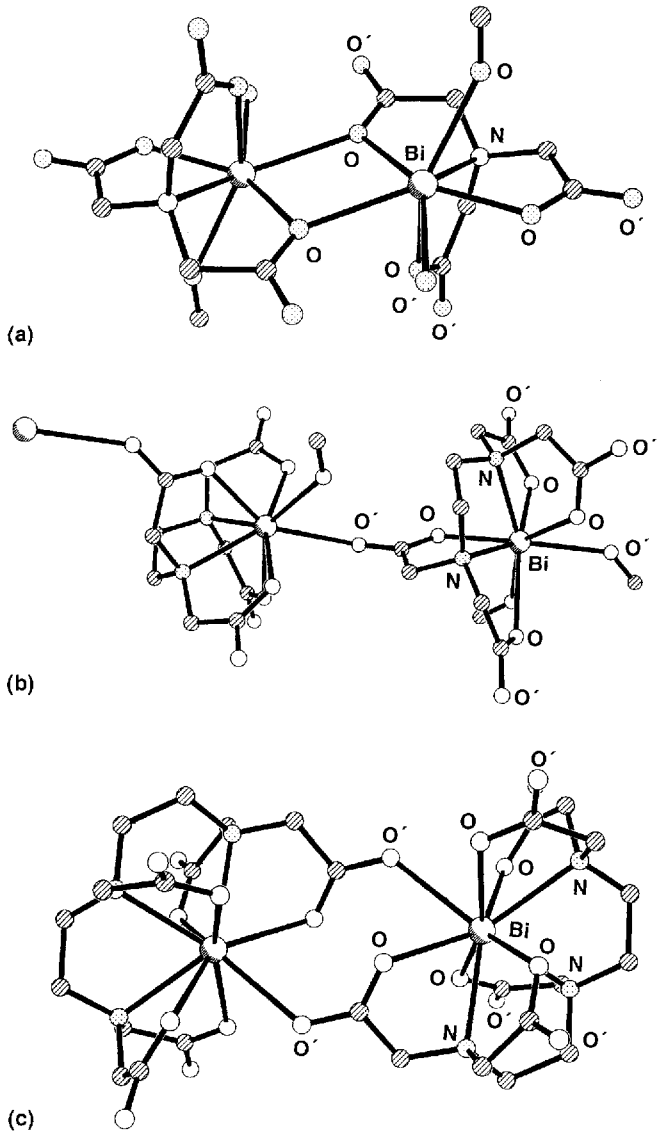
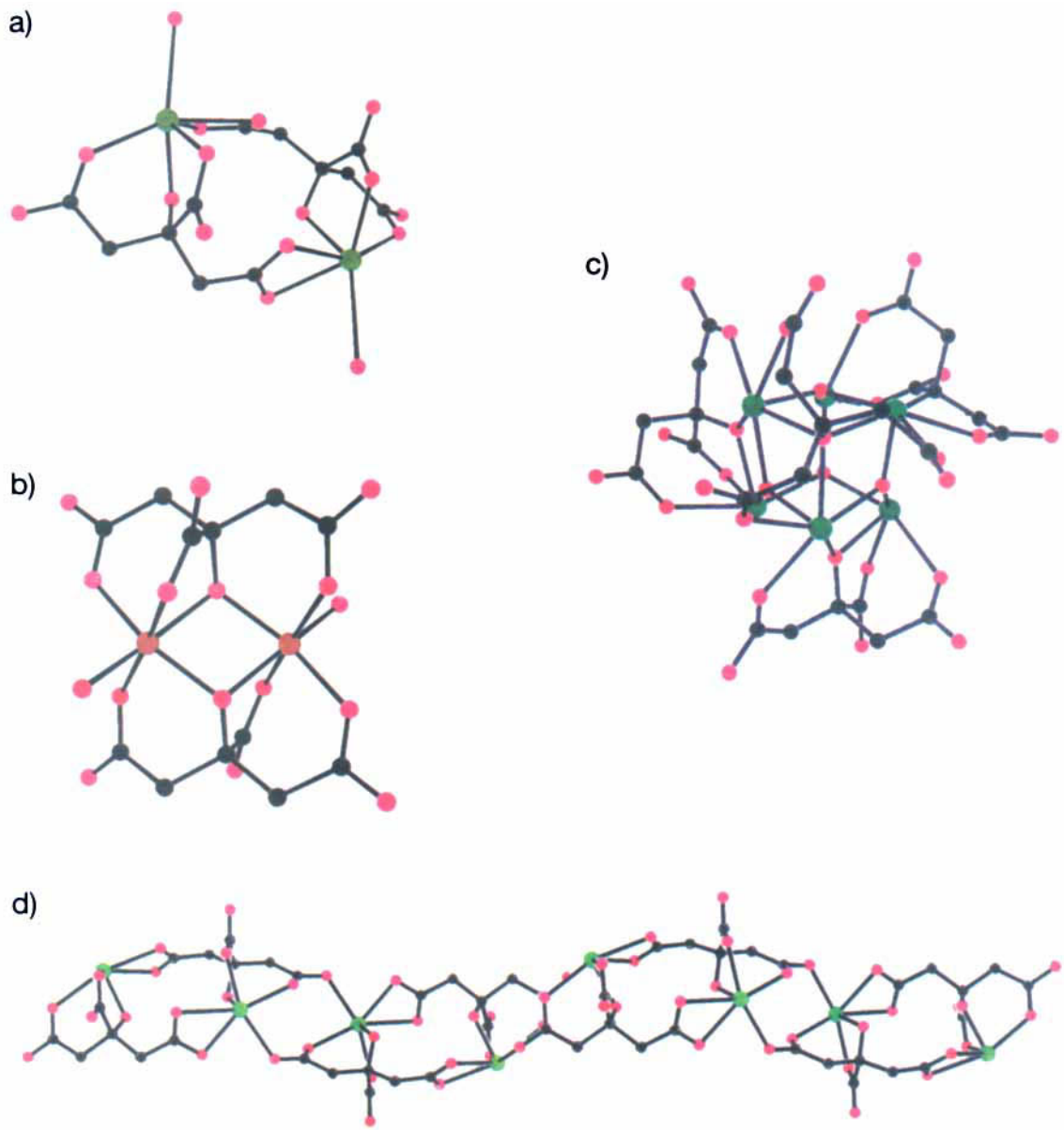


Figure 4. Structures of Bi(III) citrate complexes. (a) The basic bismuth citrate dimeric unit, the single O atom bonded to each Bi can be from H_2O or from a neighbouring carboxylate; (b) the Fe(III) citrate dimer $[Fe_2(cit)_2(H_2O)_2]^{2-}$[54] for comparison with (a); (c) hexanuclear cluster of $[Bi_6O_4(OH)_4(Hcit)_4]^{6-}$, (one of the citrate ligands was not detected)[49], and (d) polyanionic chain of $[\{Bi(cit)_2Bi\}_n]^{2n-}$. Color code: Bi: green; C: black; O: red; Fe: brown



which two Bi(III) ions are doubly bridged by citrate carboxylate oxygens as shown in Figure 4a. These dimeric units are assembled into infinite polymeric chains via double syn-anti carboxylate bridges (Figure 4d). Such polymers, formed into sheets, could be deposited on the ulcer crater to form a protective coating, or on the bacteria that can occur in ulcers. Cleavage of the double bridges between dimeric units by citrate or water can be envisaged to give singly bridged polyanionic species. In fact, various tiny crystalline species have been found in the ulcer craters of patients after treatment with colloidal bismuth subcitrate[53]. There is still no report of the structure of BSS (bismuth subsalicylate, empirical formula: $OC_6H_4COOBiO$).

It is possible to envisage dimeric bismuth citrate units inhibiting the uptake of Fe(III) into some types of bacteria, since Fe(III) also forms a dimeric citrate complex, $[Fe(cit)_2(H_2O)_2]^{2-}$ (Figure 4b)[54]. In this Fe(III) complex the double bridge is provided by alkoxide oxygens rather than carboxylates as is the case of Bi(III).

Recently some bismuth complexes with thiosemicarbazones and dithiocarbazonic acid methylester derivatives have been reported to show activity against *H. pylori*[55].

4 Bismuth Complexes with Biomolecules

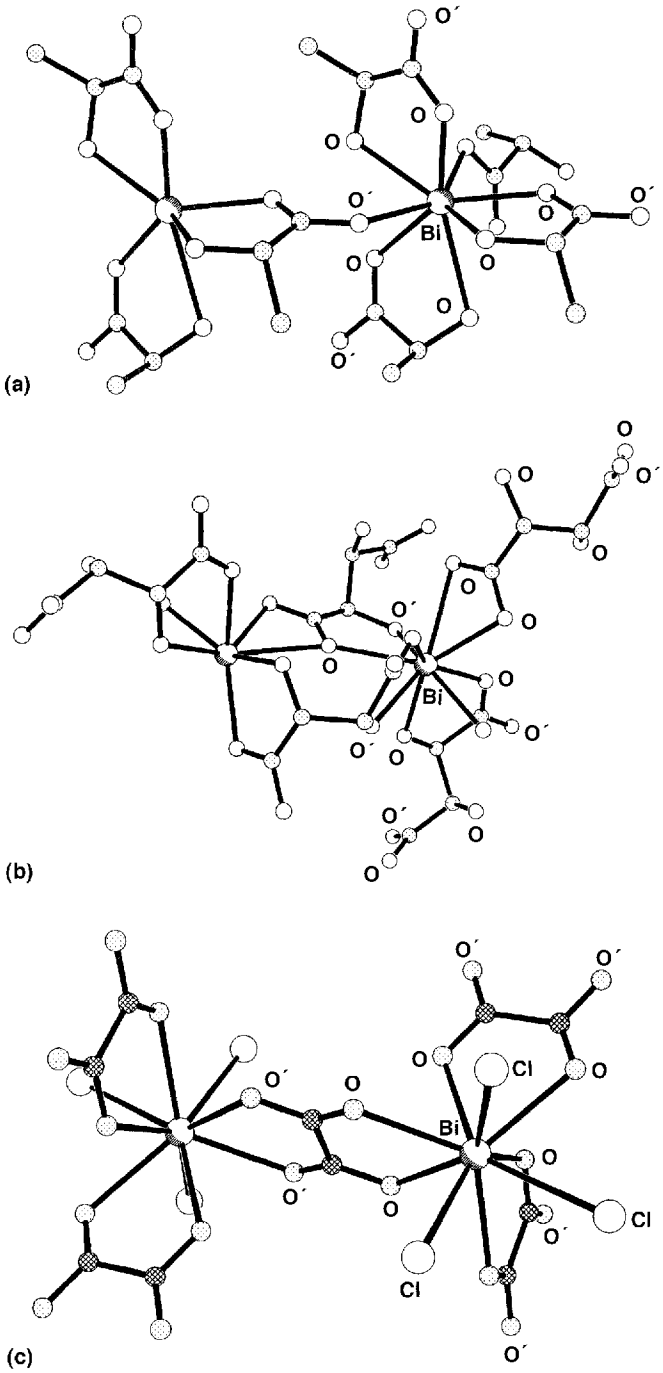
4.1 Bismuth Binding to Oxygen-Containing Molecules

Bi(III) is readily hydrolyzed, with $\log K_{OH} \approx 12.5$, and also readily forms multinuclear clusters even at pH 1.5[13]. The structures of some bismuth complexes with oxygen-containing biomolecules crystallized at acidic pH values have been solved by Herrmann and co-workers recently. These include D-lactate, L-(−)-malate, oxalate and L-(+)-tartrate complexes (Figure 5)[56]. In these complexes, bismuth exhibits a high coordination number (e.g. 8 and 9), and all the complexes are polymeric via carboxylate oxygen bridging. In the bismuth oxalate complex, the bismuth is also bridged by chloride ions. Five-membered chelate rings are preferentially formed in these complexes, probably due to the large size of Bi(III) (1.03 Å)[57]. It is not clear if these ligands are the target molecules of bismuth in biological systems.

4.2 Bismuth Complexes with Thiolate Ligands

As Bi(III) is a broadline or soft metal ion, it is expected to bind strongly to thiolate sulfur. The amino acid cysteine and tripeptide glutathione (γ-L-Glu-L-Cys-Gly, gsh) can prevent the precipitation of CBS at pH 2.0, and animal studies have shown that simultaneous oral administration of bismuth salts and thiolates produces a significant rise in the bismuth concentration in blood plasma[58,59]. IR spectroscopic studies suggest that Bi(III) coordinates only to the thiolate group of cysteine[60], while the X-ray crystal structure of the complex $\{[(SC(CH_3)_2CH(NH_2)CO_2]BiCl\}$ shows chelation of Bi(III) by sulfur, nitrogen and oxygen of tridentate D-penicillamine[61]. The interaction of bismuth with oxygen atoms of the carboxylate groups leads to a polymeric two-dimensional structure (Figure 6). Bismuth forms stable complexes with gsh and *N*-acetyl-L-cysteine (nac) with a stoichiometry $[Bi(H_{-1}gsh)_3]$ or $[Bi(H_{-1}nac)_3]$ (Scheme 5)[62], and the binding constants have been obtained from studies of competition reactions between edta and gsh (or nac) giving $\log K$ 29.6 and 31.4 (I = 0.1 M $NaNO_3$, 298 K) for $[Bi(H_{-1}gsh)_3]$ and $[Bi(H_{-1}nac)_3]$, respectively. Both NMR and EXAFS[52] suggest that the deprotonated thiolate group is the only strong binding site for Bi(III) in these ligands, and Bi(III) induces an unusually large chemical shift change of 1.4 ppm for the β-CH_2 Cys protons of gsh and nac. In spite of the extremely high thermodynamic stability of $[Bi(H_{-1}gsh)_3]$, bound gsh is kinetically labile and exchanges with free gsh slowly at pH 4 (3 s^{-1}, 298 K) (Figure 7) but faster at biological pH (ca. 1500 s^{-1}, 298 K). Bismuth passes through red cell membranes slowly ($t_{1/2} \approx 3$ h) and forms an intracellular complex with gsh, probably $[Bi(H_{-1}gsh)_3]$.

Figure 5. X-ray structures of (a) D-lactate complex ($Bi[CH_3CH(OH)CO_2]_3$); (b) malate complex ($Bi[O_2CCH_2CH(O)CO_2]H_2O$) and (c) oxalate complex ($Bi[O_2C-CO_2]_3$)[56]



4.3 Bismuth (III) Complexation with Proteins and Enzymes

Systematic studies of the binding of high molecular mass ligands to Bi(III) have not been reported[63,64]. In the stomach, bismuth has been shown to bind strongly to connective tissue proteins, mucus glycoproteins and enzymes[65], but little is known about the binding mode or kinetic behaviour. The most detailed studies on the proteins are described below.

Figure 6. X-ray crystal structure of the D-penicillamine complex $[Bi(S-C(CH_3)_2CH(NH_2)CO_2)Cl]$, showing cross-linking of molecules in the lattice via carboxylate oxygens[61]

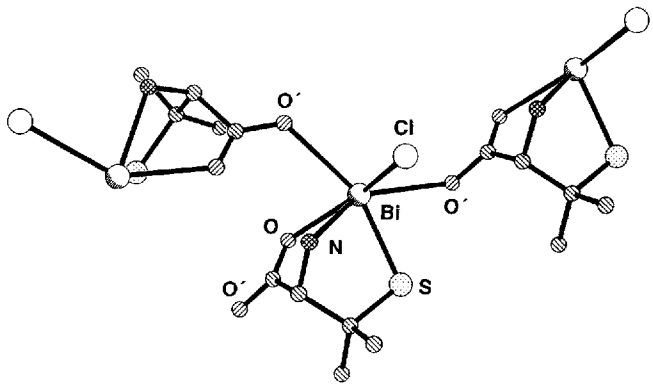
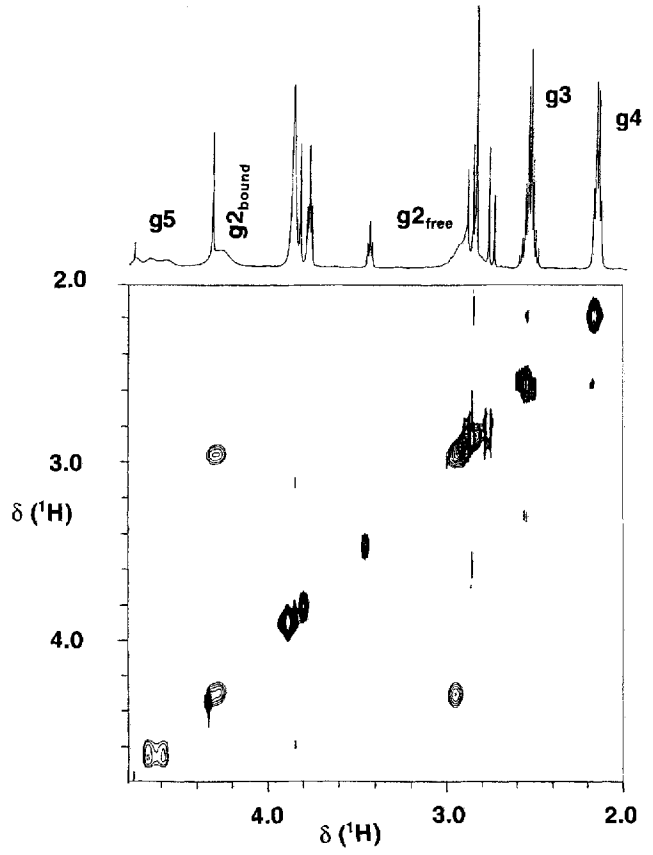
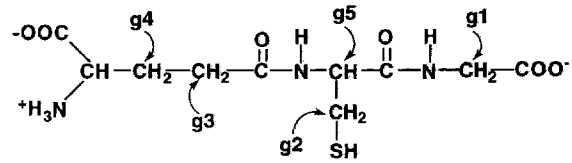


Figure 7. 500 MHz ^1H 2D-EXSY NMR spectrum showing cross-peaks for exchange between bound and free glutathione at pH* 4.0[62]. For peak labels see Scheme 5. The g2 cross-peak (β-CH_2) is clearly resolved; the difference in shifts of g5 between free and bound forms is much smaller (cross-peak close to diagonal)

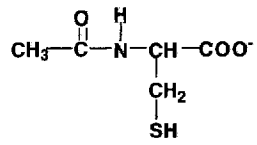


Metallothionein: Bismuth is known to be a potent inducer of renal metallothionein (MT) synthesis, and pretreatment with bismuth can protect from some of the toxic side effects induced by the anticancer drug cisplatin[66–68]. The protection probably involves Bi(III) induction of metallothionein synthesis. However, there appear to be no reported studies of the chemistry of Bi-metallothionein complexes. Strong binding of Bi(III) to metallothionein would

Scheme 5. Structures of glutathione and *N*-acetyl cysteine



Glutathione (gsh)



N-Acetyl-Cysteine (nac)

be expected, since 20 of its 61 amino acid residues are cysteines. The protein appears to be involved in the normal storage of up to seven Zn(II) ions, or ten Cu(I) ions or toxic ions such as Cd(II), Hg(II), and Au(I) from antiarthritic drugs[69]. An interaction has also been suggested between bismuth and a copper-binding protein different from metallothionein, as well as with the copper-containing blood plasma protein caeruloplasmin[70]. After administration of bismuth, a significant rise in both the copper content and the amount of MT-like proteins has been observed in the kidney. Simultaneous administration of selenium has been reported to inhibit the binding of bismuth to a Bi-binding protein in the kidney, which is similar to the effect of Se on the binding of mercury to a Hg-binding protein[71,72].

Albumin: Feldman et al. have suggested that the main binding target for Bi(III) in blood plasma is albumin, since it contains a free thiolate group at Cys34 (with $pK_a \approx 5$)[63]. But their data did not support this conclusion, since only about 2% of albumin molecules bound to Bi(III). Binding of antiarthritic gold(I) complexes to Cys34 and redox reactions with disulfides with Cys34 can be readily detected by ^1H NMR[73,74]. Our preliminary NMR data[75] suggest that Bi(III) probably does not bind to albumin at Cys34. Similar behaviour has also been found for Cd(II) (also a soft metal ion), which appears to bind at oxygen- and nitrogen-containing sites rather than Cys34[76]. Cys34 seems to be protected in a cleft in the protein and the factors which control cleft-opening are not yet understood. Proteins other than albumin may bind to Bi(III) in blood plasma and investigations of the chemical forms of bismuth present in blood plasma are urgently needed[77].

Transferrin: Transferrin is an 80-KDa glycoprotein that transports Fe(III) in blood, and is recognized by cell-surface receptors (proteins) when fully loaded with Fe(III) in both its binding sites. The diferric protein is internalized by cells, placed in vesicles (endosomes) where the pH is lowered to 5.5 and the Fe(III) is released[78]. In human blood, transferrin is only ca. 30% saturated with Fe(III), and hence there is capacity for binding to other metal ions that enter the human body. Bi(III) (1.03 Å) binds strongly to both N- and C-lobe iron binding sites of human serum transferrin

(Scheme 6)[79]. The uptake of Bi(III) by apo-transferrin from bismuth citrate complexes is very slow (hours at 310 K) and occurs in at least two steps, whereas that from bismuth nitrilotriacetate is rapid (minutes). Both UV and NMR data suggest that bismuth binds to transferrin along with bicarbonate (HCO_3^-) as synergistic anion, which is similar to Fe(III), and Bi(III) binds preferentially to the C-lobe, followed by the N-lobe. Although Bi(III) binds to transferrin relatively strongly ($\log K_1$ 19.42 and $\log K_2$ 18.58, respectively, at 310 K, 5 mM bicarbonate, pH 7.4), it can be displaced by Fe(III). Competition reactions between transferrin and citrate indicate that even in the presence of 100-fold excess of citrate, Bi(III) can still bind to transferrin. The strong binding of Bi(III) to transferrin can be correlated with its high acidity, Figure 8. The correlation suggests that the two tyrosine ligands play a dominant role in the strength of binding[80].

Scheme 6. Iron binding sites in human serum transferrin

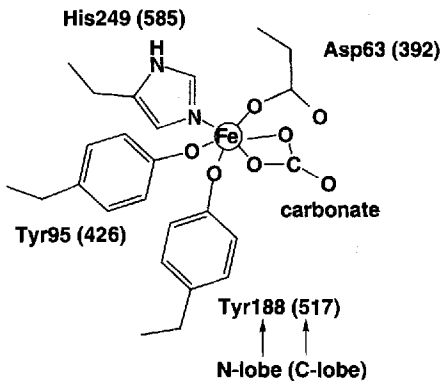
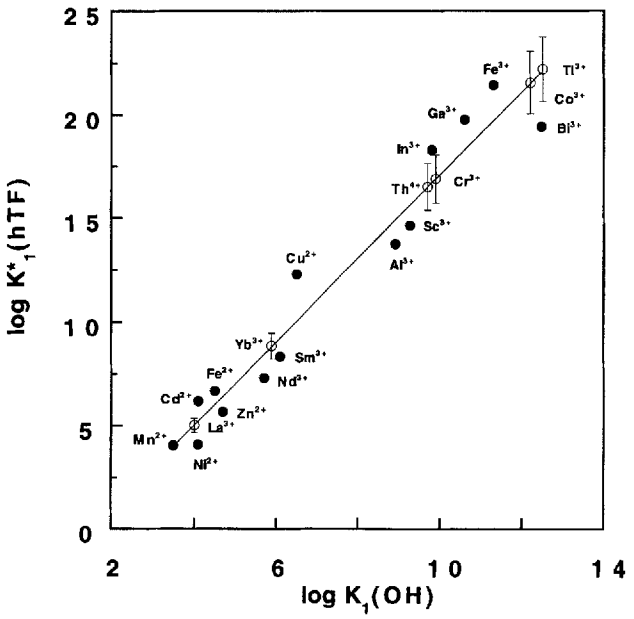


Figure 8. Correlation of the strength of metal binding to human serum transferrin with metal ion acidity ($pK_a = 14 - \log K_{OH}$)[80]. Solid circles refer to experimental values, open circles are predicted values (error bars indicate likely range of uncertainty)

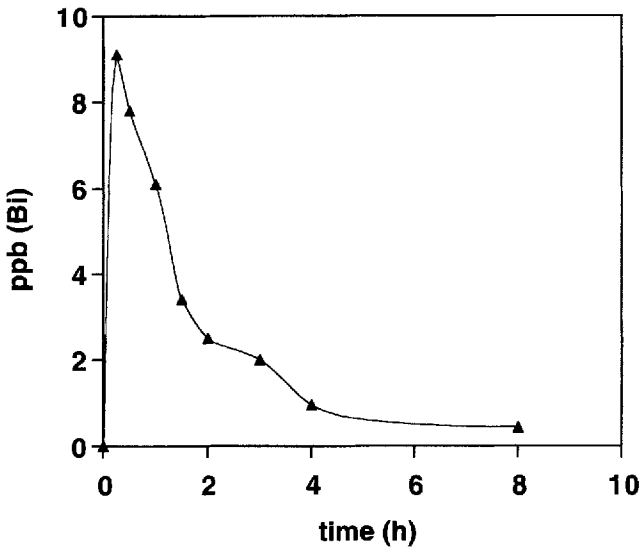


5 Pharmacology and Toxicity of Bismuth Compounds

5.1 Absorption of Bismuth into the Blood

Bismuth may act both locally within the stomach and systematically as a result of gastronintestinal absorption. After oral intake of single doses of bismuth drugs such as CBS and BSS (bismuth subsalicylate), a protective coating (probably BiOCl and Bi citrate complexes) is thought to form on the ulcer crater, and there is also a significant increase in bismuth levels in the mucosa with a peak level of 30–60 μl (0.14–0.28 μM). Concentrations of bismuth in the blood increase by 51–1483-fold. The maximum level occurs unexpectedly rapidly (15–60 min) as shown in Figure 9[81]. Intake of CBS swallowable tablets gave peak concentrations of Bi in blood plasma ranging from 25–300 μg/l (0.12–1.4 μM) after a mean time of 0.5 h[82]. Multiple-dosing with CBS shows that an apparent steady state is reached after 3–4 weeks. Despite the extremely high solubility of ranitidine bismuth citrate in water, a study in human volunteers has shown that absorption of bismuth in the blood is significantly lower than that observed after dosage of De-Nol (colloidal bismuth subcitrate)[83]. The geometric mean maximum level of Bi in plasma of 12 ng/g after a ten-day course of RBC (1 g/kg body) can be compared with 21 ng/g attained after a lower dosage of De-Nol (0.24 g/kg body). Radiobismuth studies using ^{206}Bi citrate suggest that Bi binds readily to serum components (83% after 1 h at biological pH), and that this binding is strong and not readily reversed by dialysis against phosphate buffer[84]. A gel filtration study of human blood after incubation with bismuth subgallate showed an association of bismuth with high molecular mass ligands[85].

Figure 9. Bismuth levels in human blood after intake of one CBS tablet (108 mg Bi)[81]



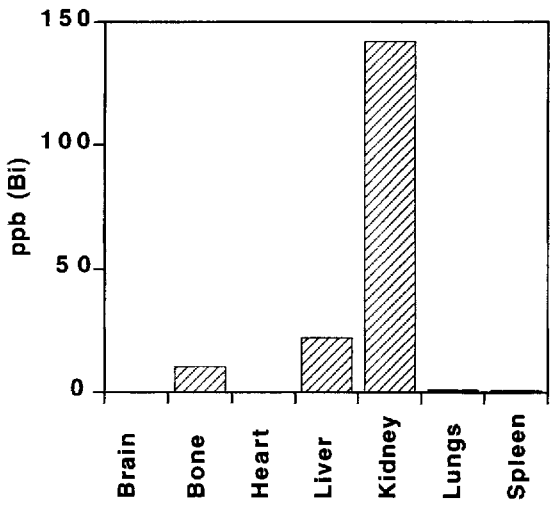
5.2 Distribution of Bismuth in Tissues

In humans and animals ca. 70–90% of bismuth is excreted into the urine after dosing with bismuth complexes. The rapid increase of bismuth concentration in the blood

of humans and animals after intake of CBS suggests gastric absorption as well. Although there are large variations in human and animal studies, the data on biodistribution of bismuth in tissues are similar; the organ with the highest content is always found to be the kidney. Dense intranuclear inclusion bodies, which probably contain bismuth–protein complexes, have been found in proximal renal tubular lining cells[86]. The retention time of bismuth in the kidney is always longer than that of any other organ. After 14 months of administration of CBS to rats, reported concentrations of bismuth range from 13.9 μg/l wet weight in the kidney, up to 0.3 μg/l in muscle. In terms of bismuth concentration, the order of bismuth levels in organs ranges from (high to low): kidney > liver > bone > lung > spleen > brain > heart (Figure 10)[87]. However, this order may be influenced by the physicochemical form of the administered bismuth complex. After oral intake of trimethylbismuth, the bismuth concentration in the liver has been found to be higher than that in the kidney, probably due to the organic character of this molecule[88].

The concentration of bismuth in brain tissue after administration of antiulcer drugs has been reported to be higher than in controls[89,90]. For patients who have died of bismuth encephalopathy, the concentration of bismuth in the grey matter was found to be about twice as high as that in white matter, with the highest concentration probably in the thalamus and cerebellar cortex[91].

Figure 10. Bismuth tissue distribution in rats after injection of CBS (630 μg/kg body mass) twice a week for 70 days[87]



5.3 Human Toxicity

Many toxic effects in humans have been attributed to bismuth complexes: encephalopathy, nephropathy, osteoarthropathy, gingivitis, stomatitis, colitis and hepatitis. Different adverse effects on the various organ systems have been associated with different bismuth complexes, as shown in Table 6. It seems that CBS and BSS are less toxic compared to previously-used complexes. Signs and symptoms of bismuth encephalopathy have been described in detail by Slikkerveer and De Wolff[31], mainly from reported cases in France and Australia. The diagnosis is generally confirmed by the detection of bismuth in e.g. blood, plasma, and serum. In patients with encephalopathy, the bismuth levels in blood usually exceed 100 μg/l (0.48 μM); most of these patients have blood levels of >500 μg/l (2.39 μM) at the time of presentation, but there is no clear correlation between clinical illness and bismuth concentration in the patient's blood. Thus, the interpretation of "Hillemand safety levels"[92] (50–100 μg/l bismuth) as a warning sign of toxicity is not reliable.

Table 6. Toxicity of bismuth complexes in humans (data from reference[77])

Tissue	Toxicity	Bismuth complexes used for treatment
Kidney	Acute reversible renal failure	Triglycollamate, thioglycollanate, diallylacetate, CBS[a]
Liver	Acute hepatitis	Thioglycollate
Bones	Osteoporosis Osteomalacia Fracture	Subnitrate Subcarbonate Subgallate
Skin	Erythroderma	Tartrate, subsalicylate
Neuropsychiatric organs	Chronic "encephalopathy"	Subnitrate, subgallate, subsalicylate, CBS[b]

[a] Prolonged high dose/overdose. – [b] Prolonged high dose, renal impairment.

6 Conclusion

Despite the widespread use of bismuth compounds in medicine for over two centuries, its chemistry and biochemistry are currently poorly understood. Recent work has begun to elucidate the structures of Bi(III) thiolates, carboxylates and aminocarboxylate complexes in particular. The occurrence of a highly variable coordination number (3–9) and coordination geometry, together with the apparent activation of a strong lone-pair effect in certain complexes (e.g. those with alkoxide ligands) is highly characteristic of Bi(III). So too is the strong acidity of Bi(III) aqua complexes. Complexes crystallized from aqueous solution commonly contain bridging carboxylate ligands and so the chemistry of Bi(III) citrate antiulcer drugs appears to be dominated by polymeric species. The interaction of these polymers with membrane surfaces may be important to their bioactivity. The rates of ligand exchange on Bi(III) are highly variable and pH-dependent.

Little is known about the interaction of Bi(III) with proteins or enzymes, although this could be very important to the biological activity. It appears that Bi(III) can bind strongly to both Fe(III) sites (e.g. N and O ligands in transferrin) and Zn(II) sites (e.g. S ligands in metallothionein). Glutathione forms a strong complex $[Bi(H_{-1}gsh)_3]$, which may be involved in transport of Bi(III) in cells. Further exploration of the chemistry and biochemistry of bismuth is now warranted particularly in view of current interest in the introduction of new Bi(III) antiulcer drugs, in the use of ^{212}Bi in radiotherapy, and the discovery of anticancer and anti HIV activity. Such studies may lead to the design of new bioactive compounds and to a better understanding of their mechanism of action.

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