7

Transition Metals and d-Block Metal Chemistry

7.1 What are d-block metals?

The elements in groups 3–12 as shown in the schematic periodic table below are defined as the so-called d-block metals. The term *transition metal* is also often used to describe this group of elements. However, the IUPAC (International Union of Pure and Applied Chemistry) defines transition metals as elements with an incomplete d subshell or elements that can form a cation with an incomplete d subshell. Therefore, the group 12 metals zinc (Zn), cadmium (Cd) and mercury (Hg) are not typically classified as transition metals (Figure 7.1) [1].

Elements in the f-block, the so-called lanthanides and actinides, have been in the past called *inner transition metals*. Nowadays, they are more often referred to as *f-block elements* and selected examples will be discussed in Chapter 11.

Each group of d-block metals is formed by three members and is therefore called a *triad*. Sometimes, elements are grouped according to their chemical behaviour. One example is the group of platinum group metals, which encompasses ruthenium (Ru), osmium (Os), palladium (Pd) and platinum (Pt). Sometimes, you can find the term *heavier d-block metals*, which refers to d-block metals of the second and third row.

7.1.1 Electronic configurations

Generally, the ground-state electronic configurations of the first, second and third-row d-block metals follows the progressive filling of the 3d, 4d and 5d atomic orbitals, respectively. Nevertheless, there are exceptions, such as the ground state of chromium, which is $[Ar]4s^1 3d^5$ rather than $[Ar]4s^2 3d^4$. The reasons are fairly complicated and will not be further discussed in this book (Table 7.1).

d-Block metals can show several oxidation states as their valence electrons can be present in more than one atomic orbital. M^{2+} and M^{3+} ions of the first-row d-block metals follow the general formula $[Ar]3d^n$. The electronic configurations for second- and third-row d-block metals are again more complicated and will not be further discussed in this book.

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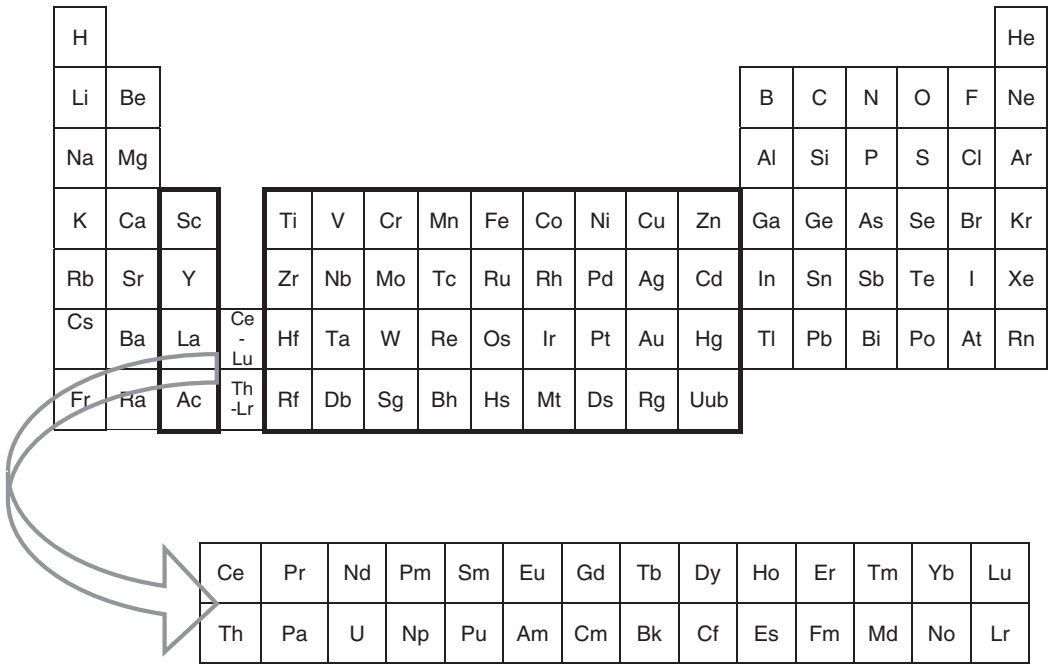


Figure 7.1 *Periodic table of elements; d-block elements are highlighted*

Table 7.1 *Examples of ground-state electronic configurations*

Sc	Ti	V	Cr	Mn	Fe	Co	Ni	Cu	Zn
d^1s^2	d^2s^2	d^3s^2	d^5s^1	d^5s^2	d^6s^2	d^7s^2	d^8s^2	$d^{10}s^1$	$d^{10}s^2$

7.1.2 Characteristic properties

Nearly all d-block metals are hard, malleable and ductile, and conduct electricity and heat. Most of them will form one of the typical metal structures. Exceptions are manganese (Mn), Zn, Cd and Hg. Only three d-block elements, namely iron (Fe), cobalt (Co) and nickel (Ni), are known to produce a magnetic field.

d-Block metals tend to readily form complexes with a characteristic colour if their ground-state electronic configuration is different from d^0 or d^{10}. Complex formation is often characterised by a colour change. For example, $[CoF_6]^{3-}$ is green, whereas $[Co(NH_3)H_2O]^{3+}$ is red and $[Co(H_2O)_6]^{3+}$ is blue.

> ***Coordination complexes*** *are defined as chemical structures that consist of a central atom or metal ion, and the surrounding molecules or anions called the ligands.*

Paramagnetism is a phenomenon that is often observed for d-block metal compounds. This is a result of the presence of unpaired electrons and can be investigated using electron paramagnetic resonance (EPR) spectroscopy. As a result, abnormalities in the NMR spectra can be observed such as broadening of the signals or unusual chemical shifts.

Paramagnetism *is defined as the phenomenon whereby some materials show magnetic properties only once they are exposed to a magnetic field. Outside this magnetic field, no magnetic properties are seen. This is in contrast to ferromagnets, which show magnetic properties independent of the environment.*

7.1.3 Coordination numbers and geometries

d-Block metal compounds readily form complexes displaying different coordination numbers and geometries. In this section, we will restrict our discussion to complexes with only one metal centre (mononuclear complex). Also, it is important to note that the discussed geometries are regular geometries, which in practice can often be distorted as a result of steric hindrances. Additionally, in reality, fluxional behaviour in solution can be observed if the energy difference between the different structures is small enough, but we will restrict the following discussion to the solid state of complexes.

In general, steric and electronic factors dictate the coordination number. Sterically demanding ligands are more likely to form complexes with a low coordination number. In contrast, complexes containing small ligands and a large metal centre favour high coordination numbers (Figure 7.2).

The *Kepert model* is typically used to describe the shape of d-block metal complexes. The metal is defined to be the centre of the complex, and the ligands are arranged freely on a sphere around the centre. Only ligands are taken into consideration when determining the geometry of the complex. This is in contrast to the valence shell electron pair repulsion (VSEPR) model, which is used to determine the structure of p-block element compounds, where also nonbonding electrons are considered.

The **η*-nomenclature for ligands*** *is used in organometallic chemistry and describes the number of atoms in a ligand that directly interact with the metal. The prefix* η *(eta) is accompanied by a number, which equals the number of atoms coordinating to the metal. This is called* ***hapticity*** *of a ligand.*

7.1.3.1 Coordination number 2: linear

A coordination number of 2 can be typically found for the metals Cu(I),[1] Ag(I), Au(I) and Hg(II). The metal forms the centre of the complex, and the two ligands are arranged at 180° to each other (Figure 7.3).

7.1.3.2 Coordination number 3: trigonal planar or trigonal pyramidal (less common arrangement)

In general, trifold coordination of a metal centre is not very common. There are examples of metals with a full d-orbital (d^{10} metals), which form trigonal planar structures. This means three ligands are arranged around the metal centre in one plane with 120° angle to each other. Examples include complexes of Cu(I), for example, in $[Cu(CN)_3]^{2-}$ and Ag(I), Au(I), Hg(II) and Pt(0) in $[Pt(PPh_3)_3]$ (Figure 7.4).

7.1.3.3 Coordination number 4: tetrahedral or square planar

The coordination number 4 is extremely common for d-block metal complexes. Most frequently, a tetrahedral arrangement can be observed. Examples include $[MnO_4]^{2-}$, $[FeCl_4]^-$ and $[CrO_4]^{2-}$ (Figure 7.5).

The square planar arrangement in which all four ligands are arranged around the metal centre in one plane is less commonly observed and often connected to d^8 metals. Nevertheless, some of these complexes are very important as a result of their medical application. For example, the square planar complex $[PtCl_4]^{2-}$

[1] Oxidation states for d-block elements are often denoted in Roman numbers.

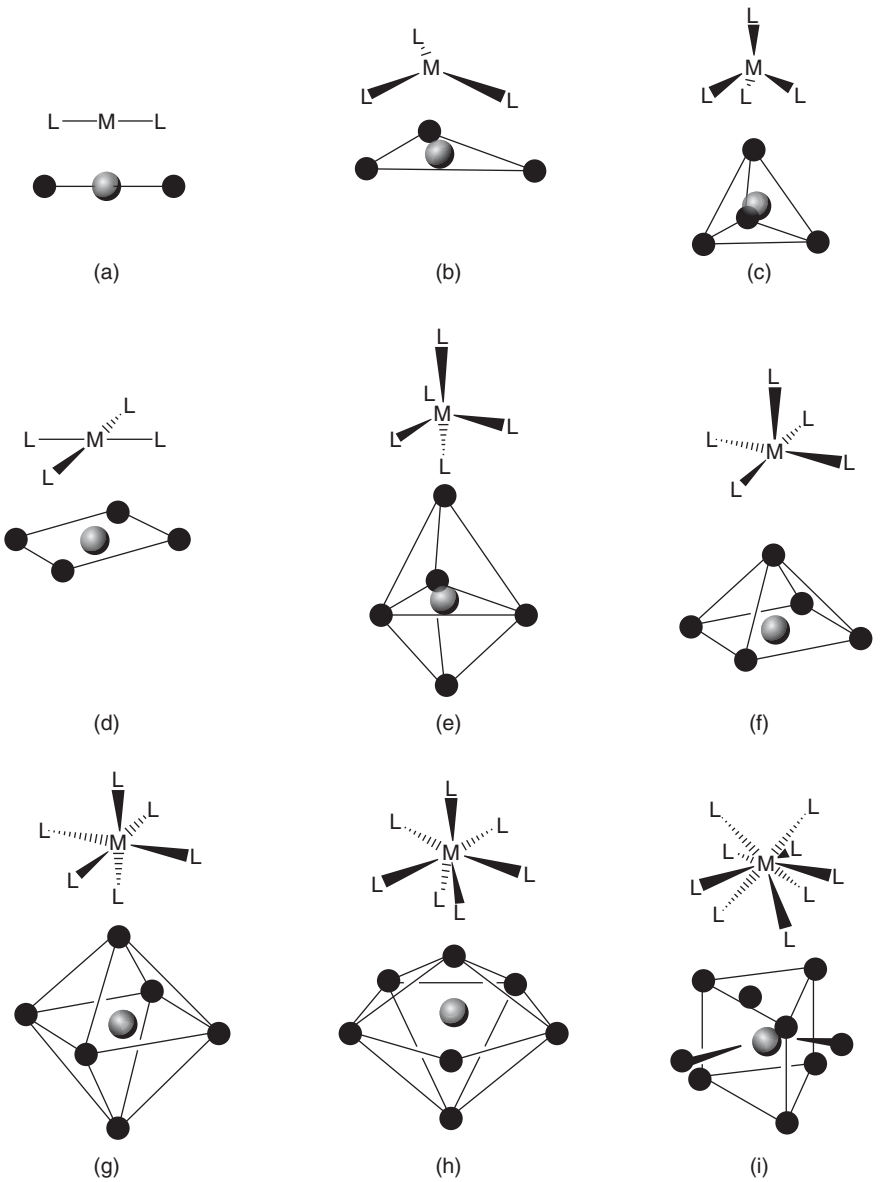


Figure 7.2 *Common geometries of metal complexes [2]. (a) Linear, (b) trigonal, (c) tetrahedran, (d) square planar, (e) trigonal bipyramid, (f) square pyramid, (g) octahedron, (h) pentagonal bipyramid and (i) tricapped trigonal prism (Reproduced with permission from [2]. Copyright © 2009, John Wiley & Sons, Ltd.)*

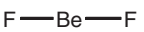


Figure 7.3 *Example of a linear complex (BeF_2 in the gas phase)*

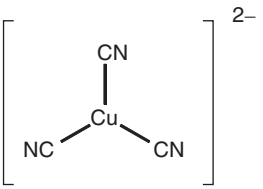


Figure 7.4 *Example of a trigonal planar complex*

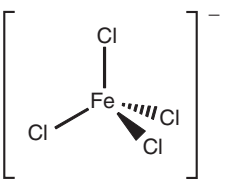


Figure 7.5 *Example of a tetrahedral complex*

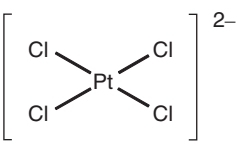


Figure 7.6 *Example of a square planar complex*

is commonly used as a precursor to the known chemotherapeutic agent cisplatin, whereas square planar complexes $[PdCl_4]^{2-}$, $[AuCl_4]^-$ and $[RhCl(PPh_3)_3]$ are all under investigation for their use in medicine (Figure 7.6).

7.1.3.4 Coordination number 5: trigonal bipyramidal or square-based pyramidal

The energy difference between the trigonal bipyramidal structure and the square-based pyramidal structure is usually fairly small and therefore many structures lie in reality between those two. Examples for simple trigonal bipyramidal structures include $[CdCl_5]^{3-}$ and $[CuCl_5]^{3-}$, whereas $[WCl(O)]^-$ and $[TcCl_4(N)]^-$ form square-based pyramidal structures typical for a series of oxo and nitrido complexes (Figure 7.7).

7.1.3.5 Coordination number 6: octahedral or trigonal prismatic (less common geometry)

Octahedral geometry is most commonly observed for the coordination number 6. Metals of all kinds of electronic configurations form octahedral complexes, for example, $[Mn(OH_2)_6]^{3+}$, $[V(OH_2)_6]^{3+}$, $[Fe(CN)_6]^{3-}$ and $[Fe(OH_2)_6]^{2+}$ (Figure 7.8).

It was long believed the octahedral geometry is the only geometry for coordination number 6 in existence, but eventually examples of trigonal prismatic coordination have been confirmed by X-ray analysis. Examples are $[ZrMe_6]^{2-}$ and $[ReMe_6]$ (Figure 7.9).

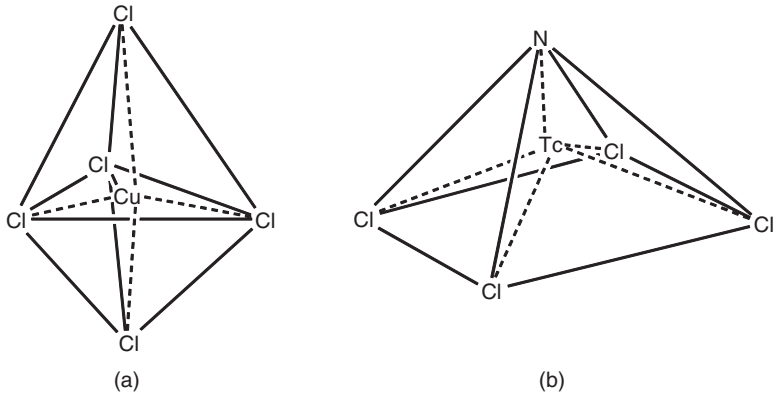


Figure 7.7 *Examples of a (a) trigonal bipyramidal and a (b) square-based pyramidal complex*

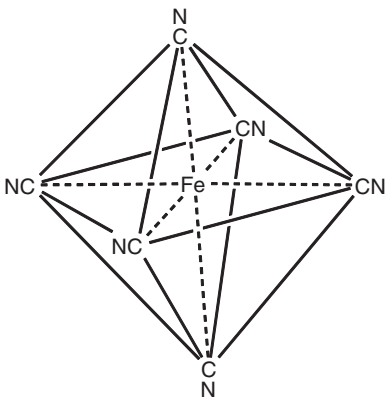


Figure 7.8 *Example of an octahedral complex*

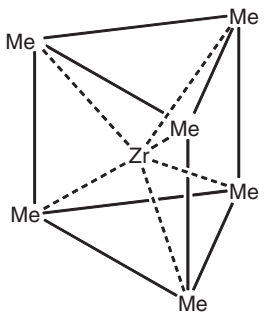


Figure 7.9 *Example of a trigonal prismatic complex*

7.1.3.6 Coordination number 7 and higher

Coordination numbers of 7 and higher are most commonly observed for d-block metals of the second and third row. Geometries can become fairly complicated and are not further discussed here.

7.1.4 Crystal field theory

Many transition-metal complexes are coloured. This and other spectroscopic properties, such as magnetism and hydration enthalpies, can be explained with the so-called crystal field theory (CFT).

The CFT describes the degeneration of the d- and f-orbitals in transition-metal complexes. It does not attempt to describe any type of chemical bonds.

CFT is based on the interaction of a positively charged cation and the nonbinding (negatively charged) electrons of the ligand. The general principle is that the five d orbitals are degenerated, meaning that they do not occupy the same energy level anymore. Once the ligands approach the central positively charged cation, the electrons of the ligands will become closer to some of the d orbitals of the metal. This results in the degeneration of the d orbitals. Electrons in d orbitals that are closer to the ligands will occupy a higher energy level as the negatively charged electrons will repel each other (Figure 7.10).

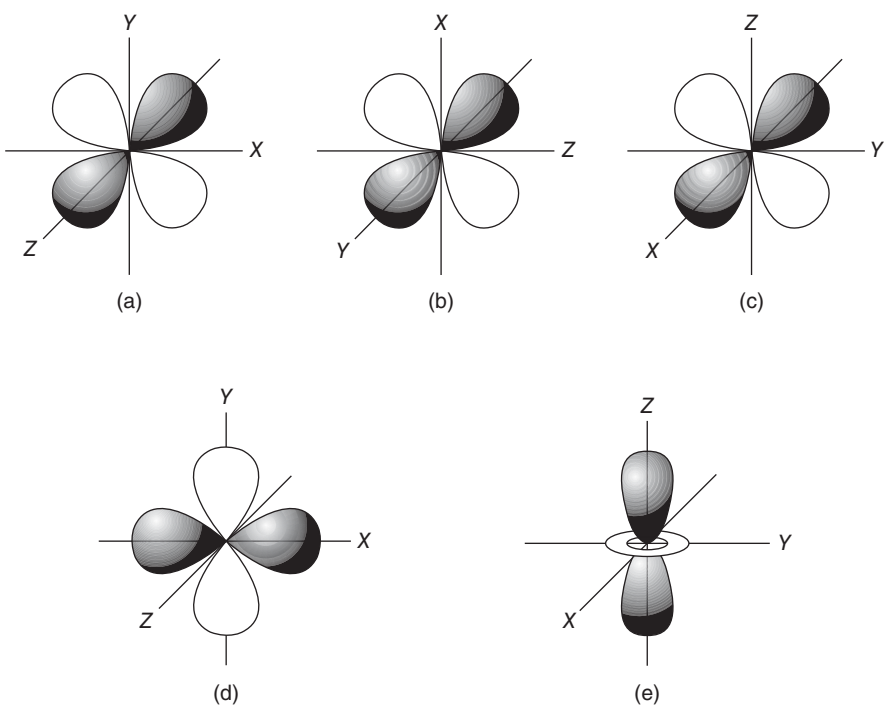


Figure 7.10 *Geometry of d orbitals: (a) d_{xy}, (b) d_{xz}, (c) d_{yz}, (d) $d_{x^2-y^2}$ and (e) d_{z^2} [2] (Reproduced with permission from [2]. Copyright © 2009, John Wiley & Sons, Ltd.)*

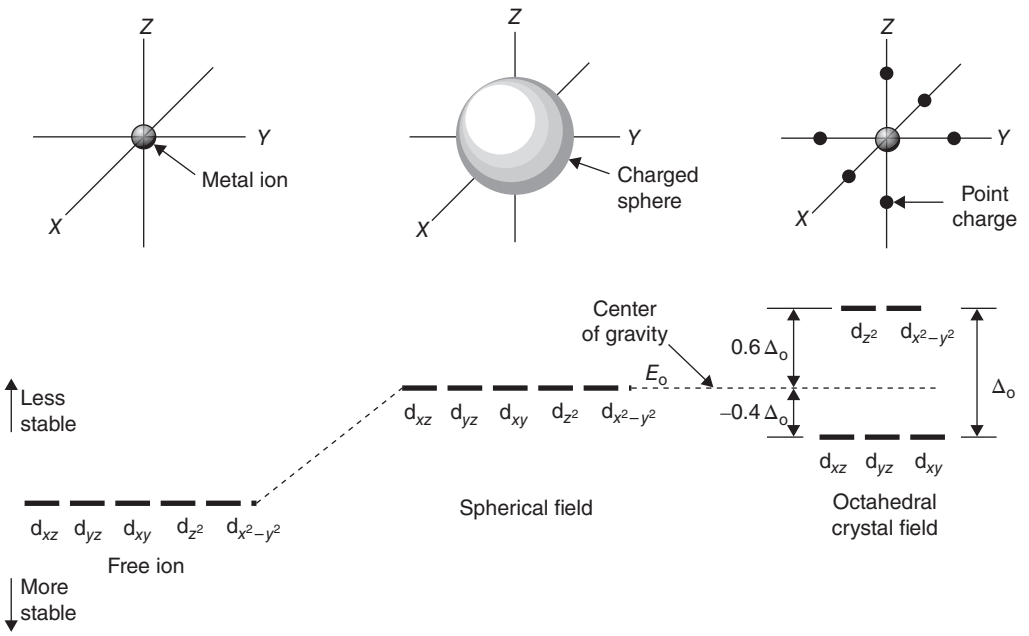


Figure 7.11 *Generation of the octahedral crystal field from the free ion [2] (Reproduced with permission from [2]. Copyright © 2009, John Wiley & Sons, Ltd.)*

The most common type of transition-metal complexes is octahedral complexes, where the central metal is coordinated by six ligands. The five d orbitals are split into two sets with the same energy level, the energy difference is called Δ_{oct}. The orbitals d_{xy}, d_{xz} and d_{yz} occupy the lower energy level as they are further away from the ligands. Ligands can be seen as approaching the central metal along the axes, where the orbitals d_{xy}, d_{xz} and d_{yz}, referred to as t_{2g}, are located in between the axes. In contrast, the two remaining d orbitals $d_{x^2-y^2}$ and d_{z^2}, referred to as e_g, are positioned along the axes (from where the ligands approach) and therefore occupy a higher energy level within an octahedral complex.

Figure 7.11 shows how energy levels of d orbitals change, ranging from the energy of d orbitals of the free metal ion, followed by the energy of d orbitals of the complex in a spherical field and the split energy levels observed in an octahedral field.

There are several factors influencing this splitting of the d orbitals:

- The metal ion itself.
- The oxidation state of the metal ion: The energy difference Δ increases with increasing oxidation state for any given metal.
- The nature of the ligand: Some ligands encourage a large value for Δ, whilst the formation of complexes with other ligands results in a small splitting of t_{2g} and e_g orbital sets. The *spectrochemical series* is a list that shows ligands in the order in which they produce a splitting, ranging from small to large Δ:

$$I^- < Br^- < S^{2-} < SCN^- < Cl^- < NO_3^- < N_3^- < F^- < OH^- < C_2O_4^{2-} < NH_3 < NO_2^- < PPh_3 < CN^- < CO$$

- The geometry of the transition-metal complex: The arrangement of energy level changes and is dependent on the geometry of the complex.

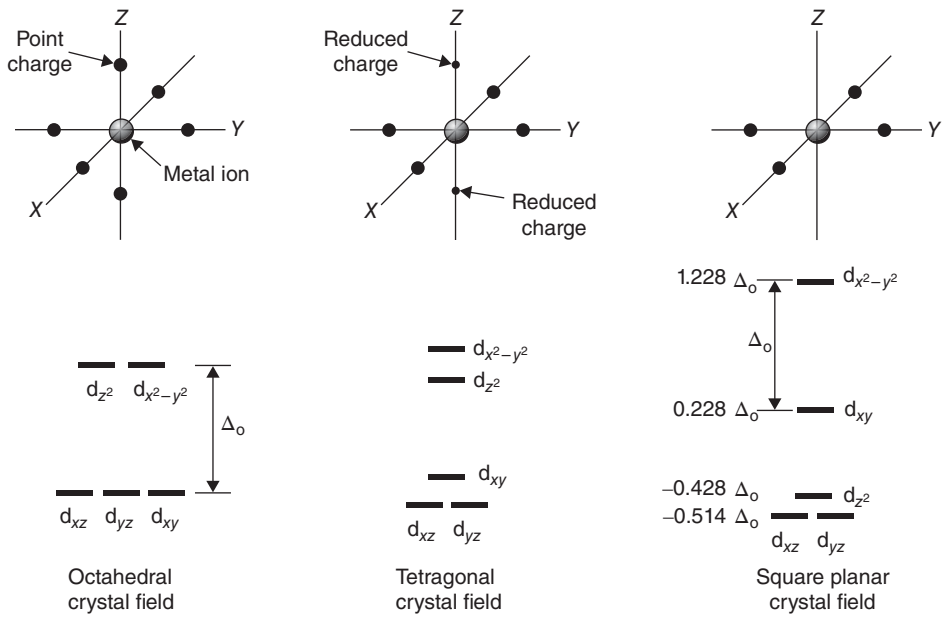


Figure 7.12 *Octahedral, tetragonal and square planar crystal fields [2] (Reproduced with permission from [2]. Copyright © 2009, John Wiley & Sons, Ltd.)*

Figure 7.11 shows the energy diagram for octahedral complexes. In Figure 7.12, the CFT splitting diagrams for other geometries, including tetrahedral, square planar and trigonal bipyramidal complexes are shown.

It is interesting to observe what happens when these orbitals are filled with electrons. The lowest energy levels will be filled first, and Hund's rule states that orbitals are filled with one electron first, before the electrons are paired. The *Pauli Exclusion Principle* states that two electrons in the same orbital are not allowed to have the same spin; nevertheless, it consumes energy to change the spin of an electron. Looking at octahedral complexes and the energy scheme shown in Figure 7.11, filling the orbitals with one, two or three electrons is straightforward. Each of the t_{2g} orbitals will be filled with one electron. The addition of the next electron leads now to two possibilities. Electron number 4 can either be added to a vacant e_g orbital, which means the energy Δ_{Oct} is needed to promote it to this level. These types of complexes are called *high-spin complexes*. Alternatively, the fourth electron can be added to one of the already occupied orbitals of t_{2g} once the electron spin has changed. These complexes are called as *low-spin complexes*. Depending on the size of Δ_{Oct}, different types of complex are formed. If Δ_{Oct} is very large, it consumes less energy to fill a t_{2g} orbital with a second electron; vice versa, if Δ_{Oct} is relatively small, it is energetically favourable to promote the fourth electron to the e_g level.

Examples of a low- and high-spin complex with four electrons in the d orbitals are shown in Figure 7.13. Both complexes have the same metal ion in the same oxidation state as the centre, and Δ_{Oct} depends only on the ligands. The same discussion follows for the addition of a fifth electron.

As mentioned at the beginning of this chapter, CFT can be used to explain the colour of these often brightly coloured transition metals. When a molecule absorbs a photon, one or more electrons are temporarily promoted within the set of split d orbitals from the lower to the higher energy level. This leads to a complex in an excited state, and the energy difference to the ground state equals the energy of the absorbed photon. The latter energy is inversely related to the wavelength of the light absorbed. Therefore the transition-metal complex can be seen in the complementary colour.

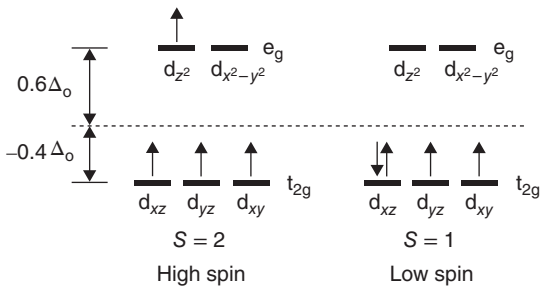


Figure 7.13 *High- and low-spin possibilities for d^4 in an octahedral crystal field [2] (Reproduced with permission from [2]. Copyright © 2009, John Wiley & Sons, Ltd.)*

7.2 Group 10: platinum anticancer agents

Group 10 of the periodic table of elements consists of the nonradioactive members nickel (Ni), palladium (Pd) and platinum (Pt) as well as the radioactive element darmstadtium (Ds) (Figure 7.14).

The noble metals palladium and platinum are resistant to corrosion and can be attacked by O_2, F_2 and Cl_2 only at very high temperatures. Palladium dissolves in hot oxidising acids, whereas platinum dissolves in only 'aqua regia' (1 : 3 mixture of HNO_3 and HCl).

Palladium is used as a hydrogenation catalyst, for $H_2/D_2/T_2$ separation and purification as well as a catalyst in the Wacker process. The Wacker process, which facilitates the oxidation of ethylene to acetaldehyde, was the first organopalladium reaction that was applied on an industrial scale. Platinum is also intensively used as a catalyst, for example, in HNO_3 production, as oxidation catalysts, in petroleum reforming, in hydrogenations and in many more chemical processes, as well as for jewellery.

All three nonradioactive elements of group 10 show a high diversity in their electronic configuration (Figure 7.15).

Most stable oxidation states are +II for all three nonradioactive elements, whereas Pt(II) and Pt(IV) are not only stable but also kinetically inert. The bromide and iodide salts of Pt(II) and Pd(II) are insoluble. Pt(II) has

H																	He
Li	Be											B	C	N	O	F	Ne
Na	Mg											Al	Si	P	S	Cl	Ar
K	Ca	Sc		Ti	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	Ga	Ge	As	Se	Br	Kr
Rb	Sr	Y		Zr	Nb	Mo	Tc	Ru	Rh	Pd	Ag	Cd	In	Sn	Sb	Te	I	Xe
Cs	Ba	La	Ce - Lu	Hf	Ta	W	Re	Os	Ir	Pt	Au	Hg	Tl	Pb	Bi	Po	At	Rn
Fr	Ra	Ac	Th -Lr	Rf	Db	Sg	Bh	Hs	Mt	Ds	Rg	Uub						

Figure 7.14 *Periodic table of elements; group 10 elements are highlighted*

Ni : $[Ar]4s^2 3d^8$

Pd : $[Kr]4d^{10}$

Pt : $[Xe]4f^{14} 5d^9 6s^1$

Figure 7.15 *Electronic configuration of group 10 metals*

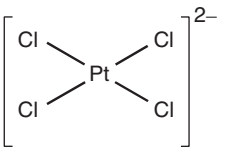


Figure 7.16 *Structure of $[PtCl_4]^{2-}$*

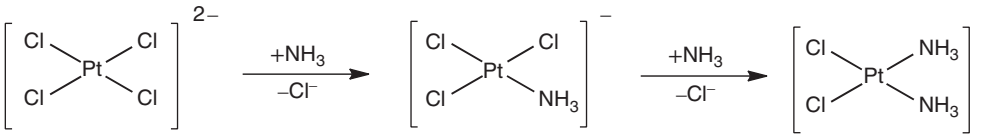


Figure 7.17 *Synthesis of cisplatin explaining the translabellising effect*

an electron configuration of d^8, and the square planar geometry is the dominant structure. The $[PtCl_4]^{2-}$ anion is an example where this square planar geometry is adapted. It is a stable anion, and indeed most platinum(II) chemistry starts with $K_2[PtCl_4]$.

$[PtCl_4]^{2-}$ is also the starting material for the synthesis of *cis*-diamminedichloroplatinum(II) (cisplatin, CDDP), a widely used chemotherapeutic drug (see Figure 7.16). The first NH_3 ligand is added to any of the four positions around the central Pt atom, as all four positions are equivalent. The second NH_3 will be directed cis to the first NH_3 group and cisplatin is obtained. The reason is that the Cl^- ligands have a larger so-called trans effect than NH_3 (Figure 7.17).

*The **trans effect** or **trans labellising effect** is mainly seen in square planar complexes and describes the ability of some ligands to direct newly added ligands into the trans position. The intensity of the trans effect increases in the following order: F^-, H_2O, $OH^- < NH_3 < py < Cl^- < Br^- < I^-$, SCN^-, $NO_2^- < SO_3^{2-} < CH_3^- < H^-$, NO, CO, CN^-.*

In comparison, if the synthesis is started from $Pt(NH_3)_4^{2+}$, transplatin is obtained. Again, the addition of the first ligand, in this case Cl^-, can occur at any of the four positions. The addition of the second Cl^- ligand will be directed into the trans position by the initial Cl^- ligand as it has a higher trans effect than the NH_3 ligand (Figure 7.18).

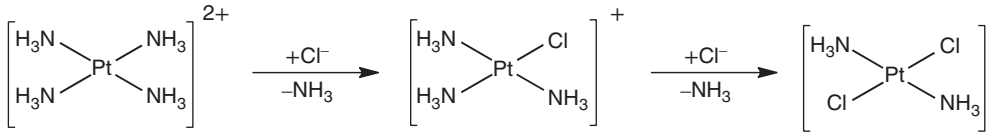


Figure 7.18 *Synthesis of transplatin explaining the translabellising effect [2] (Reproduced with permission from [2]. Copyright © 2009, John Wiley & Sons, Ltd.)*

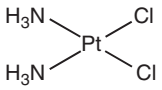


Figure 7.19 *Chemical structure of cisplatin*

7.2.1 *Cisplatin*

CDDP, also referred to as *cisplatinum* or *cisplatin*, is a yellow powder and has found widespread use a chemotherapeutic agent. The platinum complex binds to DNA and causes cross-linking, which triggers the programmed cell death (apoptosis). Cisplatin is specifically used as an effective therapeutic agent against ovarian, testicular, uterus, bladder and head and neck cancers (Figure 7.19).

7.2.1.1 Discovery

Rosenberg, a biophysicist working at Michigan State University, discovered the anticancer activity of cisplatin in 1965 serendipitously. Rosenberg devised an experiment to investigate the effect of electric fields on cell division, in which he passed an alternating electric current through two Pt electrodes immersed in a beaker containing *Escherichia coli* bacteria in a cell growth medium containing ammonium and chloride ions. During the experiment, Rosenberg discovered that the bacteria had grown in size, but not divided as was expected. On carrying out some control experiments, it became soon clear that it was not the electric current that caused this unusual cell growth. Rosenberg realised that a chemical reaction had taken place in the cell medium requiring oxygen, ammonium ions (NH_4^+) and chloride ions (Cl^-) in addition to a small amount of platinum, which was dissolved from the surface of the electrodes. A mixture of platinum salts was accidentally synthesised which contained cisplatin (*cis*-[Pt(II)$Cl_2(NH_3)_2$]). Rosenberg subsequently showed that only *cis*-[Pt(II)$Cl_2(NH_3)_2$] and not *trans*-[Pt(II)$Cl_2(NH_3)_2$] could prevent the growth of cancer cells *in vitro.* Typically, cisplatin kills cancer cells at micromolar doses.

Nevertheless, there is a lot of work necessary in between the discovery of a cytotoxic agent and the licensing of an anticancer drug. At the time Rosenberg discovered the potential of cisplatin, only organic compounds were seen to be appropriate for medicinal use in humans and certainly a heavy metal compound was seen as being too toxic for a therapeutic approach. Rosenberg convinced research institutes such as the National Cancer Institute to carry out several tests and trials. In 1979, he finally managed to file a patent on the use of cisplatin as anticancer agent. The synthesis itself had been reported 100 years ago, and cisplatin certainly was not a novel compound anymore, which could be patented. Bristol-Myers became interested in the compound, and the FDA licensed cisplatin as an anticancer drug. This discovery led to a whole new area of drug discovery, as from this point drug development was not only limited to organic compounds anymore.

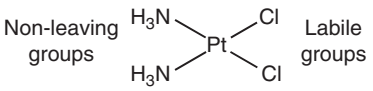


Figure 7.20 *Chemical structure of cisplatin showing the labile and the nonleaving groups*

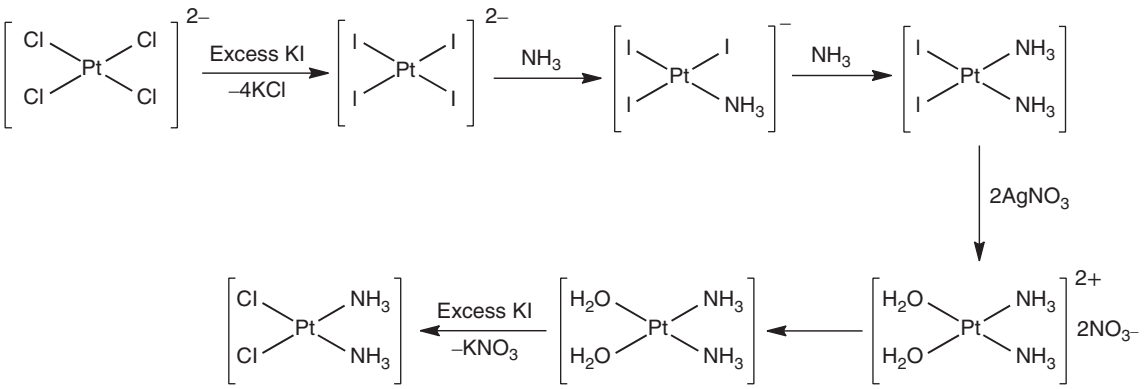


Figure 7.21 *Synthesis of cisplatin*

7.2.1.2 Mode of action

Cisplatin is a neutral, purely inorganic compound, first synthesised in 1844, containing a platinum(II) centre and two ammonia ligands and two chloride ligands. The ammonia ligands represent the nonleaving groups, whereas the chloride ligands are labile and can be exchanged by nucleophiles (Figure 7.20).

The synthesis of cisplatin starts with $K_2[PtCl_4]$, but it has undergone several improvements since it was published more than 100 years ago. The main problem is the occurrence of impurities and the synthesis of the by-product transplatin. Nowadays, the synthetic routes are mostly based on a method published in the 1970s by Dhara. In the initial step, $K_2[PtCl_4]$ is reacted with KI, and the platinum complex is converted into the tetraiodo analogue. Subsequently, NH_3 is added and *cis*-$[PtI_2(NH_3)_2]$ is obtained. *cis*-$[PtI_2(NH_3)_2]$ precipitates from the solution once $AgNO_3$ is added, and the insoluble AgI can be filtered off. KCl is added to the solution and cisplatin is formed as a yellow solid. The success of the synthesis relies on the strong translabellising effect of the iodo ligands as discussed earlier [3] (Figure 7.21).

Since the success of using cisplatin as a chemotherapeutic agent, significant research has been undertaken to establish the exact mode of action. Some areas still remain unclear, but it is clear that its anticancer ability mainly stems from its ability to form adducts with DNA. It has been generally accepted that the cytotoxic activity of cisplatin is due to the interaction between the metal complex and the genetic DNA, which is located in the cell nucleus [4].

Cisplatin is administered intravenously as the neutral complex, and transported via the blood stream to the cancer cell. The blood stream and the extracellular fluids have a high chloride concentration (>100 mM) and therefore, the platinum complex will not be hydrolysed. There is still much debate about the cellular uptake. It is believed that the neutral complex enters the cancer cell by passive and/or active transport. Apart from the passive diffusion, carrier-mediated proteins have been identified, such as the plasma membrane copper transporter, organic cation transporters and others [5] (Figure 7.22).

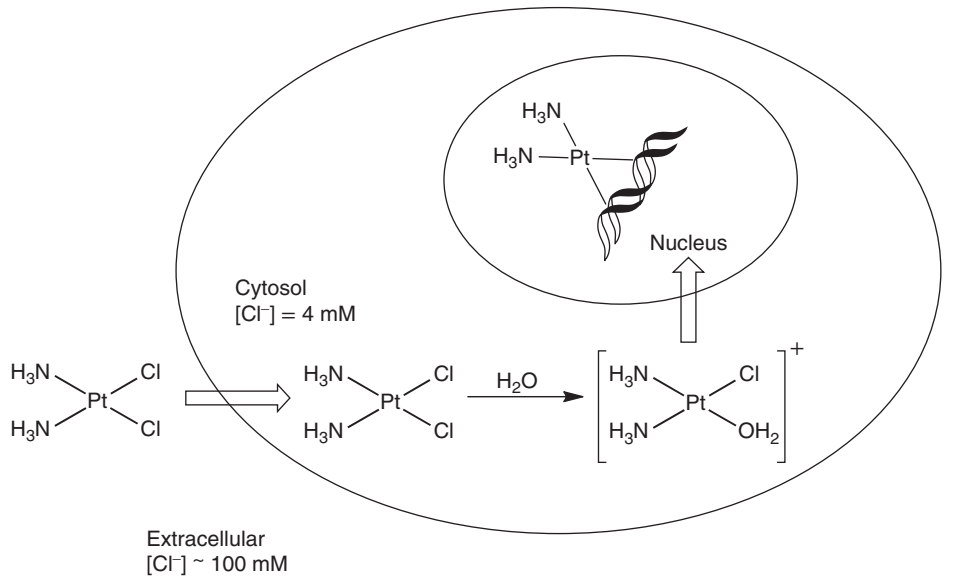


Figure 7.22 *Schematic showing the cytotoxic pathway for cisplatin (Reproduced with permission from [4]. Copyright © 2012, Royal Society of Chemistry.)*

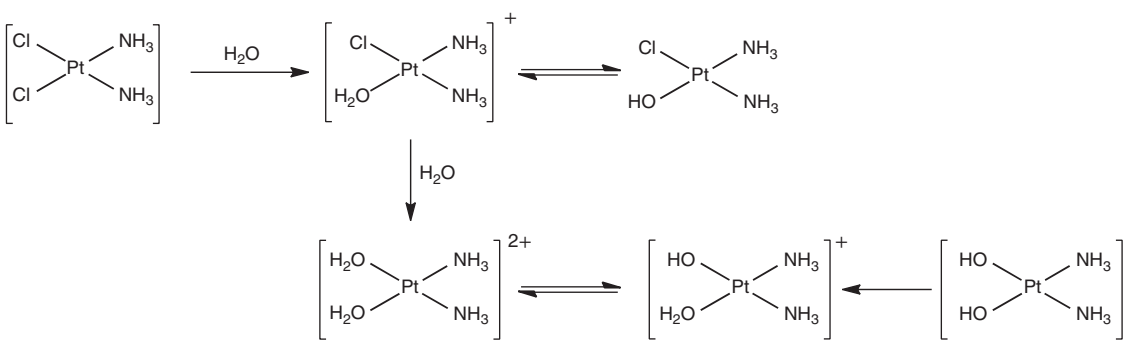


Figure 7.23 *Hydrolysis of cisplatin*

The mode of action inside the cell begins with the hydrolysis of the platinum–chloride bonds. This hydrolysis is facilitated by the significantly lower chloride concentration inside the cell (4 mM) compared to the high chloride concentration in the blood plasma, which prevents the hydrolysis of cisplatin during the transport in the blood stream. Upon entering the cell, it is proposed that cisplatin loses its chloride ligands and forms the mono and diaqua species. The hydrolysed species are good electrophiles and can bind to a variety of nucleophiles in the cell, such as nucleic acid and thiol-containing proteins (Figure 7.23).

The anticancer activity of cisplatin is based on the interaction of the platinum complex with DNA located in the nucleus. Interaction with the mitochondrial DNA is believed to be less important for the antitumour activity of cisplatin. Cisplatin binds to DNA primarily by coordination to the nitrogen (N7) atom of guanine, whereas it also can bind (to a lesser degree) to N7 and N1 of adenine and N3 of cytosine [2, 6] (Figure 7.24).

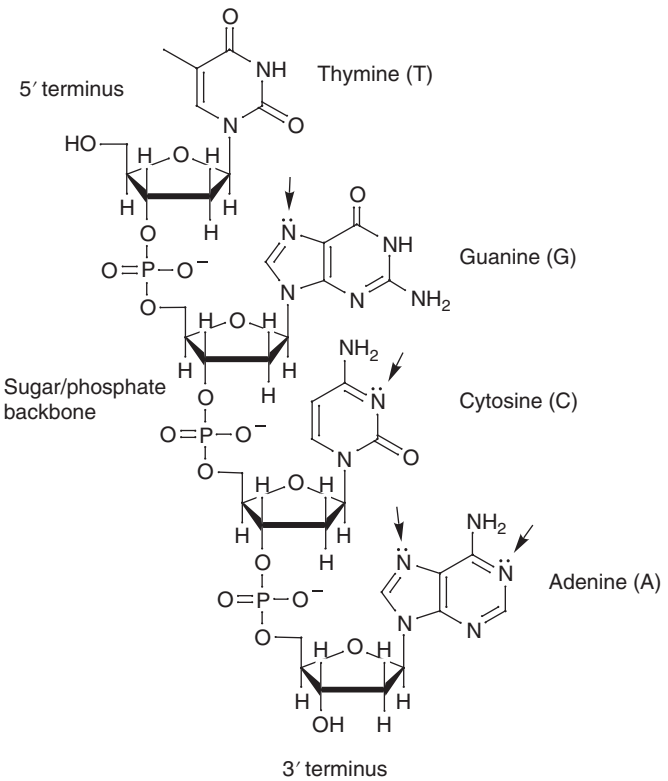


Figure 7.24 *A schematic representation of a DNA segment showing sites available for the platinum binding. Guanine N7 is the preferred binding site for cisplatin [2] (Reproduced with permission from [2]. Copyright © 2009, John Wiley & Sons, Ltd.)*

It was found that the majority of DNA–cisplatin complexes are formed by intrastrand 1,2-(GG) cross-links, which means cisplatin coordinates to two guanine bases within the same DNA strand. This form of DNA-adduct formation makes up around 65% of all DNA–cisplatin complexes. Note that the Arabic numbers refer to two adjacent nucleotides within the DNA sequence and 'intra' means that the cross-link occurs within the same DNA strand. Other binding modes include intrastrand 1,2-(AG) cross-links (25%) as well as inter-strand cross-links (between two DNA strands) and mono-functional binding to DNA [4] (Figure 7.25).

As a result of the formation of DNA–cisplatin adducts, the secondary structure of DNA is affected. In particular, the major intrastrand cross-linking of cisplatin leads to conformational alteration in the DNA. The platinum core binds to N7 of guanine bases located in the major groove and, as a result of its square planar geometry, forces the two bases to tilt towards each other and away from the parallel stacked form of DNA. This leads to a distortion of the helix axis, bending it towards the major groove. In turn, this exposes the minor groove on the opposite site and makes it accessible for other compounds. In essence, the formation of these adducts results in the DNA helix to become kinked and the DNA translation to be interrupted [2, 6, 7] (Figure 7.26).

7.2.1.3 Resistance and cytotoxicity

One of the major problems of chemotherapy with cisplatin is that after repeated use the cancer cells often become resistant to the treatment. Unfortunately, cisplatin has a narrow therapeutic window, which means

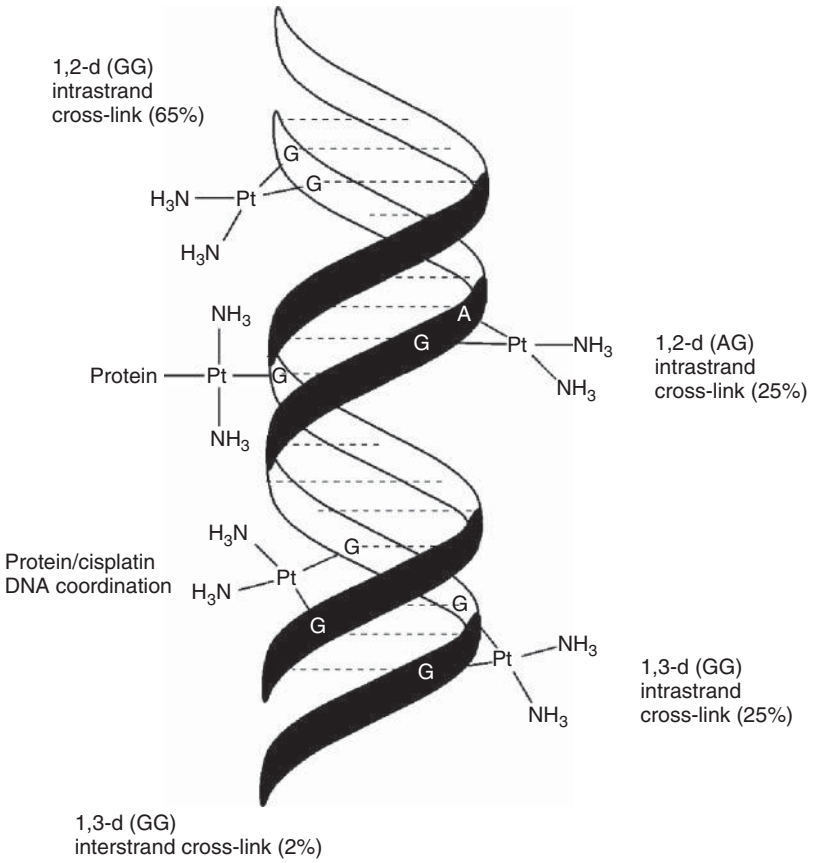


Figure 7.25 *Schematic representation of a DNA segment coordinated by cisplatin showing the different coordination modes*

that the difference between the therapeutically active and the toxic dose is fairly small. Therefore, it is not simply possibly to increase the dose once resistance is observed.

It has been observed that in platinum-resistant cells, less of the metal is bound. This can be due to the cells being more effective blocking entry to the cell, being able to move cisplatin actively out of the cell or, in the case where the agent made its way to its target, that DNA can be repaired and the metal complex is removed.

It has been shown that cancer cells that develop resistance to cisplatin have a higher concentration of the sulfur-containing proteins such as glutathione (GSH) and metallothionein (MT). Both GSH and MT can form very stable complexes with Pt^{2+} via a S—Pt bond, which will reduce the ability of the platinum complex to interact with other targets in the cell. It is actually assumed that most of the platinum drugs bind to sulfur before it reaches the DNA, as this interaction is the kinetically favoured process. There is evidence that some of the Pt–thioester complexes can be broken in the presence of DNA. Nevertheless, the Pt–thiol complexes are very stable and will therefore inactivate the platinum drug [4]. There is also evidence that the Pt(II)–S adducts could be exported out of the cell via a glutathione S-conjugate [2]. It is interesting to note that only about 1% of the administered cisplatin will complex with DNA [5].

DNA repair mechanisms are important processes within the cells and there are several different ways in which DNA can be repaired. If the DNA has been chemically modified by drugs, UV light or radicals, Nature

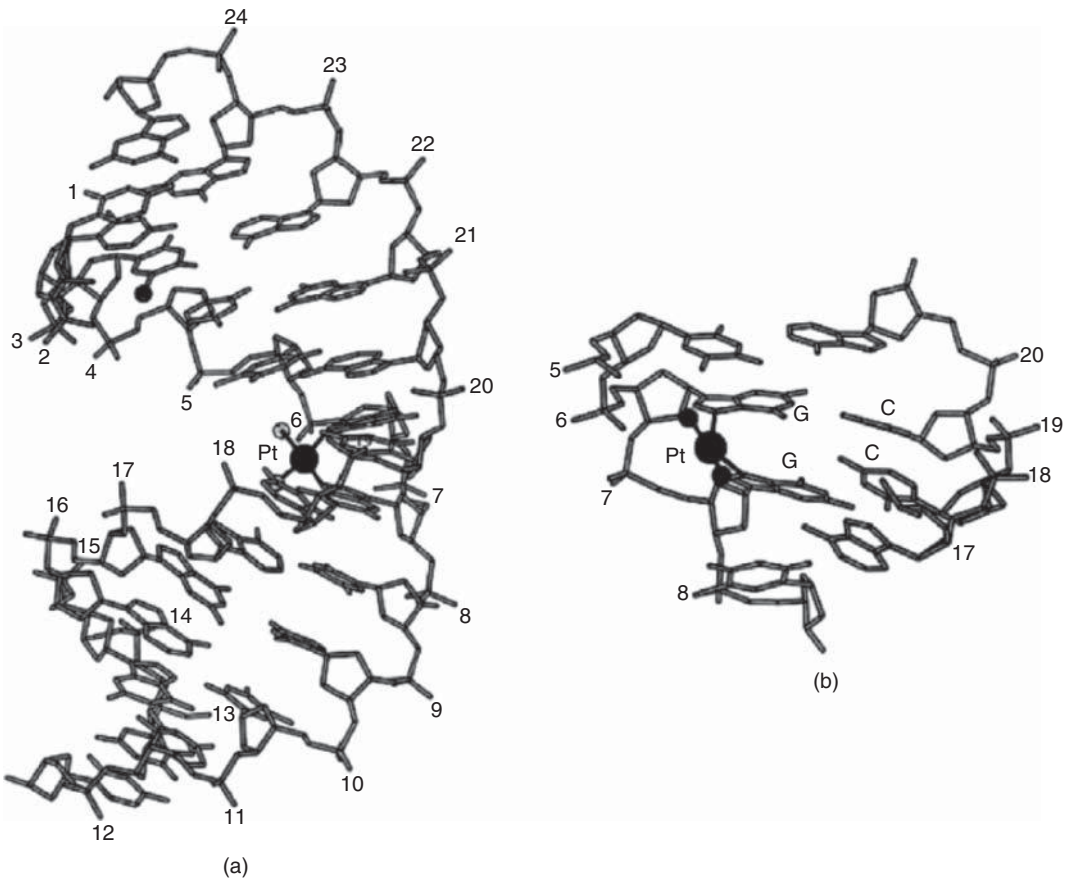


Figure 7.26 *Crystal structure of cisplatin bound to DNA (a) and to N7 of the guanine base (b) (Reprinted by permission from Macmillan Publishers Ltd: Nature [9], Copyright (1995).)*

has established a sophisticated system to check and repair the DNA and ultimately ensure the survival of the organism. One system is called *nucleotide excision repair* (*NER*) and it uses enzymes to remove the single-stranded DNA segment that has been modified. It also replaces this segment by reading the opposite DNA strand. As previously discussed, the 1,2-intrastrand cross-link represents the major Pt–DNA adduct, where cisplatin coordinates to two neighbouring guanine bases within the same DNA strand. In recent animal experiments, it has been shown that the NER system is indeed important to the removal of platinum from DNA. Mice with a compromised NER system were not able to remove the platinum-coordinated segments from their DNA in the kidney cells researched [2]. There are also other mechanisms of resistance under discussion, which are not further explained in this book.

7.2.1.4 Formulation and administration

Cisplatin is commercially available either as a powder for reconstitution or as a ready-to-use solution containing the drug in a saline concentration (NaCl solution) at a pH around 3.5–4.5. Under this condition, the majority of platinum is present as $[PtCl_2(NH_3)_2]$. Additionally, the low pH avoids the formation of any multinuclear platinum compounds.

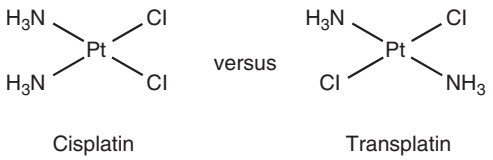


Figure 7.27 *Chemical structure of cisplatin versus transplatin*

Cisplatin is used alone or in combination for the treatment of bladder, lung, cervical, ovarian and testicular cancer. It is administered intravenously and requires pre-hydration of the patient. The dose of cisplatin depends on the body surface of the patient and ranges typically from 20 to 140 mg/m^2. It can be given as a single-dose injection or as infusion over a period of a couple of hours. Cisplatin is mainly eliminated via urine. The highest concentrations can be found in the liver, prostate and kidneys. Side effects include severe nausea, vomiting, ototoxicity and nephrotoxicity, together with myelosuppression (bone marrow suppression). In particular, nephrotoxicity is the dose-limiting factor, and close monitoring of the renal function is required. The exact mode of damage to the kidneys is unknown, but it accumulates in the kidneys and believed to damage the renal tubular cells [2, 8].

7.2.1.5 Transplatin

Soon the question arose how transplatin (*trans*-diamminedichloroplatinum (II)) differs from its stereoisomer cisplatin and how this explains the dramatic difference in efficacy. Very early, it was seen that transplatin is not active when tested in animal models, and therefore it was postulated that the cis geometry is crucial for the cytotoxic activity for which two reasons where identified (Figure 7.27).

A close look at the structure of transplatin shows that the two chloride ligands (i.e. the reactive sites) are further apart in transplatin (4.64 Å) compared to cisplatin (3.29 Å). This affects the way the metal centre Pt(II) can cross-link sites on DNA, which means that the 1,2 intrastrand cross-link between the two purine bases is stereochemically hindered for the transplatinum species (transplatin). Studies have shown that mainly the mono-functional 1,3 adducts and inter-strand adducts are formed, which are easily recognised by the NER repair system [7].

Furthermore, it has been shown that transplatin is kinetically more reactive than cisplatin and therefore more of the compound is inactivated before reaching its target, which contributes to the weaker activity of transplatin. Therefore, current research focusses on the synthesis of novel transplatin compounds in which the hydrolysis rate of the compound can be altered [2].

Recently, a range of transplatinum compounds were proved to have anticancer activity. The most notable compounds are the *trans*-Pt(IV) complexes with the general formula *trans*-Pt(IV)Cl_2X_2LL' or the *trans*-Pt(II) complexes with the general formula *trans*-Pt(II)Cl_2LL'. The ligands (L and L′) are mainly imino, aromatic amine and aliphatic amine ligands, whereas the Pt(IV) complexes contain also hydroxyl groups. Interestingly, some of those compounds are also active on cisplatin-resistant cell lines, thereby opening a new area of research for the treatment of these cancer types [9, 10] (Figure 7.28).

7.2.2 Platinum anticancer agents

Despite the success of cisplatin, there is a need to develop new platinum anticancer drugs. Cisplatin is a very toxic compound and can have severe side effects such as nephrotoxicity (kidney poisoning), ototoxicity (loss of high-frequency hearing) and peripheral neuropathy (damage to nerves of the peripheral nervous system), although it is possible to control some of these effects. Very typically, cancer cells can become resistant to

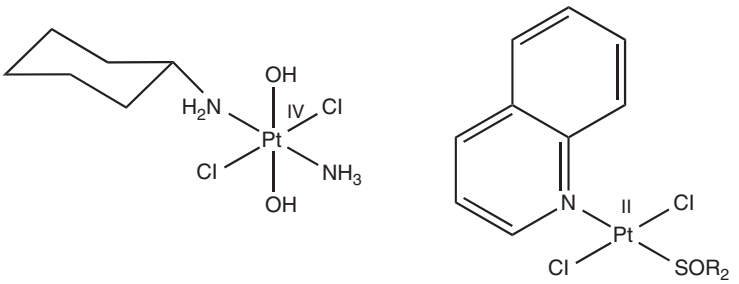


Figure 7.28 *Example of a* trans-*Pt(IV) complex (a) and a* trans-*Pt(II) complex (b)*

cisplatin after repeated administration. This is a fairly common problem experienced at the repeat treatment with cisplatin. Furthermore, compounds active against a variety of cancer types are required to combat cancer.

Two second-generation platinum drugs are so far successfully registered worldwide – carboplatin and oxaliplatin. There are others such as nedaplatin, which is registered in Japan for the treatment of head and neck, testicular, lung, cervical, ovarian and nonsmall-cell lung cancer. In South Korea, heptaplatin is used against gastric cancer, whereas lobaplatin is licensed in China for the treatment of cancers including metastatic breast cancer, small-cell lung cancer and myelogenous leukaemia [10].

Nevertheless, the development of new platinum-based drugs has been less successful than expected. The majority of compounds are not used in a clinical setting because their efficacy is too low, toxicity is too high or the compounds showed a poor aqueous solubility, a fairly common problem for transition-metal-based compounds.

7.2.2.1 Carboplatin

Carboplatin, *cis*-diammine(1,1-cyclobutanedicarboxylato)platinum(II), is a second-generation platinum drug. Its structure is based on cisplatin with the difference that the chloride ligands are exchanged for a bidentate chelating ligand. A consequence is that carboplatin is less reactive than cisplatin and therefore is less nephrotoxic and orthotoxic than the parent compound. Unfortunately, it is more myelosuppressive than cisplatin, which reduces the patients' white blood cell count and makes them susceptible to infections [8]. Carboplatin was licensed by the FDA in 1989 under the brand name Paraplatin and has since then gained worldwide recognition. Carboplatin on its own or in combination with other anticancer agents is used in the treatment of a variety of cancer types including head and neck, ovarian, small-cell lung, testicular cancer and others [2] (Figure 7.29).

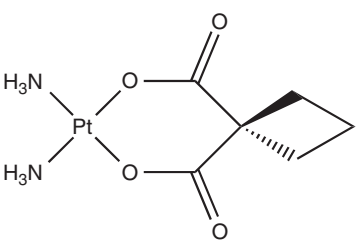


Figure 7.29 *Chemical structure of carboplatin*

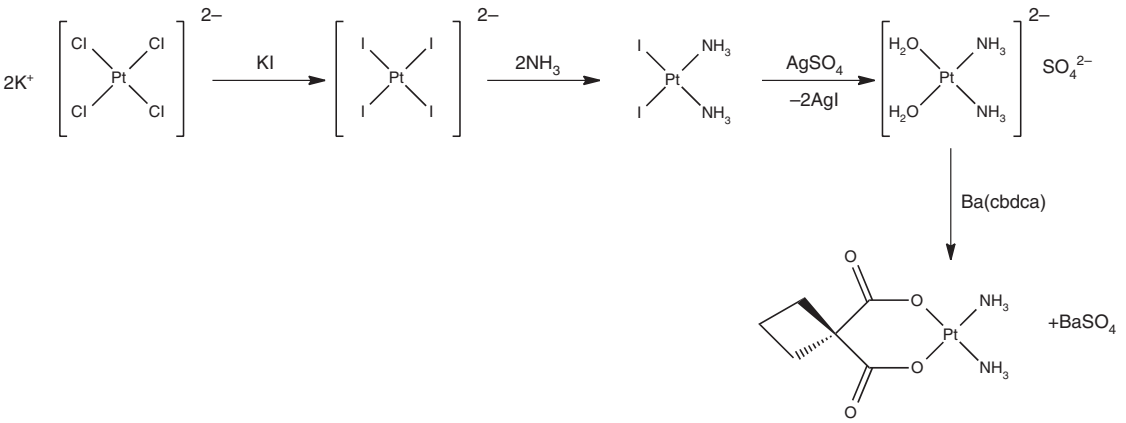


Figure 7.30 *Synthesis of carboplatin*

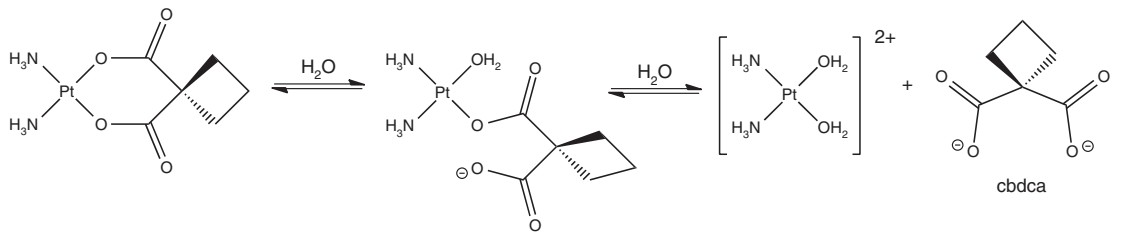


Figure 7.31 *Hydrolysis of carboplatin [2]*

Carboplatin is a pale-white solid showing good aqueous solubility. The synthesis starts with potassium tetrachloroplatinate, which is reacted to the orange $[PtI_4]^{2-}$ anion. Analogous to the synthesis of cisplatin, in the following steps the anion is reacted with ammonia (due to the translabellizing effect of iodide, the ammonia ligands are directed into the cis position) and converted to *cis*-$[Pt(NH_3)(H_2O)_2]SO_4$. In the final step, the complex is reacted with the chelating agent Ba(cbda) (cbdca, cyclobutane-1,1-dicarboxylate) and carboplatin is formed [2] (Figure 7.30).

Carboplatin is administered by intravenous (IV) injection. A typical solution contains the drug in a high concentration in water or in a mannitol or dextrose solution. The dose is determined either on the basis of the body surface area of the patient or according to their renal function. The doses are typically three to six times higher than cisplatin, which reflects the lower chemical reactivity and toxicity of this drug. Carboplatin can be given on an outpatient basis, as it is better tolerated than cisplatin.

The mode of action relies on the ring-opening of the cbdca chelate ring and interaction of the platinum centre with DNA. Carboplatin is seen as a prodrug, which itself is not very reactive within the human body but once activated shows its full potential. Hydrolysis of carboplatin and removal of the chelate ligand or at least the opening of the ring makes this compound much more cytotoxic than the parent compound itself (Figure 7.31).

In vitro studies have shown that the drug binds to DNA and forms initially a mono-functional adduct, which over time is converted into the di-functional platinum–DNA adduct. There are indications that carboplatin forms DNA intrastrand cross-links analogous to cisplatin, but its reactivity towards DNA is reduced [2].

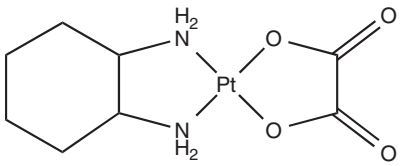


Figure 7.32 *Chemical structure of oxaliplatin*

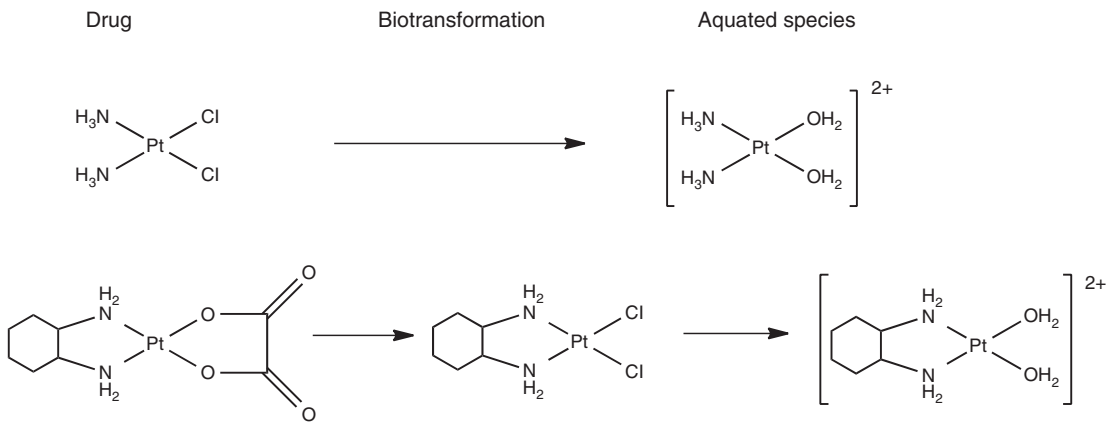


Figure 7.33 *Hydrolysis products of cisplatin and oxaliplatin*

7.2.2.2 Oxaliplatin

Oxaliplatin (*cis*-[oxalato] *trans*-1,2-diaminocyclohexane platinum(II)), for example, marketed under the trade name Eloxatin, is considered as a third-generation platinum-based anticancer drug. Its structure differs from previously synthesised platinum compounds by the configuration of its amino substituents. Its platinum centre is coordinated by two chelating ligands, namely an oxalate ligand and a so-called DACH (1,2-diaminocyclohexane) ligand. In comparison to cisplatin, the two chlorine leaving groups are replaced by an oxalato leaving group. The simple amino groups are replaced by the DACH ligand, which is the nonleaving group (Figure 7.32).

Cisplatin and carboplatin are hydrolysed to a common diamino-platinum species, whereas the hydrolysis product of oxaliplatin contains the bulky DACH group, which sterically hinders the DNA repair mechanism. These mismatch repair enzymes are particularly active in colon cancer and, not surprisingly, oxaliplatin shows excellent activity in the treatment of colon and rectal cancers [11] (Figure 7.33).

The clinical use of oxaliplatin was approved by the European Union in 1999 and by the FDA in 2002. It is most effective in combination with 5-fluorouracil and leucovorin (5-FU/LV) in the treatment of metastatic carcinomas of the colon or rectum [11]. Oxaliplatin induces less side effects than cisplatin; for example, it is less nephrotoxic and ototoxic and leads to less myelosuppression. Unfortunately, treatment with oxaliplatin can lead to nerve damage, which may not be reversible in the case of chronic exposure of the patient to the drug. Oxaliplatin is usually administered intravenously as infusion over a period of 2–6 h in doses similar to cisplatin. The neurotoxic side effects are dose-limiting [8].

The synthesis of oxaliplatin starts with $K_2[PtCl_4]$, the same starting material used for the synthesis of carboplatin. This is reacted with water and 1 equiv of the nonleaving ligand, 1*R*,2*R*-DACH ligand. Note that

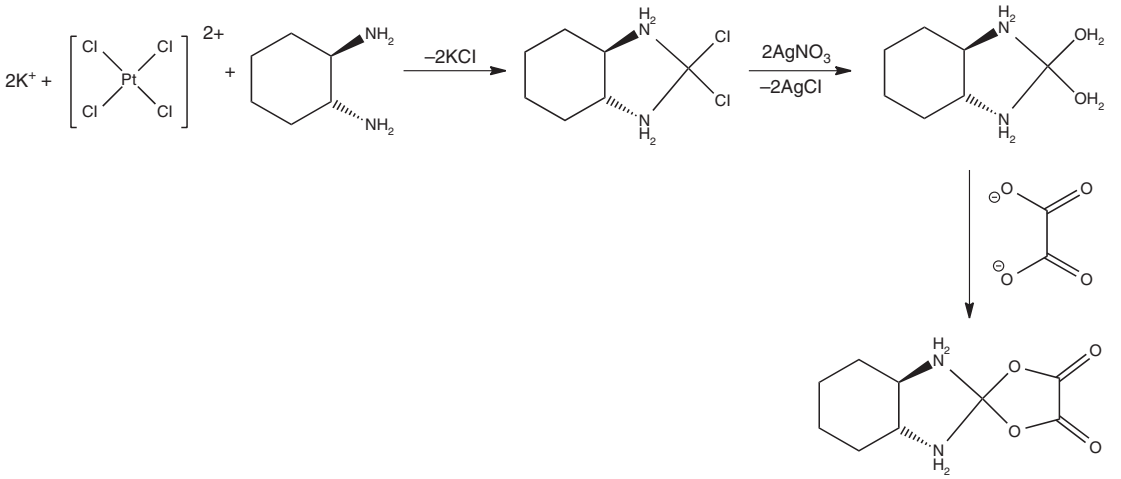


Figure 7.34 *Synthesis of oxaliplatin [2]*

there are different stereoisomers of the DACH ligand, and cytotoxicity studies have shown that the use of this specific stereoisomer 1*R*,2*R*-DACH leads to the most potent compound. Upon treatment with silver nitrate, the diaqua complex is formed. Any excess of silver ions can be removed by adding potassium iodide. This leads to the formation of the insoluble silver iodide, which can be filtered off. The diaquo platinum complex is subsequently treated with 1 equiv of oxalic acid, and oxaliplatin is formed as a solid (Figure 7.34).

It is believed that DNA is the major cellular target of oxaliplatin, as researchers have shown that it forms intrastrand cross-links similar to cisplatin. The oxaliplatin–DNA adduct also leads to a bending of the DNA similar to the cisplatin–DNA adduct. Nevertheless, there are significant differences to the cisplatin–DNA adduct. The oxaliplatin–DNA adduct forces a narrow minor groove bend (helix bend of 31°), whereas the equivalent cisplatin–DNA adduct leads to a wide minor groove (60–80°). Also, it has been observed in the solid state structure of the oxaliplatin–DNA adduct that there is a hydrogen bond formed between the NH of the DACH group and the oxygen atom of guanine base, which interacts with the platinum centre [2].

7.2.2.3 Other platinum drug candidates

There are numerous platinum compounds under research for their potential use as anticancer agents. Only a few of them have found their way into the clinic so far, with cisplatin, carboplatin and oxaliplatin being the most successful ones.

One example is nedaplatin, *cis*-diammineglycolatoplatinum(II), which is structurally similar to carboplatin. The chemical structure consists of a central platinum(II) atom with two *cis*-ammonia groups as nonleaving groups and – in contrast to carboplatin – the dianionic form of glycolic acid as the leaving group. Nedaplatin has been approved for the clinical use in the Japanese market for the treatment of head and neck, testicular, ovarian, lung and cervical cancer. It is typically administered by IV injection and its dose-limiting side effect is myelosuppression (Figure 7.35).

Lobaplatin and heptaplatin are further examples of platinum-based agents being used in China and South Korea, respectively. Lobaplatin is used in the treatment of nonsmall-cell lung cancer and breast cancer. Heptaplatin is used in South Korea to treat gastric cancer. Both drugs show the typical side effects such as myelosuppression and mild hepatotoxicity. Their success is limited and has not led to approval in the EU or by the FDA (Figure 7.36).

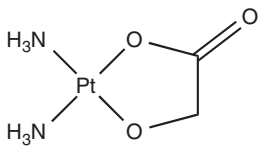


Figure 7.35 *Chemical structure of nedaplatin*

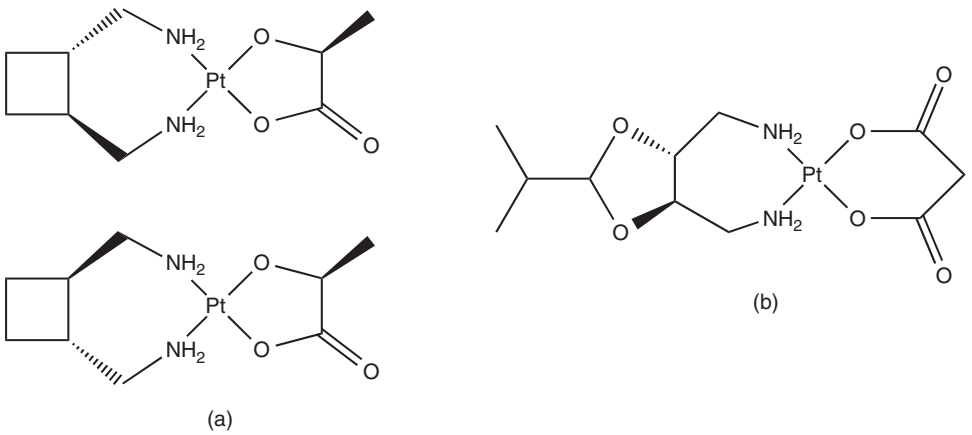


Figure 7.36 *Chemical structures of (a) lobaplatin and (b) heptaplatin*

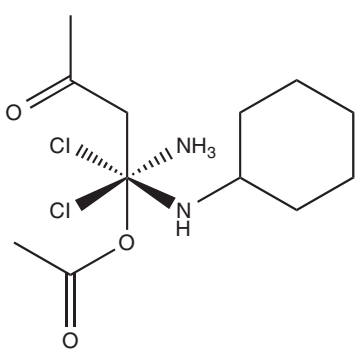


Figure 7.37 *Chemical structure of satraplatin*

Satraplatin (JM216, *cis*,*trans*,*cis*-[$PtCl_2(OAc)_2(NH_3)(C_6H_5NH_2)$]) is a Pt(IV) or Pt^{4+} complex, which is active by oral administration, as it is more hydrophobic than cisplatin. This form of administration is very attractive because of the convenience and freedom it provides to the patient. Satraplatin also has a milder toxicity profile and is shows no cross-resistance with cisplatin. Satraplatin in combination with prednisone has completed phase III clinical trials against hormone-refractory prostate cancer. The results were very encouraging, but the overall survival rate did not improve significantly enough. As a result, the fast-track approval of the FDA was not granted [12] (Figure 7.37).

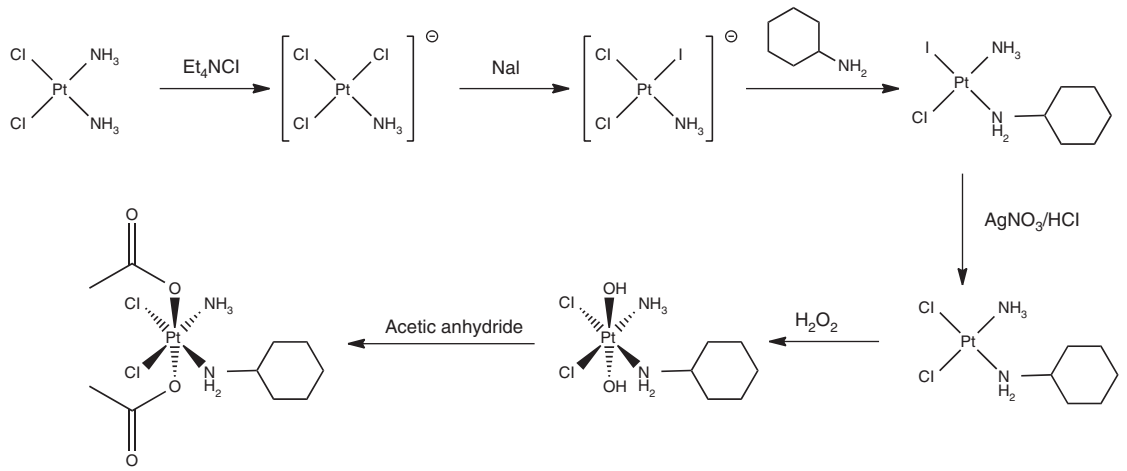


Figure 7.38 *Synthesis of satraplatin [2]*

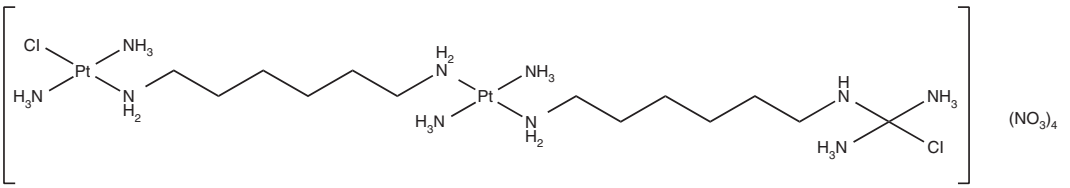


Figure 7.39 *Chemical structure of BBR3464*

Structurally, satraplatin consists of a Pt(IV) centre, which is coordinated by six ligands forming a close to octahedral geometry. In general, octahedral Pt(IV) complexes (low-spin d^6) are much more kinetically inert than square planar Pt(II) complexes. Pt(IV) complexes can be readily reduced *in vivo* to Pt(II) by reductants such as ascorbate or thiols (e.g. cysteine, GSH).

The synthesis of satraplatin starts with cisplatin, which is reacted with tetraethylammonium chloride (Et_4NCl) – a source of Cl^-. As a result of the trans-directing effect, the iodide ligand is introduced in a second step adjacent to the ammonia group. Subsequently, 1 equiv of cyclohexylamine is added, which coordinates to the platinum centre trans to the iodide. Silver nitrate is used to remove the iodide ligand, as no further 'trans-directing' action is required. The Pt^{2+} is finally oxidised to Pt^{4+}, which expands the coordination sphere from 4 to 6 – octahedral geometry. In the last step, the acetate ligands are introduced (Figure 7.38).

Satraplatin is the only orally administered platinum-based drug that has entered clinical trials so far. The difficulty for this administration route lies in the aggressive conditions that are present in the stomach. In general, metal complexes do not survive the acidic conditions in the stomach and therefore will not reach the gastrointestinal (GI) tract unchanged. The advantage of satraplatin is that the complex is relatively inert to any exchange reactions and therefore has an increased chance of reaching the pH-neutral GI tract unchanged. From here, the drug enters the blood stream. *In vitro* studies with fresh human blood have shown that within minutes the reduction of the platinum centre to Pt^{2+} takes place in the red blood cells. This may be facilitated

by haemoglobin, cytochrome c and NADH, and leads to a square planar Pt^{2+} complex containing the chloride and ammonia ligands [2].

Further research has led to the development of multi-platinum complexes. This is against the 'rules' set out for platinum-based anticancer drugs, which state that a successful drug candidate should consist of only one platinum centre with amine-based nonleaving groups in cis position and two leaving groups, also in cis position. Clearly, polynuclear platinum compounds fall outside these rules, but researchers have synthesised the unusual trinuclear complex BBR3464, which was very successful in *in vitro* studies and even reached clinical trials against melanoma and metastatic lung and pancreatic cancer [2] (Figure 7.39).

7.3 Iron and ruthenium

Group 8 of the periodic table of elements consists of the nonradioactive members iron (Fe), ruthenium (Ru) and osmium (Os), as well as the radioactive element Hassium (Hs) (Figure 7.40).

All three nonradioactive elements are silvery white, hard transition metals with a high melting point. Iron has been classified as the most common element within the entire earth, as most of the earth's core is iron. In contrast, ruthenium and osmium are two of the rarest elements on earth. The radioactive element hassium has not been isolated in pure form yet and therefore its exact properties have not been established. It has only been produced in nuclear reactors and never has been isolated.

The electronic configuration of group 8 metals is shown in Figure 7.41.

H																	He
Li	Be											B	C	N	O	F	Ne
Na	Mg											Al	Si	P	S	Cl	Ar
K	Ca	Sc		Ti	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	Ga	Ge	As	Se	Br	Kr
Rb	Sr	Y		Zr	Nb	Mo	Tc	Ru	Rh	Pd	Ag	Cd	In	Sn	Sb	Te	I	Xe
Cs	Ba	La	Ce - Lu	Hf	Ta	W	Re	Os	Ir	Pt	Au	Hg	Tl	Pb	Bi	Po	At	Rn
Fr	Ra	Ac	Th -Lr	Rf	Db	Sg	Bh	Hs	Mt	Ds	Rg	Uub						

Figure 7.40 *Periodic table of elements; group 8 elements are highlighted*

Fe : $[Ar]3d^6 4s^2$

Ru : $[Kr]4d^7 5s^1$

Os : $[Xe]4f^{14} 5d^6 6s^2$

Figure 7.41 *Electronic configuration of group 8 metals*

7.3.1 Iron

Iron is the chemical element with the symbol Fe (Latin: ferrum) and atomic number 26. It is one of the most used metals because of the relatively low production costs and its high strength. Iron can be found in many everyday items, from food containers to screw drivers or any type of machinery. Steel is a form of iron, which is alloyed with carbon and a variety of other metals.

Iron ions are a necessary trace element used by almost all living organisms with the only exceptions being a few prokaryotic organisms that live in iron-poor conditions. As an example, the lactobacilli in iron-poor milk use manganese for their catalysis processes. Iron-containing enzymes, usually containing haeme prosthetic groups, participate in the catalysis of oxidation reactions in biology and in the transport of a number of soluble gases.

More than other metals, metallic iron has long been associated with health. Chalybeate springs, the iron-containing waters, have been well known for centuries for their healing properties. In the nineteenth century, the 'veritable pills of Blaud', which contain ferrous sulfate and K_2CO_3, were used to 'cure everything'. In the 1930s, the relationship between iron-deficiency anaemia and the lack of dietary iron was established. Nowadays, iron deficiency is the most frequent nutritional deficiency in the world.

7.3.1.1 Chemistry of iron

Iron is a metal extracted from iron ore, and is almost never found in the free elemental state. In order to obtain elemental iron, the impurities must be removed by chemical reduction. Iron is the main component of steel, and it is used in the production of alloys or solid solutions of various metals.

Finely divided iron powder is pyrophoric in air. There is a difference between when elemental iron is heated in dry air or in the presence of humidity. Fe heated in dry air will oxidise, whilst when heated in moist air it will rust characterised by the formation of $Fe_2O_3 \cdot xH_2O$. The formation of rust is an electrochemical process occurring in the presence of oxygen, water and an electrolyte such as NaCl.

$$2Fe \rightarrow 2Fe^{2+} + 4e^-$$

$$O_2 + 2H_2O + 4e^- \rightarrow 4[OH]^-$$

$$Fe(OH)_2 \text{ oxidizes to } Fe_2O_3 \cdot xH_2O$$

The highest oxidation states of iron are Fe(VI) and Fe(IV), whilst Fe(V) is very rare. Examples of compounds where Fe occupies this oxidation state include $[FeO_4]^{2-}$, $[FeO_4]^{3-}$, $[FeO_4]^{4-}$ and $[FeO_3]^{2-}$. Oxidation states +2 and +3 are the most commonly occurring oxidation states for Fe. The old name for Fe(III) is ferric and for Fe(II) is ferrous – this is still reflected in many drug names. Fe reacts with halogens under heat with the formation of FeF_3, $FeCl_3$, $FeBr_3$ and FeI_2. FeF_3 is a white solid, whilst $FeCl_3$ is a dark-green hygroscopic solid. $FeCl_3$ is an important precursor for any Fe(III) chemistry.

Fe(II) halogens are typically synthesised by reacting Fe with the relevant acid, HX, with the exception of FeI_2, which can be synthesised from the elements directly. FeF_2 is a white solid, sparingly soluble in water, whilst $FeCl_2$ forms white, water-soluble, hygroscopic crystals.

$$Fe + 2HX \rightarrow FeX_2 + H_2$$

7.3.1.2 Iron performs many vital functions in the human body

Iron is an essential trace element for the human body. Haemoglobin is the oxygen-transport metalloprotein in the red blood cells; myoglobin facilitates the oxygen use and storage in the muscles; and cytochromes transport electrons. Iron is also an integral part of enzymes in various tissues. The average 70-kg adult body

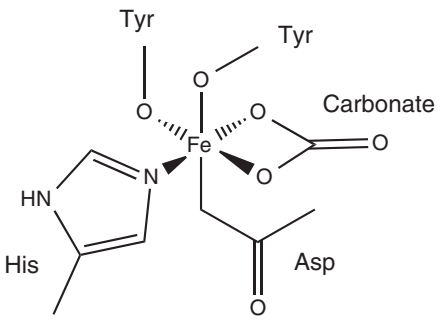


Figure 7.42 *Structure of transferrin showing the coordination of Fe^{3+} [2]*

contains around 4200 mg of iron ions. The majority (65%) can be found as haemoglobin or myoglobin, which is classified as the functional iron [13].

Iron will pass the stomach and is absorbed predominantly in the duodenum and upper jejunum. Beyond this point, intestinal bicarbonate elevates the pH, rendering iron insoluble. Free iron is, as most metal ions, highly toxic to the human body. Therefore, nature has created a sophisticated transport and storage system which ensures that no free iron ions are present in the blood stream. Iron ions absorbed from the GI tract are transported via transferrins, which are Fe^{3+}-containing metalloproteins, to the storage vessels or until it is incorporated into haemoglobin. In the human body, Fe^{3+} is stored mainly in the liver and spleen in form of ferritin, which is a water-soluble metalloprotein and stores Fe^{3+}.

Transferrins include the so-called serum transferrins, for example, ovotransferrin (present in egg white) and lactoferrin (present in milk), which can transport ~40 mg of iron ions per day in humans. The glycoprotein protein is folded in such a way that there are two pockets suitable for the coordination of Fe^{3+}. Figure 7.42 shows how Fe^{3+} is coordinated within these pockets. Note a CO_3^{2-} ligand is essential for the binding mechanism.

Transferrins *are a group of proteins that are abundant in blood and their primary role is to transport Fe^{3+}, as free Fe^{3+} would be toxic to most organisms. Transferrin consists of a single polypeptide chain independently binding a maximum of two Fe^{3+} ions. Fe^{3+} is bound in a hexa-coordinated high-spin complex within the protein. The metal is coordinated by the N atom from the imidazole residue, two deprotonated phenol groups, a carboxylate and a carbonato ligand.*

Haemoglobin and myoglobin are so-called haeme-iron proteins characterised by the presence of a haeme group (a protoporphyrin group). The main function of haemoglobin is the transport of oxygen to the tissue in need. Myoglobin is the oxygen storage protein present in the tissue.

Myoglobin consists of a monomeric protein chain containing one protoporphyrin group as the functional unit. Within myoglobin, the iron centre is coordinated by the four nitrogen groups of the porphyrin in addition to the coordination of a fifth nitrogen centre from a histidine (His) group. The functional unit containing the Fe(II) centre is called a *haeme group* and is a square-based pyramidal complex. During the oxygen binding mechanism, O_2 will enter trans to the His group to give an octahedrally coordinated iron species (Figure 7.43).

Haemoglobin, the oxygen-transport protein in the red blood cells, is a tetramer and each of the four chains contains a haeme group. It is interesting to note that the four haeme groups in haemoglobin do not operate independently. The release (and binding) of oxygen is a cooperative process, which means that the loss (uptake) of the first oxygen molecule triggers the release of the remaining three.

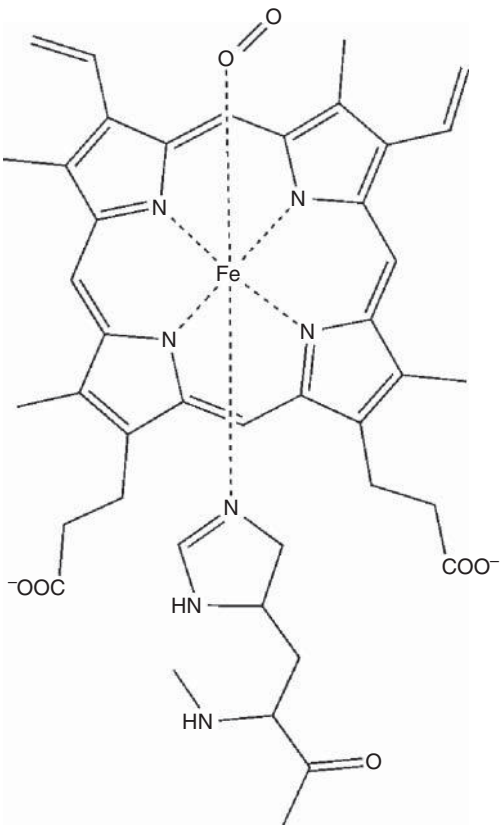


Figure 7.43 *Structure of haemoglobin*

The current model for oxygen binding in haemoglobin and myoglobin can be explained in the following way. The deoxy form contains a high-spin Fe(II) centre, which, because of its size, does not form a plane with its four nitrogen donor atoms. Instead, it is located slightly above the plane, drawn towards the His residue. Once oxygen enters trans to the His residue, the iron centre is oxidised to a low-spin Fe^{3+} centre and O_2 is reduced to $[O_2]^-$. Both species contain an unpaired electron. The low-spin Fe^{3+} moves into the plane and pulls the His residue down. This affects the remaining protein chain and triggers the uptake/release of oxygen in the other three haeme groups [13] (Figures 7.44 and 7.45).

Cytochromes are part of the mitochondrial electron-transfer chain but also found in the chloroplasts of plants (involved in photosynthesis). There are many different cytochromes; all are involved in reduction–oxidation processes and are grouped into families – cytochromes a, cytochromes b and cytochromes c. They contain a haeme group with an iron centre, which has the ability to change reversibly from Fe(III) to Fe(II), and vice versa. In contrast to the oxygen-coordinated iron centre in haemoglobin, the iron in cytochromes is always six-coordinated. Cytochrome c, for example, is involved in the mitochondrial electron-transfer chain and accepts an electron from cytochrome c1 and transfers it to cytochrome c oxidase. This electron is subsequently used in the reduction of oxygen, where four electrons are needed. This means that actually four cytochrome c transfer an electron to cytochrome c oxidase, where one molecule of O_2 is converted to two molecules of water. Note that Fe(III) forms the core of the oxidised cytochromes whereas Fe(II) is present in the reduced form [13] (Figure 7.46).

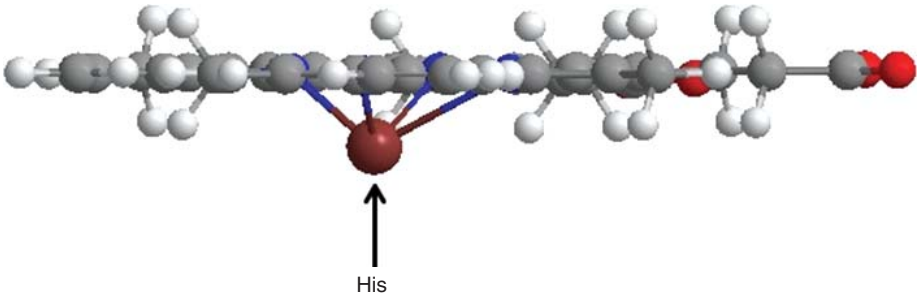


Figure 7.44 *Structure of deoxyhaemoglobin, with the iron atom lowered from the haeme plane*

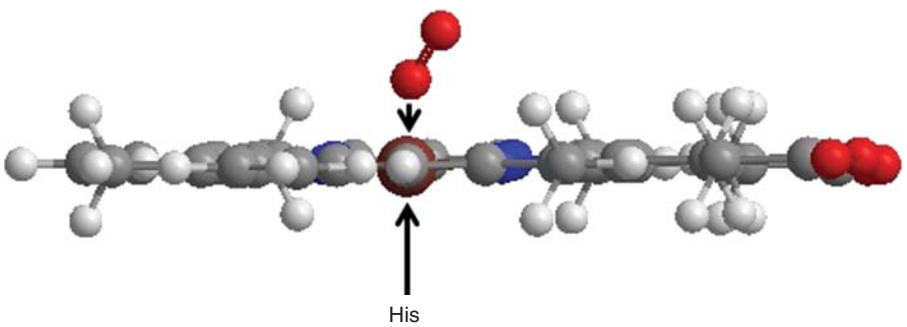


Figure 7.45 *Structure of oxyhaemoglobin, with the iron atom located within the haeme plane and coordinated to the O_2*

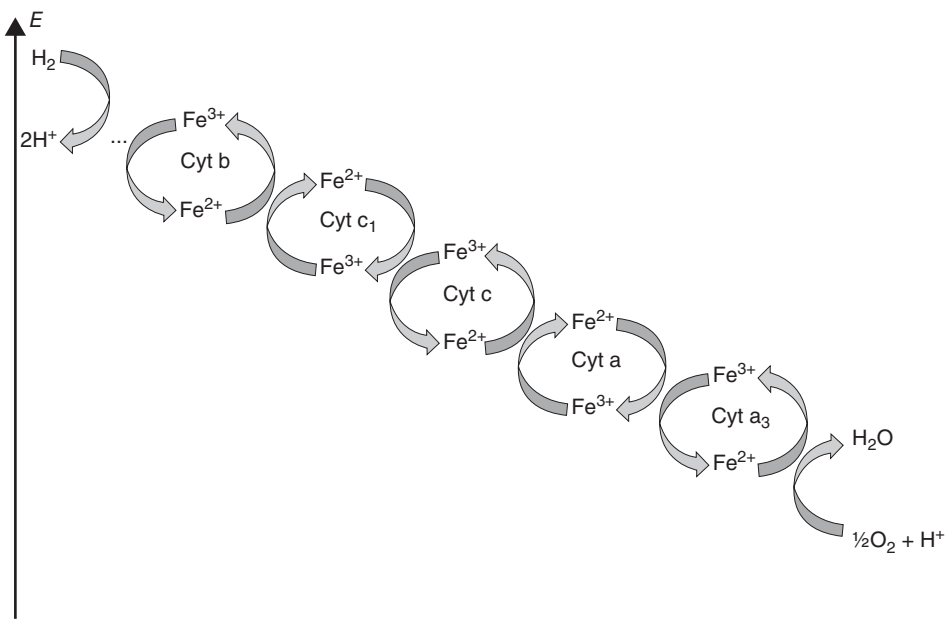


Figure 7.46 *Electron transport chain for cytochromes*

7.3.1.3 Iron uptake and metabolism

The normal diet contains around 15 mg of iron per day and only around 10% is actually absorbed. The absorption is dependent on a variety of factors, such as its bioavailability, the amount of iron present in the food and the body's need for iron. There are phases within a human's life where the body requires an increased amount of iron, such as in pregnancy or growth spurts. The absorption rate can be adjusted accordingly. The bioavailability of iron is highly dependent on the food source. Meat, poultry and fish are rich in easy-to-absorb iron. Absorption can also decrease depending on other dietary components. Some compounds, such as polyphenols (in vegetables) or tannins (in tea), can form chelates with iron.

Iron is actually excreted only in small amounts if there are no major blood losses. The human body regulates the uptake of iron precisely, only replacing the amount of iron lost in order to prevent an iron overload. Typical examples for iron loss would be through superficial GI tract bleeding or menstruation bleeding in women. Nevertheless, typically not more than 3 mg of iron per day is lost.

7.3.1.4 Medicinal use

As discussed above, iron plays a vital role in the human body. The lack of functional iron leads to anaemia, which is characterised by lethargy and weakness. Usually, iron is administered orally as Fe^{2+} or Fe^{3+} salts. Fe^{2+} compounds are more soluble at physiological pH. The advantage of Fe^{3+} salts is that they are not prone to oxidation in aqueous solutions. The most common medicinal preparations include $FeCl_3$, $FeSO_4$, Fe(II) fumarate, Fe(II) succinate and Fe(II) gluconate [13] (Figure 7.47).

The oral dose of Fe^{2+} for the treatment of iron-deficiency anaemia is typically recommended as 100–200 mg/day. In the case of ferrous sulfate ($FeSO_4$), this is equal to 65 mg of Fe^{2+}, which is given three times per day. As a therapeutic response, the haemoglobin concentration should raise about 100–200 mg/100 ml/day. It is recommended continuing the treatment for 3 months once the normal range

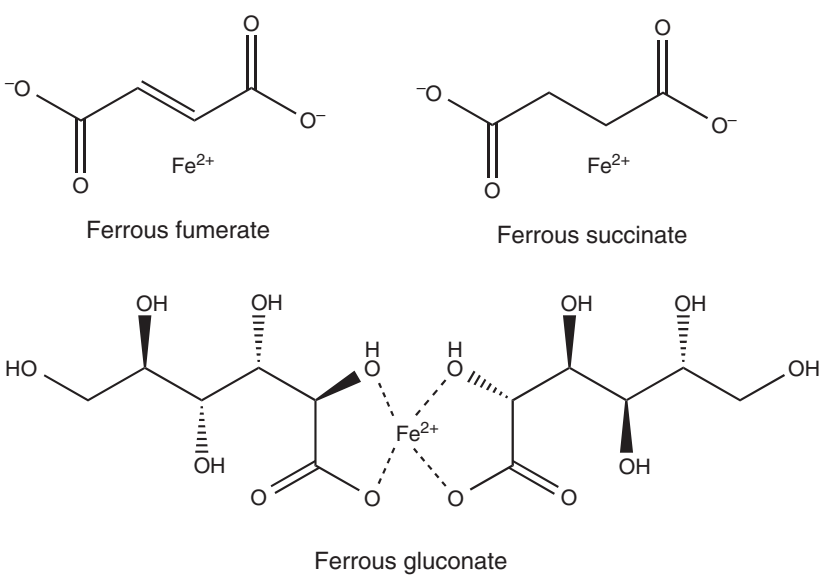


Figure 7.47 *Chemical structure of Fe(II) fumarate, Fe(II) succinate and Fe(II) gluconate*

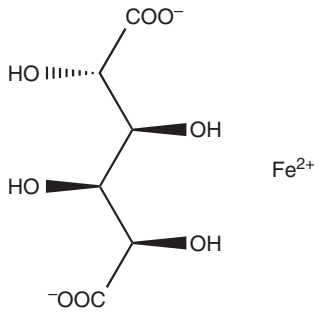


Figure 7.48 *Chemical structures of ferrous sucrose*

is reached. It is known that oral treatment with iron salts can lead to GI irritations [8]. There is very little difference between the efficiency and absorbance rate of the above-mentioned iron salts. The choice of the preparation is influenced by side effects and cost.

Iron salts such as iron dextran and iron sucrose can be administered by IV infusion or IV injection. This administration route should be chosen only when oral therapy is not successful, as there is a risk of anaphylactic reactions. Patients with chronic renal failure who are on haemodialysis treatment often require IV iron supplementation (Figure 7.48).

7.3.1.5 Bleomycin

The drug Bleomycin (BLM) is successfully used as an anticancer agent, and is known to cause fragmentation of the DNA. The drug is used for the treatment of testicular cancer, non-Hodgkin's lymphoma, Hodgkin's lymphoma and cancers of the head and neck area (Cancer research UK). The name Bleomycin describes a family of water-soluble antibiotics that can be isolated from the bacterium *Streptomyces verticillus*. All family members contain the same core structure, a sulfur-containing polypeptide chain, and are only differentiated by a small side group and the sugar moiety [14] (Figure 7.49).

BLM was discovered 1966 by Umezawa *et al.* when they screened the filtrate of *S. verticillus* for cytotoxic activity. The therapeutically active forms of BLM are BLM A_2 and B_2, which differ only in the side chain [2]. BLM is believed to exhibit its anticancer activity by DNA degradation, a process that is dependent on the presence of molecular oxygen, and the binding of a metal to BLM to form the so-called 'activated BLM complex' [15].

The structure of BLM consists of several biologically important units, each contributing to its anticancer activity. Two structural units of importance to highlight are the metal-binding site and the DNA-binding site. It is believed that the intercalation of DNA by BLM occurs via the C-terminus, which contains two thiazole rings and the positively charged sulfonium salt. The positive charges of the sulfur atom can interact with the negatively charged phosphate backbones of the DNA. The metal-binding site can be found at the N-terminus and contains deprotonated amide and histidine groups. The metal is coordinated in a square planar complex, where a primary amine group occupies the axial position [15]. It can coordinate to a variety of metals such as Cu^{2+}, Co^{2+}, Zn^{2+} and Fe^{2+}, but it shows the highest binding affinity to Fe^{2+}. The metal chelation and subsequent activation of molecular oxygen is crucial to the antiproliferative activity of BLM. The carbohydrate core seems to be less involved in the direct anticancer activity. Nevertheless, it has been suggested that it regulates the cellular uptake and indirectly regulates the anticancer activity [14, 15].

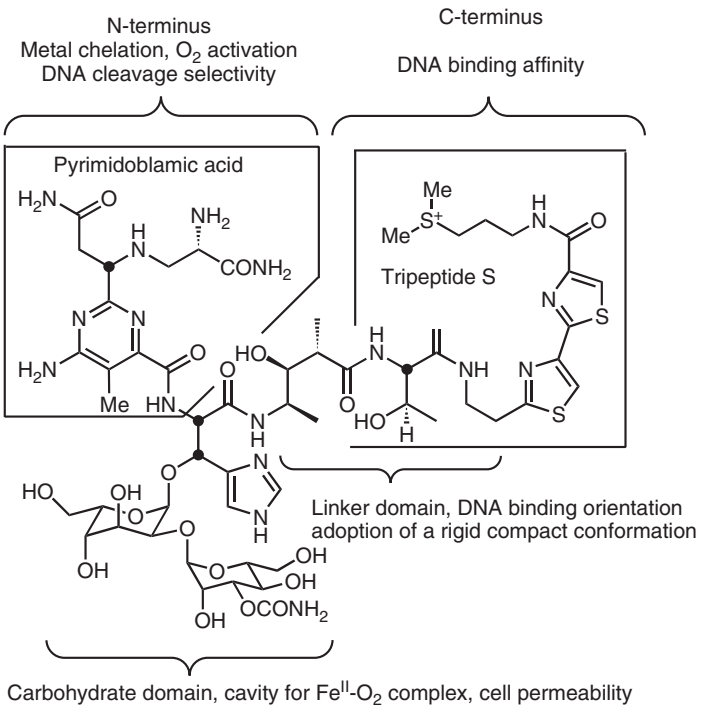


Figure 7.49 *Structure of Bleomycin [15]*

With regard to the mode of action of BLM as an anticancer agent, it is believed to be based on a unique radical mechanism that leads to DNA fragmentation. In the presence of molecular oxygen, the 'activated' BLM complex, HOO–Fe(III)BLM, is formed. This complex is known to cleave double-stranded DNA at the 5′-GC or 5′-GT sites [15].

In the next step, the homolytic splitting of the O—O bond of the HOO–Fe(III)BLM complex produces a radical that is able to degrade DNA. This complex is known to cleave double-stranded DNA at the 5′-GC or 5′-GT sites by abstraction of the C4′-H atom followed by a fragmentation of the deoxyribose backbone [15]. It is believed that the homolytic splitting of the O—O bond and the cleavage of DNA take place in a concerted manner, which means simultaneously. There are also other modes of action known for the DNA degeneration by BLM, but the above-mentioned one is the most prominent one [16].

For a clinical application, usually a mixture of BLM A_2 (~60–70%) and BLM B_2 (~20–30%) and others in combination with other antiproliferative agents are administered J. C. Dabrowiak, Metals in medicine, Wiley-Blackwell, Oxford, 2009. The most commonly used drug Blenoxane (70% BLM A_2) has been developed and marketed by GlaxoSmithKline and is used in the treatment of Hodgkin's lymphoma, testicular cancer and carcinomas of the skin and head and neck [15]. BLM is usually administered intravenously or intramuscularly. It is known to cause very little bone marrow suppression, but has a high dermatological toxicity causing increased pigmentation. The major side effect with the use of BLM is the occurrence of pulmonary fibrosis, which is dose-related. It is therefore important to regularly check the lungs by X-ray and respiratory function in general [8].

7.3.2 Ruthenium

Ruthenium is the chemical element with the symbol Ru and atomic number 44. It occurs as a minor side product in the mining of platinum. Ruthenium is relatively inert to most chemicals. Its main applications are in the area of specialised electrical parts.

The success of cisplatin, together with the occurrence of dose-limiting resistances and severe side effects such as nausea and nephrotoxicity, encouraged the research into other metal-based anticancer agents. Ruthenium is one of those metals under intense research, and first results look very promising, with two candidates – NAMI-A and KP1019 – having entered clinical trials.

7.3.2.1 Ruthenium properties and its biology

Ruthenium has mainly four properties that make it an interesting candidate for drug development: (i) the range of oxidation states, (ii) low toxicity compared to cisplatin, (iii) rate of ligand exchange and (iv) ability to mimic iron binding in biological targets.

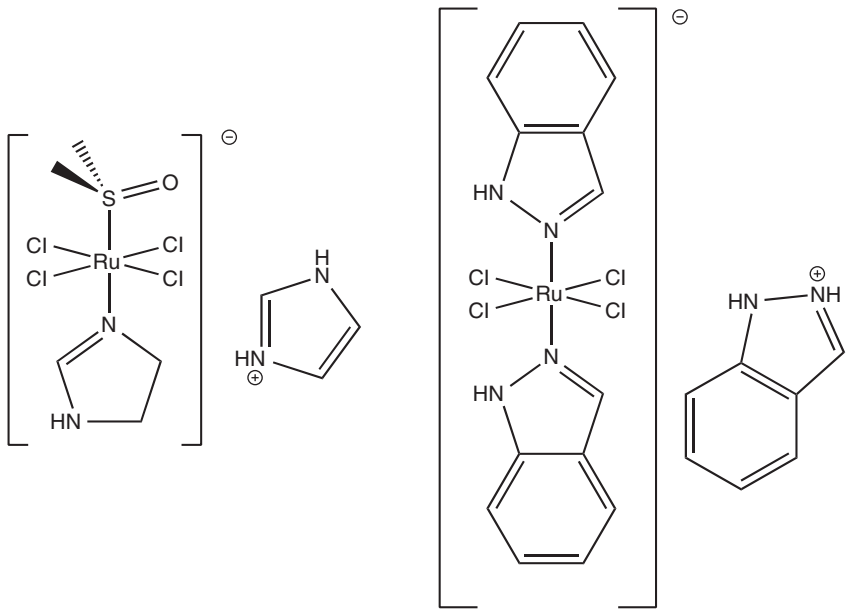
Under physiological conditions, ruthenium can be found in several oxidation states such as II, III and IV, and the energy barrier for conversion between these oxidation states is fairly low. Ru(II) and Ru(III) have been extensively used in drug design, and they preferentially form six-coordinated octahedral complexes. Ru(III) species are the most inert biological species compared to the Ru(II) and Ru(IV) compounds. The redox potential of a complex depends on the ligands and can therefore be modified. For pharmaceutical applications, a Ru(III) complex can be so engineered that it easily reduces to the Ru(II) compound. In a biological environment, Ru(III) and Ru(IV) can be reduced by biomolecules such as GSH and ascorbate. In turn, Ru(II) species can be oxidised by molecular oxygen or enzymes such as cytochrome oxidase. Cancer cells are known to have a generally reducing environment. Therefore, ruthenium complexes can be administered as inert Ru(III) compounds, which are then *in situ* reduced to the active Ru(II) species [17]. This would mean that minimal damage is caused to healthy cells whilst cancerous cells become the target of the active ruthenium species; the result is that ruthenium compounds show a significantly lower toxicity than platinum complexes. Nevertheless, this theory, called *activation by reduction* has been questioned in recent years.

Ligand exchange is an important factor for the activity of metal-based drugs, especially anticancer agents. Only very few metal drugs reach their target in the form they have been administered. Most undergo rapid ligand exchange as soon as there are interactions with macromolecules or water. Some interactions are crucial for the activation of metal-based drugs, but often they hinder their activity. Ruthenium complexes have similar ligand exchange kinetics as cisplatin, with Ru(III) compounds being the most inert complexes. In contrast to cisplatin, which forms square planar complexes, ruthenium complexes are octahedral and therefore there is room for two more ligands compared to cisplatin to engineer the potential drug.

Iron and ruthenium are both members of group 8 within the periodic table of elements, and therefore researchers have inquired whether ruthenium can utilise the transport mechanisms normally used by iron. Iron is a key element for many biological processes; nevertheless, it is toxic for biological systems in its isolated form. It is believed that the low toxicity of ruthenium is a result of its iron-mimicking ability. There have been several hypotheses suggesting that ruthenium could use transferrin and albumin as transport mechanisms instead of iron or in a 'piggy-back' mechanism where ruthenium binds to the outside of transferrin when it is loaded with iron.

7.3.2.2 Ruthenium-based anticancer agents

KP1019 and NAMI-A are two ruthenium compounds that have entered clinical trials. They are classical coordination compounds in which the central ruthenium atom is coordinated by Lewis bases in an octahedral



(a) (b)

Figure 7.50 *Chemical structures of and (a) NAMI-A and (b) KP1019*

arrangement. Both complexes are based on a Ru(III) core, and the ligands are typically chloride and organic groups. This means that the complex can easily be hydrolysed and converted into its active form. Despite their similar structures, both ruthenium compounds present a different anticancer activity. KP1019 is active against primary cancers, whereas NAMI-A is active against metastasis, secondary tumours that have moved to other areas. The treatment of metastasis is currently an area where improvement is urgently needed (Figure 7.50).

NAMI-A has shown activity in the treatment of metastatic cancer and has completed phase I clinical trials in the Netherlands. It has been shown that the complex is relatively nontoxic. Significantly higher doses than cisplatin (above 500 mg/m^2/day) lead to side effects such as blisters on the extremities. Within the study, the ruthenium complex was administered intravenously over a period of 3 h in a 0.9% saline solution (pH ~ 4) [2]. The NAMI-A is synthesised by reacting $RuCl_3 \cdot 3H_20$ with HCl and DMSO (dimethylsulfoxide). This reaction results in the *trans* complex Imidazolium *trans*-imidazoledimethyl sulfoxide-tetrachlororuthenate(III) (NAMI-A) [2] (Figure 7.51).

It is interesting to note that Imidazolium *trans*-imidazoledimethyl sulfoxide-tetrachlororuthenate(III) is a paramagnetic compound and the complex is quickly hydrolysed in water. Initially, one chloride ligand is replaced by an aquo ligand, but the DMSO ligand is quickly replaced as well. As previously mentioned, it has been suggested that the complex is activated by bio-reduction of the Ru(III) centre to Ru(II) in the hypoxic environment of cancer cells. There is not much knowledge at present about the biological target for Imidazolium *trans*-imidazoledimethyl sulfoxide-tetrachlororuthenate(III). It is known that it interacts with the imidazoles of proteins and that the interaction with DNA is only weak, questioning DNA as a primary target for the ruthenium drug.

KP1019, *trans*-[tetrachloro-bis(1*H*-indazole)ruthenate(III)], was tested in phase I clinical trials as a possible treatment option against colon cancer. Administered doses ranged from 25 to 600 mg twice weekly, and no significant side effects were noticed. The synthesis of KP1019 also starts with $RuCl_3 \cdot 3H_20$, which is dissolved in ethanolic HCl and reacted with an excess of indazole (Figure 7.52).

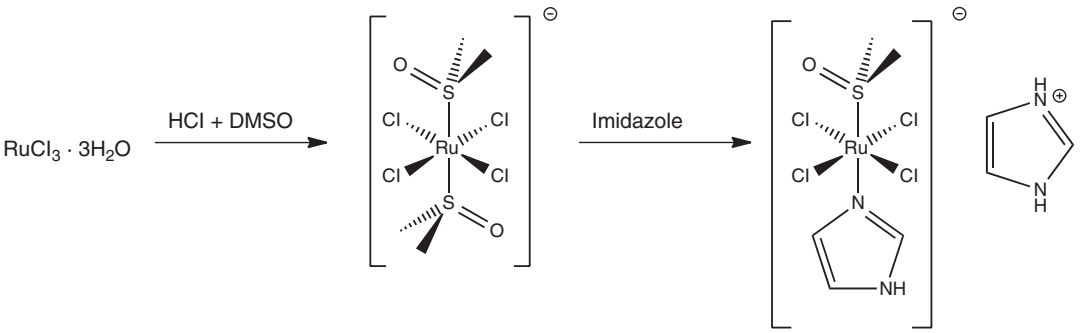


Figure 7.51 *Synthesis of NAMI-A [2]*

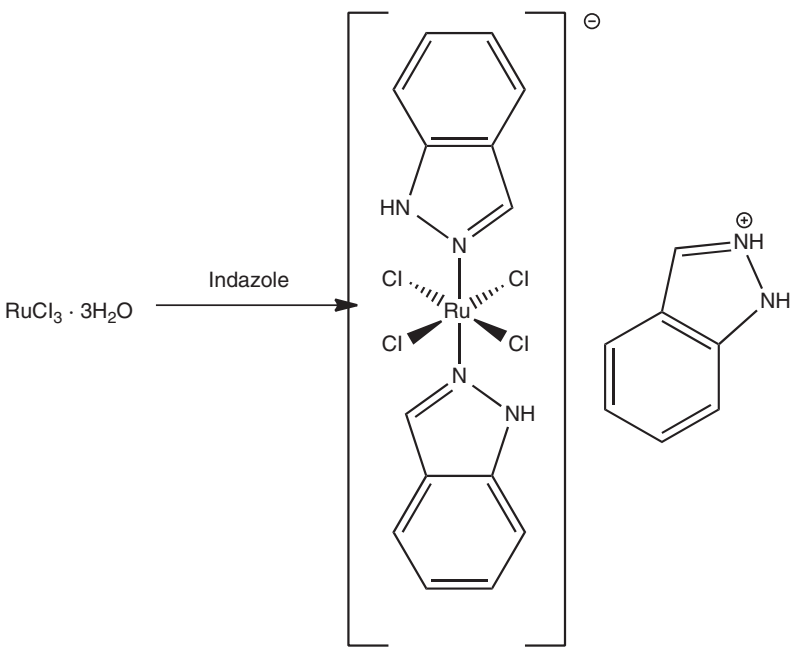


Figure 7.52 *Synthesis of KP1019 [2]*

The ruthenium complex hydrolyses quickly in water and induces apoptosis in cancer cells, potentially by blocking the mitochondrial function. KP1019 is believed to be transported by the protein transferrin to the tumour cells which are known to have a greater number of transferrin receptor on their cell surface.

RAPTA is an organometallic ruthenium complex (see Chapter 8 for the definition of organometallic complex) which is highly water-soluble. The Ru(II) centre is coordinated by chloride, an aromatic ring and 1,3,5-triaza-7-phosphatricyclo-[3.3.1.1]decane which is a form of phosphaadamantane. Interestingly, it shows similar biological activity as Imidazolium *trans*-imidazoledimethyl sulfoxide-tetrachlororuthenate(III) despite the differences in geometry, ligands and oxidation state. This shows that the active species in the cancer cell is different from the administered active pharmaceutical ingredient (API) (Figure 7.53).

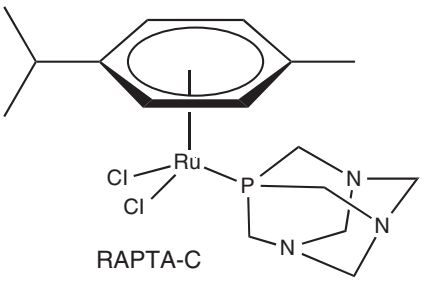


Figure 7.53 *Chemical structure of RAPTA*

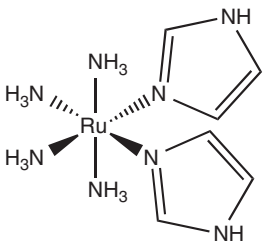


Figure 7.54 *Chemical structure of Ru(III) imidazole*

In summary, the mode of action for ruthenium complexes is still under investigation, and there are a variety of biological targets. It is believed that interaction with proteins significantly contributes to their anticancer activity. Nevertheless, a number of ruthenium complexes have been shown to bind to DNA. The mode of binding to DNA differs from cisplatin, as ruthenium complexes form cross-links between DNA strands probably due to steric hindrance by their octahedral geometry. It has also been shown that Ru(II) species are much more reactive towards DNA than Ru(III) and Ru(IV) compounds, the latter two can potentially be seen as less toxic prodrugs.

7.3.2.3 Further medical applications of ruthenium-based complexes

Ruthenium complexes have been under investigation as immunosuppressants. Cyclosporin A has severe side effects, such as hypertension, nephrotoxicity and nausea; hence there is a drive to search for new drugs. Ru(III) imidazole $[Ru(NH_3)_4(Im)_2]$ is a fairly stable complex that has been shown to inhibit the T-cell proliferation at nanomolar level [17] (Figure 7.54).

Ruthenium complexes have also shown promising results when initially tested as antimicrobials and antibiotics. Especially in the fight against malaria, new compounds are desperately needed as the Plasmodium parasite has become resistant to many traditional treatment options, mainly chloroquine. Research has shown that the Ru(II) chloroquine complex is significantly more effective, and it is suggested that the uptake of the metal complex follows a different route. Similar results have been seen when ruthenium compounds were tested as antibiotics [18].

H																	He
Li	Be											B	C	N	O	F	Ne
Na	Mg											Al	Si	P	S	Cl	Ar
K	Ca	Sc		Ti	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	Ga	Ge	As	Se	Br	Kr
Rb	Sr	Y		Zr	Nb	Mo	Tc	Ru	Rh	Pd	Ag	Cd	In	Sn	Sb	Te	I	Xe
Cs	Ba	La	Ce-Lu	Hf	Ta	W	Re	Os	Ir	Pt	Au	Hg	Tl	Pb	Bi	Po	At	Rn
Fr	Ra	Ac	Th-Lr	Rf	Db	Sg	Bh	Hs	Mt	Ds	Rg	Uub						

Figure 7.55 *Periodic table of elements; the coinage metals are highlighted*

7.4 The coinage metals

Historically, the three nonradioactive members of group 11 of the periodic table (11th vertical column) are designated as coinage metals, consisting of copper (Cu), silver (Ag) and gold (Au) (Figure 7.55).

Metals used to make coins have to fulfil special requirements, considering that a coin may be in circulation for several decades. Therefore, a coin needs to have anticorrosive properties and should not show any significant signs of wear. Very often, coinage metals are therefore mixed with other metals to form a so-called alloy. These alloys are harder and often more resistant to everyday use than the metals themselves.

Quite often, there is a problem that for low denomination coins the face value is significantly lower than the value of the metal itself. For example, the modern British penny is made out of steel plated with copper, whereas the American penny is made out of zinc with a copper covering.

7.4.1 General chemistry

Group 11 metals, such as Cu, Ag and Au, are known as the *noble metals* and belong to the d-block or transition metals. They are all relatively inert and corrosion-resistant metals and therefore are useful for the production of coins. All three metals are excellent conductors of electricity and heat. The most conductive metal for electricity is Ag, followed by Cu and then Au. Silver is the most thermally conductive element and the most light-reflecting element. Copper is widely used in electrical wiring and circuitry. In precision equipment, where the risk of corrosion needs to be kept as low as possible, gold is quite often used. Silver is widely used in mission-critical applications such as electrical contacts as well as in agriculture, medicine and scientific applications. Probably, one of the best known applications of silver ions and silver is the use in photography. Upon exposure, the silver nitrate in the film reverts to metallic silver itself.

Silver, gold and copper are all quite soft metals and therefore are not very useful as weapons or tools. Nevertheless, because of their malleability, they have been and still are used to make ornaments and jewellery.

The electronic configuration of group 11 metals is shown in Figure 7.56.

Cu : $[Ar]3d^{10}4s^1$

Ag : $[Kr]4d^{10}5s^1$

Au : $[Xe]4f^{14}5d^{10}6s^1$

Figure 7.56 *Electronic configuration of group 11 metals*

Silver and gold are generally inert and not attacked by oxygen or nonoxidising acids. Silver dissolves in salpetric acid, and in the presence of H_2S it forms Ag_2S. Gold dissolves in concentrated hydrochloric acid, forming $[AuCl_4]^-$ in the presence of an oxidising agent.

Both metals react with halogens. Ag(I), Au(III) and Au(I) are the dominant oxidation states, whereas the dominant oxidation states of copper are Cu(II) and Cu(I). Typical examples for the oxidation states III include AuF_3, $AuCl_3$ and $AuBr_3$, whereas there is only AgF_3. There are many examples for silver and gold salts where the metal takes the oxidation state I.

7.4.2 Copper-containing drugs

Copper is a valuable metal and has been mined for more than 2000 years. It has had many uses throughout history. Initially, copper was mainly used to make alloys such as brass and bronze, which are harder and stronger than copper itself. Nowadays, copper is mainly used because it conducts heat and electricity (e.g. wiring) and it is corrosion-resistant (e.g. as roofing material).

Historically, copper was used for the treatment of a variety of diseases, including chronic ulcers, headaches, ear infections, rheumatoid arthritis (RA), and so on. In 1832, copper workers were found to be immune to an outbreak of cholera in Paris, which stimulated further research into the medicinal use of copper. Almost every cell in the human body uses copper, as most contain copper-dependent enzymes. Unfortunately, excessive amounts of copper are toxic for the human body, whereas low amounts of copper also lead to health problems, manifested in Menkes disease.

Copper ions from food sources are processed by the liver, and transported and excreted in a safe manner. Inorganic metallic copper from sources such as drinking water mainly enters the blood directly and can be toxic as it can penetrate the blood–brain barrier. Typically, 50% of the daily copper intake is absorbed in the GI tract and transported to the liver from where it is transported to the peripheral tissue bound to ceruloplasmin, a copper-binding glycoprotein. A smaller amount of copper is also bound to albumin. Excess copper is mainly excreted in bile into the gut and then the faeces.

Copper is an essential trace metal, and copper ions are incorporated into a number of metalloenzymes – so called *cuproenzymes*. In the human body, the majority of copper ions can be found as Cu^{2+}; nevertheless, the oxidation state shifts between the cuprous (Cu^+) and cupric (Cu^{2+}) forms. It is important that copper can accept and donate electrons easily for its role in a variety of cuproenzymes, and examples are outlined in the following. Lysyl oxidase, a cuproenzyme, is responsible for the cross-linking of collagen and elastin, which forms strong and at the same time elastic tissue connections. These tissues are used, for example, for the formation of blood vessels and the heart. Ceruloplasmin (ferroxidase I) and ferroxidase II, two copper-based enzymes, can oxidise ferrous iron to ferric iron. Ferric iron can then be transported with the help of transferrin, for example, to form red blood cells. Furthermore, a variety of copper-dependent enzymes, such as cytochrome c and superoxide dismutase, work as antioxidants and are involved in the reduction of reactive oxygen species (ROS). There are also a variety of cuproenzymes that are involved in the synthesis (dopamine-b-monooxygenase) and metabolism (monoamine oxidase) of neurotransmitters [19].

7.4.2.1 Wilson disease

Wilson disease is a genetic disorder in which excessive amounts of copper build up in the human body. The copper is mainly stored in the liver and brain, and therefore causes liver cirrhosis and damage to the brain tissue. The damage to the brain tissue occurs mainly at the lenticular nucleus and a typical brown ring is visible around the iris; therefore Wilson disease is also called *hepatolenticular degeneration*.

Wilson disease was first described in 1912 by Wilson, pointing out the 'progressive lenticular degeneration' accompanied by chronic liver disease, which was leading to cirrhosis. It was Kayser and Fleischer who later made the association between these symptoms and a deposition of copper in the human body. Later on, it was established that Wilson disease is a genetic disorder with an autosomal recessive inheritance pattern. The abnormal gene was identified to be ATP7B, a metal-transporting adenosine triphosphatase (ATPase) mainly expressed in hepatocytes, which has the main function of transporting copper across the membrane. This means that, in the absence of this gene, the excretion of copper via the liver is reduced. This leads to copper accumulation in the liver, which damages the liver and eventually releases copper into the blood stream from where it can further poison the organs. Mainly the brain, kidneys and the cornea are affected by the copper accumulation.

Wilson disease presents itself mostly as a liver disease or a psychological illness and was fatal until treatments were developed. The main route of treatment is chelation therapy (see Chapter 11), with British anti-lewisite (BAL) being the first chelating agent used in 1951. In 1956, the orally available chelating agent D-penicillamine simplified the treatment of Wilson disease. Further treatment options include liver transplant, which has the potential to cure these patients [20]. Zinc supplementation can also be used in patients with Wilson's disease, as it prevents the absorption of copper ions (see Section 7.5). It is important to note that this therapy has a slow onset time and chelating treatments have to be continued for 2–3 weeks after the start with zinc supplementation [8].

7.4.2.2 Copper and wound healing

Glycyl-L-histidyl-L-lysine (GHK) is a tripeptide known for its high binding affinity to Cu^{2+} and its complex role in wound healing. The GHK–Cu(II) complex was isolated from human plasma in the 1970s and it was shown to be an activator for wound healing. GHK–Cu(II) has two main functions: as an anti-inflammatory agent to protect the tissue from oxidative damage after the injury, and as an activator for wound healing itself as it activates the tissue remodelling [21].

The structure of GHK is very similar to that of common drugs used to treat ulcers (Figures 7.57–7.59).

After the initial stages of wound healing are activated, such as blood coagulation and neutrophil invasion, a second stage of wound healing begins, which includes the population of GHK at the wound, which has a high affinity to Cu^{2+}. Mast cells, which are located in the skin, secrete GHK, which accumulates Cu^{2+} and forms the copper complex GHK–Cu(II) and therefore increases the metal–tripeptide concentration at the wound. First, GHK–Cu(II) has an anti-inflammatory effect by protecting the tissue from oxidative damage and by suppressing local inflammatory signals (i.e. cytokine interleukin-1 (IL-1)). Second, GHK–Cu(II) is released into the blood stream and encourages the production of wound macrophages that support the wound repair by removing the damaged tissue and secreting a family of several growth factor proteins. GHK–Cu(II) also hinders fibroblast production of TGF-β-1 and therefore suppresses the scar development. The GHK–Cu(II) complex also stimulates the growth of blood vessels, neurons and elastin, and, in general, supports most processes of wound healing [7].

Because of its versatile properties during the wound-healing process, it is not surprising that researchers tried to design commercial products based on GHK–Cu(II). Initial results were promising, but unfortunately the stability of the tripeptide GHK was not sufficient enough resulting in rapid breakdown. In the human body, GHK is permanently reproduced and therefore stability issues are not a major problem [22].

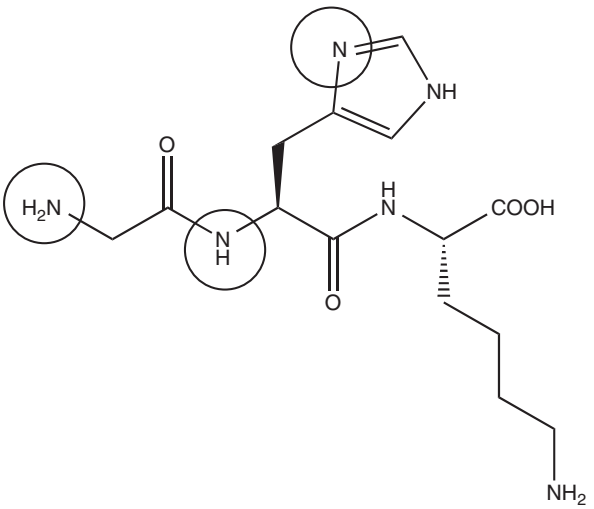


Figure 7.57 *Chemical structure of GHK; potential atoms for coordination to Cu^{2+} are circled*

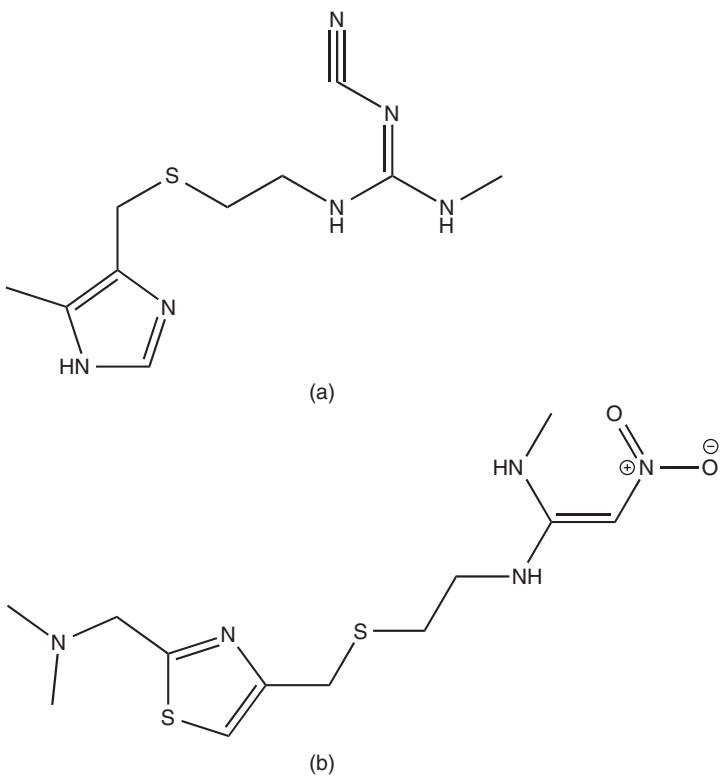


Figure 7.58 *Chemical structure of commonly used antiulcer drugs: (a) cimetidine and (b) nizatidine*

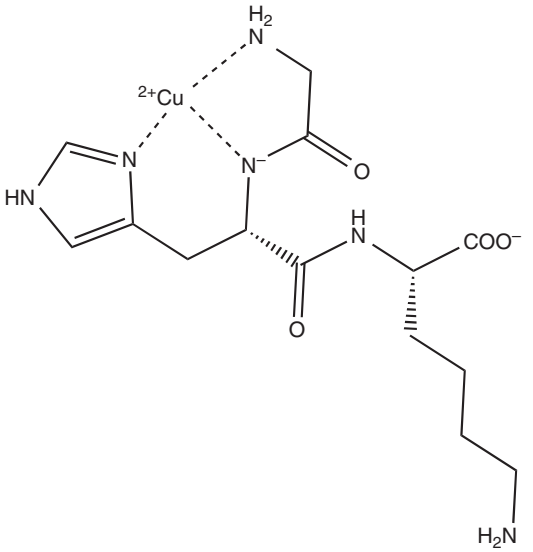


Figure 7.59 *Chemical structure of the GHK–Cu(II) complex*

7.4.2.3 Copper and cancer

Cancer progression has been linked to increased ceruloplasmin and copper levels in a variety of tissues. Copper deficiency has been considered as an anticancer strategy, but several clinical studies have not been encouraging. The exact role of copper in cancer is not yet fully understood, but it is possible to be involved via oxidation processes and the production of ROS and its involvement in angiogenic processes, as copper is believed to stimulate proliferation of endothelial cells [23].

7.4.3 Silver: the future of antimicrobial agents?

The name silver is derived from the Saxon word 'siloflur', which has been subsequently transformed into the German word 'Silabar' followed by 'Silber' and the English word 'silver'. Romans called the element 'argentum', and this is where the symbol Ag derives from.

Silver is widely distributed in nature. It can be found in its native form and in various ores such as argentite (Ag_2S), which is the most important ore mineral for silver, and horn silver (AgCl). The principal sources of silver are copper, copper–nickel, gold, lead and lead–zinc ores, which can be mainly found in Peru, Mexico, China and Australia.

Silver has no known active biological role in the human body, and the levels of Ag^+ within the body are below detection limits. The metal has been used for thousands of years mainly as ornamental metal or for coins.

Furthermore, silver has been used for medicinal purposes since 1000 BC. It was known that water would keep fresh if it was kept in a silver pitcher; for example, Alexander the Great (356–323 BC) used to transport his water supplies in silver pitchers during the Persian War. A piece of silver was also used, for example, to keep milk fresh, before any household refrigeration was developed. In 1869, Ravelin proved that silver in low doses acts as an antimicrobial. Around the same time, the Swiss botanist von Nägeli showed that already at very low concentration Ag^+ can kill the green algae spirogyra in fresh water. This work inspired the gynaecologist Crede to recommended use of $AgNO_3$ drops on new born children with conjunctivitis.

In 1884, Crede introduced the application of a 1% silver nitrate solution for the prevention of blindness in newborn, and the results were so impressive that this still used nowadays in America [24].

Today, airlines and NASA rely on silver filters to guarantee good water quality on board their aircrafts. A contact time of hours is necessary to see a disinfectant against coliforms and viruses of silver in water. In the concentrations normally applied, silver does not show any impact on the taste, odour or colour of the water. There is also no negative effect on human cells known. The only negative health effect known is called *argyria*, which is an irreversible darkening of the skin as a result of a prolonged application of silver. The existence of silver-resistant organisms is probably one of the major drawbacks for silver-based therapies.

7.4.3.1 Silver ions and their medicinal use

The antibacterial activity of silver and silver ions has long been known and led to many applications. This is mainly due to the fact that its toxicity to human cells is considerably lower than to bacteria. Most commonly, it is used for the prophylactic treatment of burns and in water disinfection. Unfortunately, the mechanisms and the chemistry of silver ions in biological organisms are so far not entirely clear. von Nägeli proposed the so-called oligodynamic effect, which describes the toxic effect of metals on organisms. It has been proposed that silver ions irreversibly damage key enzymes in the cell membrane of bacteria. This would lead to an inactivation of the pathogen. Silver reacts, as many other transition metals, preferentially with the thiol groups and also amino, carboxyl, phosphate and imidazole groups.

Silver nitrate ($AgNO_3$), after salicylic acid, is widely used for the treatment of warts. $AgNO_3$ is a highly water-soluble salt, which readily precipitates as AgCl, black in colour, when in contact with the skin. Warts are caused by a human papillomavirus, and mostly hands, feet and the anogenital areas are affected. The treatment is based on the destruction of the local tissue, and the silver salt is applied via a caustic pen to the affected area. Silver nitrate is highly corrosive and is known to destroy these types of tissue growth. Care has to be taken when this treatment option is used, as the resulting AgCl stains any skin or fabric which it has been in contact with.

7.4.3.2 Silver(I) sulfadiazine

Silver sulfadiazine is indicated for the prophylaxis and treatment of infections in burn wounds. Silver sulfadiazine is highly insoluble in water, and as a result, it does not cause hypochloraemia in burns in contrast to silver nitrate.

The active ingredient silver sulfadiazine is a sulfur-derived topical antibacterial used primarily on second- and third-degree burns. It is known to be active against many Gram-negative and Gram-positive bacteria as well as against yeast. The cream is kept applied to the burned skin at all times for the duration of the healing period or until a graft is applied. It prevents the growth of a wide array of bacteria, as well as yeast, on the damaged skin. Caution has to be given to large-area application as sulfadiazine levels in the plasma may well reach therapeutic levels that can cause side effects (Figure 7.60).

7.4.3.3 Silver dressings

All kinds of dressings containing silver ions have become more and more popular because of their antimicrobial effect. Nevertheless, the effect of silver nanoparticles on wound healing is still under discussion. Also, the use of dressings containing silver sulfadiazine and hydrocolloid dressings containing silver for the treatment of foot ulcers is still under discussion [25].

So far, clinical recommendation suggests that antimicrobial dressings containing silver should be used only when there are clinical signs of an infection present. They should not be routinely used for wound dressing

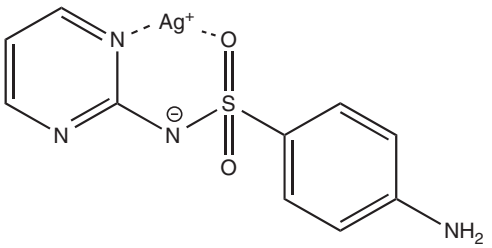


Figure 7.60 *Chemical structure of silvadene*

or the treatment of ulcers. Silver dressing will work only in the presence of wound secretion, as only then the silver ions will show an antimicrobial effect. There is also some evidence that silver dressings delay the healing process of acute wounds and therefore silver dressings are not recommended for use in those cases [8].

7.4.4 Gold: the fight against rheumatoid arthritis

From ancient times, gold has been regarded as one of the most beautiful metals and ever since treasured by man. The metal was first used to make tools, weapons and jewellery but was soon used for trade and as coins. Gold is a soft yellow metal, which is characterised by its high ductility. Very often, gold is alloyed with other metals to give it more strength. For example, white gold is gold alloyed with palladium.

Gold has also a long-standing tradition in medicine, as it has been used by many nations for thousands of years. From as early as 2500 BC, Arabians, Chinese and Indians used gold compounds for medicinal purposes. In mediaeval times, the elixir 'aurum potabile', which was an alcoholic mixture of herbs with some gold flakes, was sold by medicine men travelling around Europe and this elixir was supposed to cure most diseases. In the nineteenth century, $Na[AuCl_4]$ was reported to treat syphilis, whilst others used it to cure alcoholism. On a more serious note, Koch discovered in 1890 the antibacterial properties of gold cyanide. *In vitro* experiments with the *Mycobacterium tuberculosis* showed that gold cyanide has the potential as a tuberculosis therapy. Gold compounds were also investigated for the treatment of RA, when it was believed that RA was caused by bacteria, and many other health problems [7].

7.4.4.1 Gold therapy for rheumatoid arthritis

RA is a chronic, inflammatory, progressive autoimmune disease that primarily affects the joints. The disease is characterised by swelling of the joints and increasing pain leading to stiffness. Inflammation occurs initially in the synovial membrane surrounding the joints and then spreads to the synovium. An irreversible erosion of the articular cartilage on the bone joints means that bones will directly rub against each other and cause severe pain. The peak period of onset is between 35 and 55 years of age, with premenopausal women more often affected than men (ratio of 3 : 1). There is no obvious inheritance pattern to be found, but a genetic predisposition seems to be underlying. Patients are diagnosed on mainly three factors: painful joints, inflammation and the presence of the so-called rheumatoid factor.

Treatment options include anti-inflammatory drugs and/or disease-modifying antirheumatic drugs (DMARDs). Anti-inflammatory drugs comprise mostly nonsteroidal anti-inflammatory drugs (NSAIDs) or corticosteroids. NSAIDs are the first drugs of choice for the treatment of mild RA, as they possess analgesic and anti-inflammatory properties and also can be given in conjunction with DMARDs. Patients respond and tolerate NSAIDs quite variably, but generally the onset is rather quick. If there is no relief within 2–3 weeks,

the treatment options are reconsidered. Corticosteroids are the most potent anti-inflammatory agents and additionally can be used as immunosuppressants. Corticosteroids are very commonly used in RA despite their severe side effects, which really limit their application. Additionally, corticosteroids have very little influence on the disease itself, but they are invaluable when the pain gets intolerable and mobility is severely decreased.

DMARDs are a category of unrelated drugs that are used in RA to slow down the progression of the disease. In contrast, NSAIDs treat only the inflammation, and corticosteroids are also insufficient to slow down the progression of RA. DMARDs are comprised of immune modulators, sulfasalazine and gold compounds amongst others, and they are used when the RA progresses from a mild to a more severe form. The onset time for DMARDs is significantly longer than for NSAIDs and it can take 2–6 months until any effect is seen. Typically, irreversible joint damage is already observed in the 2 years following diagnosis of RA, and therefore it is recommended to consider DMARDs as soon as moderate to severe RA is diagnosed. Some patients respond well to a combination therapy of NSAIDs and DMARDs, but patient response varies from individual to individual. In general, DMARDs have a higher toxicity and therefore come with significantly more side effects than NSAIDs. Gold salts are long known for their therapeutic effects in RA. In general, the application of gold compounds to treat diseases is called *chrysotherapy*, with RA being the main application area. Gold salts are clinically available as oral and intramuscular formulations.

> ***Chrysotherapy*** *is the treatment of certain diseases, especially RA, with gold compounds. Side effects can be quite severe and dose-limiting and include discolouration of skin, diarrhoea, nausea, flushing, vomiting, metallic taste in mouth and even damage of the kidneys and liver* [26].

7.4.4.2 Examples of gold-containing DMARDs

Au(I)thiolates, such as aurothioglucose, disodium aurothiomalate and trisodium bis(thiosulfato)gold, are the first-generation gold-based DMARDs. They feature linear, two-coordinated Au(I) thiolates and are polymeric with the exception of trisodium bis(thiosulfato)gold. The thiolate group stabilises the oxidation state of +1 for the gold atom, and therefore hinders disproportionation to Au(O) and Au(II).

> ***Disproportionation*** *is a redox reaction in which a species is oxidised and reduced at the same time and two different products are formed.*

The first-generation gold drugs are water soluble because of their hydrophilic groups and they are therefore commonly administered by intramuscular injection. The injection has to be done by a healthcare professional, which means the patient requires regular visits to the clinic. These gold-based compounds typically accumulate in the kidneys, where they are nephrotoxic and cause a leakage of proteins at the glomerulus. Typically, chrysotherapy is discontinued if there is no beneficial effect seen after 6 months, but side effects often last longer.

Sodium aurothiomalate is a commonly used gold-based DMARD and is indicated for active progressive RA. It is administered by deep intramuscular injection. Administration is started with a test dose of 10 mg followed by weekly intervals of 50 mg doses. An improvement is expected to be seen once 300–500 mg is administered. Treatment should be discontinued if there is no improvement after administering 1 g or 2 months. Intervals of administration should be gradually increased to 4 weeks in patients in whom an effect

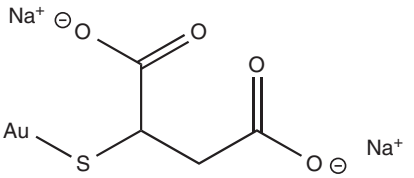


Figure 7.61 *Chemical structure of sodium aurothiomalate*

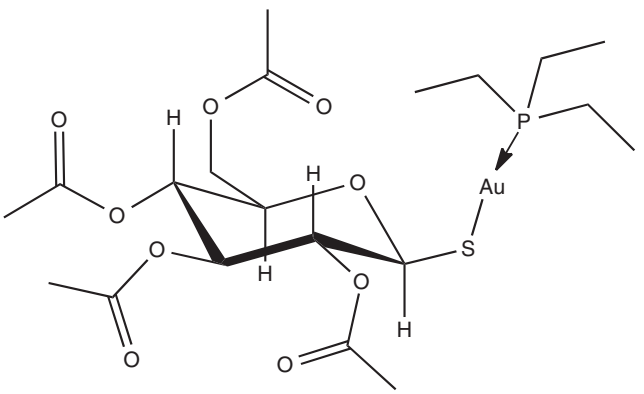


Figure 7.62 *Chemical structure of Auranofin*

can be seen. If any blood disorders or other side effects such as GI bleedings or proteinuria are observed, sodium aurothiomalate should be discontinued [8, 27] (Figure 7.61).

A second-generation gold-based drug came on the market, called *Auranofin* – ([tetra-*O*-acetyl-β-D-(glucopyranosyl)thio]-triethylphosphine)gold(I) – licensed as an orally available gold drug for the treatment of RA. It features a linear S–Au–P geometry, as shown by X-ray analysis. It is more lipophilic than the first-generation drugs, which makes oral administration possible. Treatment with Auranofin requires less visits to the clinic, but it is believed to be less successful in the treatment of RA compared to gold drugs being administered intramuscularly (Figure 7.62).

7.4.4.3 Metabolism of gold drugs

The clinically used gold drugs can be considered as prodrugs, which undergo rapid metabolism *in vivo* to form active pharmacological species. The precise mechanism of action is not known, but most probably it involves a thiol exchange. This means that the thiolate ligand will be replaced by a biological thiol such as albumin, which is a major protein in the serum and is sulfur-rich. Cysteine-34, one of the cysteine residues in albumin, is likely to be deprotonated at physiological pH and is a likely target for the gold compounds. In the case of Auranofin, the triethylphosphine (Et_3P) is oxidised, whilst the disulfide link in the albumin is reduced, and the harmless oxidised species $Et_3P{=}O$ is excreted via the kidneys.

The water-soluble first-generation gold drugs are injected intramuscularly and interact with the cells directly. They do not enter the cells but bind to the cell membranes via thiol groups on the cell surface and interfere with normal cell signalling pathways. Orally available gold drugs, such as Auranofin, enter the cells by a so-called thiol shuttle. Auranofin is transported to the cell, where it reacts with sulfhydryl-dependent

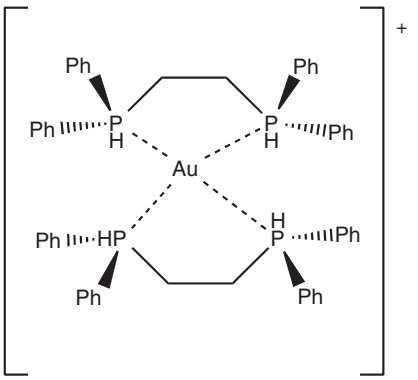


Figure 7.63 *Chemical structure of $[Au(dppe)_2]^+$*

membrane transport proteins (MSH), which are present on the cell membrane. The Et_3PAu^+ moiety binds to MSH and is transported into the cell, where the phosphine is oxidised as explained earlier and the Au moiety binds to the proteins. The thiolate ligand remains outside the cell. The gold cation can also leave the cell using the same mechanism, independent of the phosphine ligand.

7.4.4.4 Further pharmacological potential of gold complexes

The major clinical use of gold complexes relates to RA. Nevertheless, screening for the *antitumour activity* of antiarthritic gold drugs has encouraged studies towards the use as anticancer drugs. Au(III) species are isoelectronic to cisplatin (the most widely used metal-based anticancer drug).

> *The term **isoelectronic** describes two or more molecular entities that have the same number of valence electrons and the same chemical structure independent of the actual elements present.*

The Au(I) complex of bis(diphenylphosphino)ethane (dppe) has an exciting cytotoxic profile when tested *in vitro* and *in vivo*. Experiments have shown that it was more effective when co-administered with cisplatin than each of the compounds individually. Unfortunately, no further studies were undertaken; especially, clinical trials could not proceed, as an acute toxicity to heart, liver and lungs was detected. Furthermore, some *antimicrobial* activity and some *antimalarial* activity of Au(I) complexes have been reported [7] (Figure 7.63).

7.5 Group 12 elements: zinc and its role in biological systems

The three natural occurring elements of group 12 of the periodic table (12th vertical column) are zinc (Zn), cadmium (Cd) and mercury (Hg) (Figure 7.64).

There is no significant abundance of the metals zinc, cadmium and mercury in the earth's crust, but they can be obtained from the respective ores. Zinc blende (ZnS) and sphalerite [(ZnFe)S] are the main sources of zinc, whereas CdS-containing ores are the only ores of importance for cadmium extraction. In order to obtain the pure metal, the relevant ores are roasted and the metal oxides are isolated. The corresponding metal is then extracted under high temperatures in the presence of carbon.

H																	He
Li	Be											B	C	N	O	F	Ne
Na	Mg											Al	Si	P	S	Cl	Ar
K	Ca	Sc		Ti	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	Ga	Ge	As	Se	Br	Kr
Rb	Sr	Y		Zr	Nb	Mo	Tc	Ru	Rh	Pd	Ag	Cd	In	Sn	Sb	Te	I	Xe
Cs	Ba	La	Ce - Lu	Hf	Ta	W	Re	Os	Ir	Pt	Au	Hg	Tl	Pb	Bi	Po	At	Rn
Fr	Ra	Ac	Th -Lr	Rf	Db	Sg	Bh	Hs	Mt	Ds	Rg	Uub						

Figure 7.64 *Periodic table of elements; group 12 metals are highlighted*

Mercury is liquid at room temperature, the only metal that shows this behaviour. Therefore, it was and still is often used in thermometers and barometers despite its toxicity. Mercury and cadmium are both highly toxic elements. Cadmium can often be found in batteries. Mercury is also well known for its formation of amalgams with other metals. Amalgams are formed by reacting mercury with other metals (except silver) and have been generally widely used as dental fillings, which mostly contain mercury and variable amounts of silver together with other metals such as tin and copper. Nowadays, there is an increased concern about the safety of those dental amalgams and alternative fillings are more and more used. Mercury(II) nitrate was used in the eighteenth and nineteenth century to cure felts for the production of hats. Hat makers who were exposed for long periods to the mercury compound showed symptoms of mercury poisoning including uncontrollable muscle tremors, confused speech patterns and, in extreme cases, hallucinations. This was the inspiration for the term *mad as a hatter*.

Zinc has many applications (e.g. for galvanisation or in batteries) and is often used in alloys. Zinc is also an essential element for living organisms, plays a vital role in their biochemistry and is often found as the active centre in many enzymes. Cadmium and mercury can compete with zinc at these enzyme-binding sites, which leads to their characteristic toxicity.

7.5.1 General chemistry

The elements of group 12 and their chemical behaviour differ from other d-block metals as they have a completely filled valence shell (d shell) and two electrons in the s shell. The latter two electrons are easily removed, leading to the divalent cation. Group 12 elements do not form ions with oxidation states higher than +2 as a result of the closed full d shell. The electronic configuration of group 12 elements is shown in Figure 7.65.

The chemistry of Zn^{2+} and Cd^{2+} is expected to be fairly similar to that of Mg^{2+} and Be^{2+}. Indeed, there are some overlaps in regard to biological targets, but the chemical behaviour itself differs between members of group 2 and group 12 as a result of their different electronic configurations. It is important to note that mercury has some properties that are unique to this element and cannot be compared with the chemical behaviour of zinc or cadmium. Therefore, the chemistry of mercury will be discussed in less detail in this book.

Zinc and cadmium both dissolve in a variety of acids with the formation of hydrogen gas and the relevant metal cation M^{2+}. In contrast, mercury is inert to reactions with acids. A similar trend is seen for the formation

Zn : $[Ar]3d^{10}4s^2$

Cd : $[Kr]4d^{10}5s^2$

Hg : $[Xe]4f^{14}5d^{10}6s^2$

Figure 7.65 *Electronic configuration of group 12 elements*

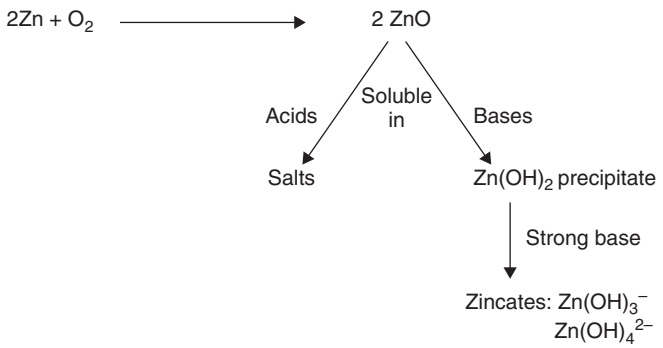


Figure 7.66 *Formation of zinc oxide and its chemical behaviour in acids and bases*

of oxides. Zinc and cadmium form the corresponding oxide when heated under oxygen. Mercury can also form the oxide, but the process is fairly slow. The resulting oxides, ZnO and CdO, are soluble in both acids and bases. ZnO will form salts when dissolved in acids, and the precipitate $Zn(OH)_2$ when dissolved in a base. $Zn(OH)_2$ can be dissolved in a strong base and the so-called zincates [$Zn(OH)_3^-$, $Zn(OH)_4^{2-}$] can be obtained. Cadmium oxides can also be dissolved in acids and bases, but the obtained $Cd(OH)_2$ is insoluble in even strong base solutions, but the hydroxide can be dissolved in ammonia. Halides of the metals (M) zinc and cadmium follow the general formula MX_2 and are either insoluble (X = F) in water or show a low aqueous solubility (Figure 7.66).

7.5.2 The role of zinc in biological systems

The average human body contains around 2 g of Zn^{2+}. Therefore, zinc (after iron) is the second most abundant d-block metal in the human body. Zinc occurs in the human body as Zn^{2+} (closed d^{10} shell configuration), which forms diamagnetic and mainly colourless complexes. In biological systems, zinc ions are often found as the active centre of enzymes, which can catalyse metabolism or degradation processes, and are known to be essential for stabilising certain protein structures that are important for a variety of biological processes.

Already from ancient times, Zn^{2+} was known to have important biological properties. Zinc-based ointments were traditionally used for wound healing. Low Zn^{2+} concentrations can lead to a variety of health-related problems especially in connection with biological systems of high Zn^{2+} demand such as the reproductive system. The daily requirement for Zn^{2+} is between 3 and 25 mg, depending on the age and circumstances [28].

The enzymatic function of Zn^{2+} is based on its Lewis acid activity, which are electron-deficient species (see Chapter 4). In the following chapters, examples will be shown to further explain this. Carboanhydrase (CA),

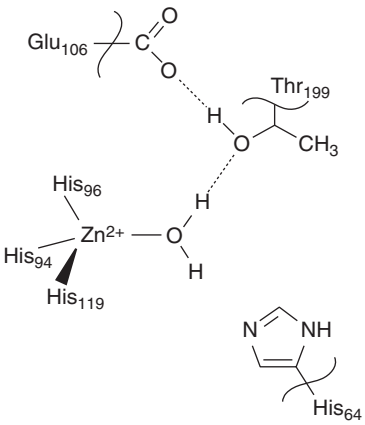


Figure 7.67 *Scheme depicting the Zn^{2+} site of CA [28]*

carboxypeptidase and superoxide dismutase are some examples for well-studied zinc-containing enzymes. The so-called zinc fingers have been discovered because of the crucial role of Zn^{2+} in the growth of organisms. Within the zinc finger, Zn^{2+} stabilises the protein structure and therefore enables its biological function.

7.5.2.1 Carboanhydrase (CA)

CAs are enzymes that catalyse the hydrolysis of carbon dioxide. These enzymes are involved in many biological processes such as photosynthesis (CO_2 uptake), respiration (CO_2 release) and pH control.

$$H_2O + CO_2 \leftrightarrow HCO_3^- + H^+$$

The human CA, form II(c), consists of 259 amino acids with a molecular weight of around 30 kDa. The catalytic site contains a Zn^{2+} ion which is coordinated by three neutral histidine (His) residues and a water molecule. The water molecule is believed to be important for structural reasons and enzymatic functionality (Figure 7.67).

A hypothetical mechanism for the mode of action for CA is shown in Figure 7.68. In a first step (i), a proton is transferred to His_{64} from the coordinated water molecule. In a second step (ii), a buffer molecule (B) coordinates this proton and transports it away from the active site. The remaining hydroxide ligand reacts quickly and forms a transition state via hydrogen bonding with CO_2 (iii). Following some more transformations, HCO_3^- is released as it is replaced by another molecule of water (iv) and (v) [28].

7.5.2.2 Carboxypeptidase A (CPA)

Carboxypeptidase A (CPA) is an enzyme of the digestive system that is known to cleave amino acids favouring the C-terminal end as well as certain esters. This enzymatic activity depends on the metal at the catalytic site. Zn^{2+} and some Co^{2+}-containing CPAs exhibit peptidase function, whilst esterase function has been seen by CPAs containing a variety of divalent d-block metals. CPA has a size similar to CA, consisting of about 300 amino acids and a molecular mass of 34 kDa. The metal centre is coordinated by two neutral histidine residues and one deprotonated glutamate residue as well as a water molecule.

At physiological conditions, the hydrolysis of proteins and peptides is a fairly sophisticated and slow chemical process. Within the catalysed reaction, the peptide or protein is attacked by an electrophile and

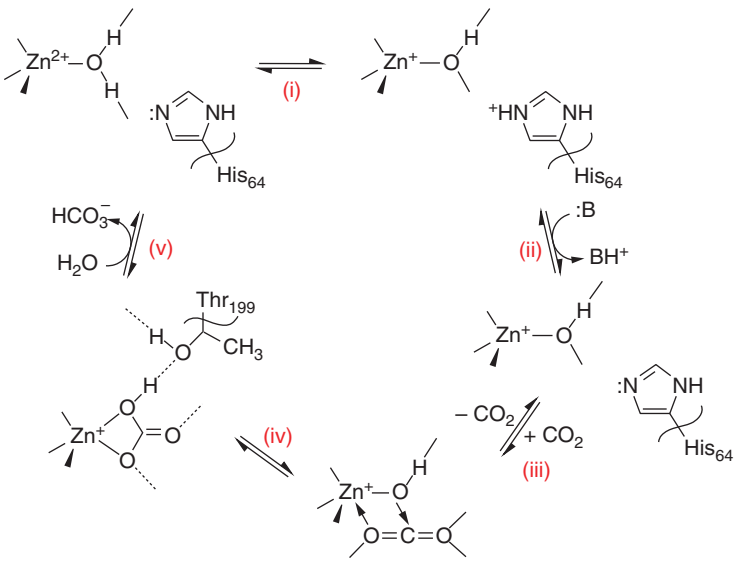


Figure 7.68 *Scheme showing a hypothetical mechanism for the mode of action of CA [28]*

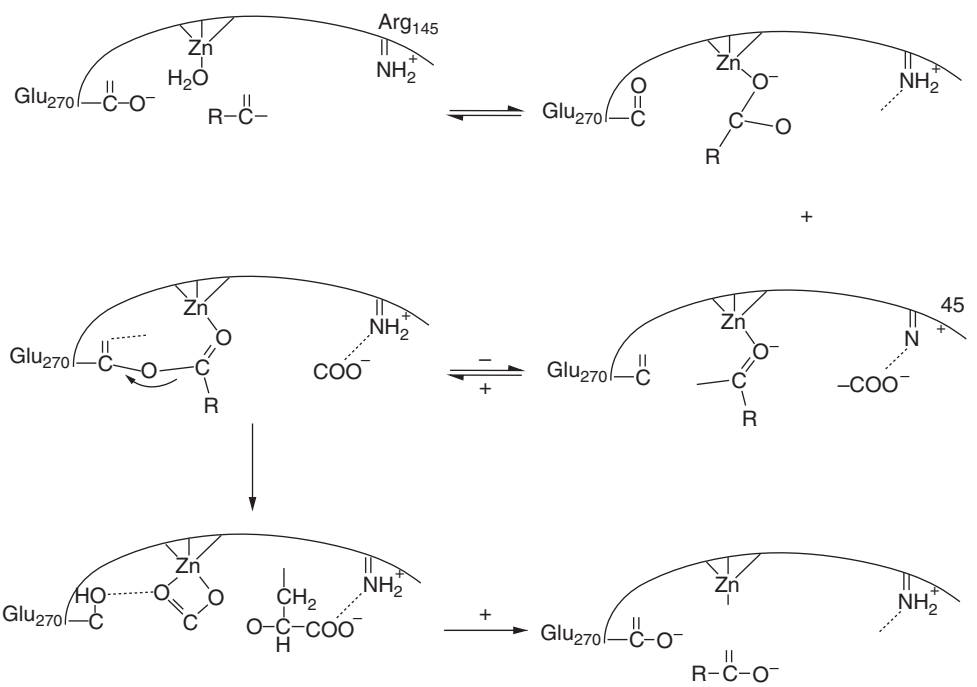


Figure 7.69 *Mode of action at the catalytic site of Zn^{2+}-containing CPAs [28] (Reproduced with permission from [28]. Copyright © 1994, John Wiley & Sons, Ltd.)*

a nucleophile, leading to a fast reaction. Several theories describing the mode of action have been published, and one of those is described below in order to give an idea about the catalytic processes. The electrophile (metal centre) is coordinated to the oxygen of the carbonyl group and therefore allows the glutamate-270 to attack the activated carbonyl group. A mixed anhydride is formed, which subsequently is hydrolysed to form the desired products [28] (Figure 7.69).

Another theory describes the CPA-catalysed hydrolysis of a peptide bond analogous to the CA mechanism. The Zn^{2+} ion is coordinated by one molecule of water, which acts as a nucleophile and can attack the carbonyl group of esters or peptide bonds. A complicated system of hydrogen bonding further facilitates the substrate binding and final steps of this hydrolysis process (Figure 7.70).

Angiotensin converting enzymes (ACEs) are zinc-containing CPAs that convert angiotensin I into angiotensin II. Angiotensin II regulates the reabsorption of water and sodium ions in the kidneys and contracts the blood vessels leading to an increase of the blood pressure. ACE inhibitors are a class of drugs that block the activity of these enzymes. These drugs are used to lower the blood pressure in patients with hypertension and there are a variety of drug examples, such as captopril and lisinopril. The mode of action of these ACE inhibitors is based on their ability to bind to the Zn^{2+} centre and the active site of these CPAs and thereby blocking their enzyme activity. Captopril contains a thiol (SH) group, which coordinates directly to Zn^{2+}, whilst the carbonyl and carboxyl groups interact with the amino acid residues mainly via hydrogen bonding (Figure 7.71).

7.5.2.3 Zinc finger

It is well known that Zn^{2+} is essential for the growth of organisms and transcription of genetic material. It has been shown that there are special proteins that recognise certain DNA segments leading to the activation or regulation of genetic transcription. These proteins contain residues that can coordinate Zn^{2+}. This coordination leads to folding and a specific conformation, and they are called *zinc fingers*. Typically Zn^{2+} is coordinated by two neutral histidine (His) and two deprotonated cysteine (Cys) residues (Figures 7.72 and 7.73).

7.5.3 Zinc: clinical applications and toxicity

Clinical applications of Zn^{2+} range from its use in barrier creams and as a treatment option for Wilson disease to the use of zinc ions for the stabilisation of insulin. Long-acting human or porcine insulin is usually on the market as insulin zinc suspension. It is a sterile solution of usually human or porcine insulin, which is complexed by Zn^{2+}.

Zinc sulfate in the form of either injection or tablets can be used to treat zinc deficiency and as supplementation in conditions with an increased zinc loss. Zinc acetate is one treatment option for Wilson disease, as the zinc supplementation prevents the absorption of copper. It is important to note that zinc treatment has a slow onset time, which is crucial to take into account when switching from another therapy such as chelation therapy. Zinc acetate is usually offered to the market in an oral delivery form, mainly in capsules [8] (Figure 7.74).

Zinc ions are can also be found in barrier creams and lotions. Zinc oxide is present in barrier creams, for example, in creams used against nappy rash, often formulated with paraffin and cod-liver oil. Calamine lotion and creams are indicated for the treatment of pruritus, and both contain zinc oxide. It is interesting to note that the application of zinc oxide may affect the quality of X-ray images and it is therefore recommended not to apply these creams or lotions before X-ray tests [8].

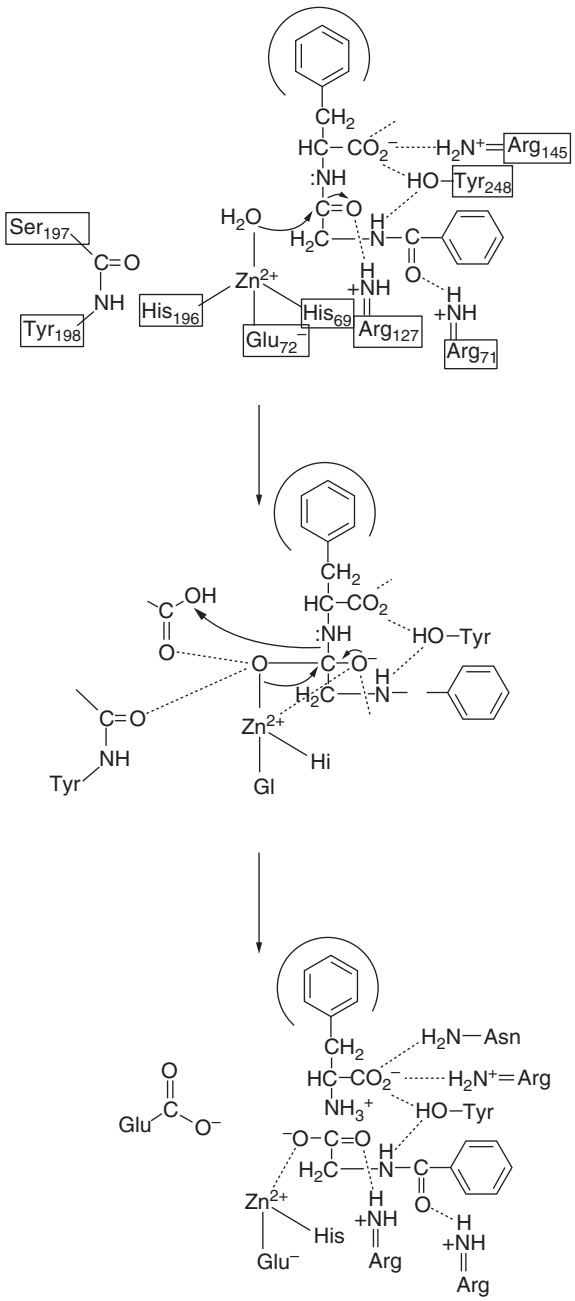


Figure 7.70 *Alternative mode of action at the catalytic site of Zn^{2+}-containing CPAs [28] (Reproduced with permission from [28]. Copyright © 1994, John Wiley & Sons, Ltd.)*

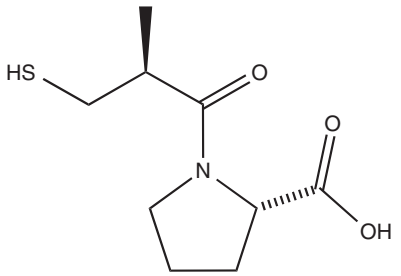


Figure 7.71 *Chemical structure of the antihypertensive drug captopril*

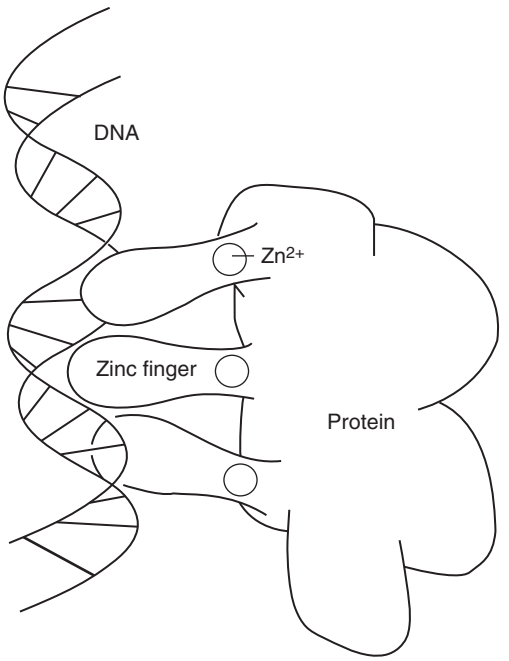


Figure 7.72 *Schematic representation of the interaction between DNA and a zinc finger protein [28] (Reproduced with permission from [28]. Copyright © 1994, John Wiley & Sons, Ltd.)*

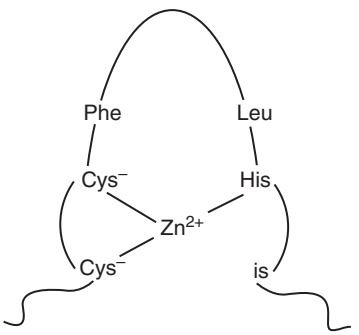


Figure 7.73 *Zinc finger motif from TF IIIA [28] (Reproduced with permission from [28]. Copyright © 1994, John Wiley & Sons, Ltd.)*

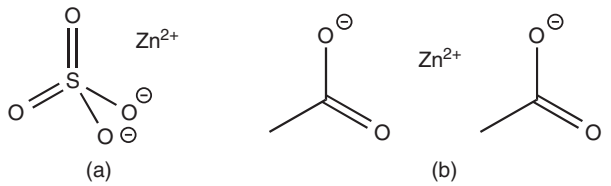


Figure 7.74 *Lewis formula of zinc sulfate (a) and zinc acetate (b)*

Zinc is an essential element, but excess zinc can have a negative impact on human health. Zinc toxicity might be seen if the intake exceeds 225 mg. Typical symptoms include nausea, vomiting, diarrhoea and cramps. The so-called zinc-shakes are seen in workers, such as welders, who inhale freshly formed zinc oxide. Ingested metallic zinc dissolves in the stomach acid and zinc chloride ($ZnCl_2$) is formed. Zinc chloride is toxic to most organisms, depending on the concentration [29].

7.6 Exercises

7.6.1 What is the oxidation state of the central metal atom in the following complexes?

(a) $[Au(CN)_4]^-$
(b) $[V(CO)_6]$
(c) $[Fe(H_2O)_6]^{2+}$
(d) $[PdCl_6]^{2-}$

7.6.2 What is the charge of a complex formed by

(a) Co^{2+} and $3C_2O_4{}^{2-}$
(b) Pt^{4+} and $3H_2O$ and $3Br^-$
(c) Au^+ and $2CN^-$

7.6.3 Draw the energy diagrams displaying the d-orbital splitting for the low and high-spin complexes of the following examples assuming the formation of an octahedral complex:

(a) Fe^{3+}
(b) Zn^{2+}
(c) Cu^{2+}
(d) Cr^{3+}
(e) Cr^{2+}

7.6.4 Predict the geometry of the following complexes:

(a) $Zn(NH_3)_4$
(b) $[Mn(OH_2)_6]^{2+}$
(c) $Ag(NH_3)_2{}^+$

7.7 Case studies

7.7.1 Silver nitrate solution

Your pharmaceutical analysis company has been contacted by an important client and asked to analyse a batch containing silver nitrate ($AgNO_3$) solution for topical use. The description of your brief states that you are supposed to analyse the API in these solutions following standard quality assurance guidelines.

Typical analysis methods used for quality purposes are based on titration reactions. A certain amount of solution is diluted and treated with nitric acid and ammonium iron(III) sulfate. The solution is then titrated with ammonium thiocyanate [30].

(a) Research the type of titration described. Describe the chemical structure of any reagents involved in the analysis.
(b) Formulate the relevant chemical equations. What is the role of ammonium iron(III) sulfate?
(c) The package states that each the solution contains 10% silver nitrate. A volume containing 50 mg of silver nitrate is diluted with water and 2 ml of nitric acid and 2 ml of ammonium iron(III) sulfate solution are added. The solution is then titrated with 0.02 M ammonium thiocyanate solution until a red colour appears [30].
(d) For each titration, the following volume of ammonium thiocyanate solution has been used:

14.5 ml	14.8 ml	15.0 ml

Calculate the amount of $AgNO_3$ present in your sample. Express your answer in grams and moles.
(e) Critically discuss your result in context with the stated value for the API.
(f) Research the typically accepted error margins.

7.7.2 Ferrous sulfate tablets

Your pharmaceutical analysis company has been contacted by an important client and asked to analyse a batch of tablets containing dried ferrous sulfate ($FeSO_4$) tablets. The description of your brief states that you are supposed to analyse the API in these tablets following standard quality assurance guidelines.

Typical analysis methods used for quality purposes are based on titration reactions. A certain amount of ferrous sulfate tablets are crushed and dissolved. The solution is then titrated with cerium(IV)sulfate under acidic conditions using ferroin as the indicator British Pharmacopoeia.

(a) Research the type of titration described. Describe the chemical structure of any reagents involved in the analysis.
(b) Formulate the relevant chemical equations.
(c) The package states that each tablet contains 200 mg ferrous sulfate. The weight of 20 tablets has been determined to be 6.24 g. A certain amount of powder containing 0.5 g of dried ferrous sulfate is dissolved in water and sulfuric acid, and ferroin is added as an indicator. The solution is then titrated with 0.1 M ammonium cerium(IV)sulfate solution [30].

For each titration, the following volume of ammonium cerium(IV)sulfate solution has been used:

32.5 ml	33.3 ml	32.9 ml

Calculate the amount of $FeSO_4$ present in your sample. Express your answer in grams and moles.

(d) Critically discuss your result in context with the stated value for the API.

(e) Research the typically accepted error margins.

7.7.3 Zinc sulfate eye drops

Zinc sulfate solution has been traditionally used as an astringent and a mild antiseptic. Nowadays, its use is limited. Your pharmaceutical analysis company has been contacted by an important client and asked to analyse a batch of eye drops containing zinc sulfate ($ZnSO_4$). The description of your brief states that you are supposed to analyse the API in these tablets following standard quality assurance guidelines.

Typical analysis methods used for quality purposes are based on titration reactions. A certain volume of the eye drops is diluted and typically titrated with ammonium edetate using morbant black as indicator [30].

(a) Research the type of titration described. Describe the chemical structure of any reagents involved in the analysis. You might want to familiarise yourself with the concept of chelation (see Chapter 11).

(b) Formulate the relevant chemical equations.

(c) The package states that each sterile solution contains 0.25% w/v of zinc sulfate heptahydrate. For the titration, 5 ml of the zinc sulfate solution is diluted and an ammonia buffer is added to achieve pH 10.9. This solution is titrated with 0.01 M disodium edetate and mordant black as indicator [30].

For each titration, the following volume of edetate solution has been used:

5.0 ml	4.6 ml	4.3 ml

Calculate the amount of $ZnSO_4$ present in your sample. Express your answer in grams and moles.

(d) Critically discuss your result in context with the stated value for the API.

(e) Research the typically accepted error margins.

References

1. N. G. Connelly, *Nomenclature of inorganic chemistry: IUPAC recommendations 2005*, Royal Society of Chemistry, Cambridge, **2005**.
2. J. C. Dabrowiak, *Metals in medicine*, Wiley-Blackwell, Oxford, **2009**.
3. R. A. Alderden, M. D. Hall, T. W. Hambley, *J. Chem. Educ.* **2006**, *83*, 728–734.
4. Z. D. Bugarcic, J. Bogojeski, B. Petrovic, S. Hochreuther, R. van Eldik, *Dalton Trans.* **2012**, *41*, 12329–12345.
5. F. Arnesano, G. Natile, *Coord. Chem. Rev.* **2009**, *253*, 2070–2081.
6. P. M. Takahara, A. C. Rosenzweig, C. A. Frederick, S. J. Lippard, *Nature* **1995**, *377*, 649–652.
7. E. R. Tiekink, M. Gielen, *Metallotherapeutic drugs and metal-based diagnostic agents: the use of metals in medicine*, Wiley, Chichester, **2005**.
8. *British national formulary*, British Medical Association and Pharmaceutical Society of Great Britain, London.
9. M. Coluccia, G. Natile, *Anti-Cancer Agent Med.* **2007**, *7*, 111–123.
10. I. Kostova, *Recent Pat. Anti-Cancer Drug Discovery* **2006**, *1*, 1–22.
11. (a) H. Hochster, A. Chachoua, J. Speyer, J. Escalon, A. Zeleniuch-Jacquotte, F. Muggia, *J. Clin. Oncol.* **2003**, *21*, 2703–2707; (b) S. Goel, A. Bulgaru, H. Hochster, S. Wadler, W. Zamboni, M. Egorin, P. Ivy, L. Leibes, F. Muggia, G. Lockwood, E. Harvey, G. Renshaw, S. Mani, *Ann. Oncol.* **2003**, *14*, 1682–1687.
12. H. Choy, C. Park, M. Yao, *Clin. Cancer Res.* **2008**, *14*, 1633–1638.
13. C. E. Housecroft, A. G. Sharpe, *Inorganic chemistry*, 3rd ed., Pearson Prentice Hall, Harlow, **2008**.
14. R. H. Blum, S. K. Carter, K. Agre, *Cancer* **1973**, *31*, 903–914.
15. D. L. Boger, H. Cai, *Angew. Chem. Int. Ed.* **1999**, *38*, 448–476.
16. E. Alessio, *Bioinorganic medicinal chemistry*, Wiley, Weinheim, **2011**.
17. C. S. Allardyce, P. J. Dyson, *Platinum Met. Rev.* **2001**, *45*, 62–69.
18. Y. K. Yan, M. Melchart, A. Habtemariam, P. J. Sadler, *Chem. Commun.* **2005**, 4764–4776.
19. (a) M. C. Linder, M. HazeghAzam, *Am. J. Clin. Nutr.* **1996**, *63*, S797–S811; (b) R. Uauy, M. Olivares, M. Gonzalez, *Am. J. Clin. Nutr.* **1998**, *67*, 952S–959S.
20. (a) A. K. Wernimont, D. L. Huffman, A. L. Lamb, T. V. O'Halloran, A. C. Rosenzweig, *Nat. Struct. Biol.* **2000**, *7*, 766–771; (b) E. A. Roberts, M. L. Schilsky, *Hepatology* **2003**, *38*, 536.
21. C. K. Sen, S. Khanna, M. Venojarvi, P. Trikha, E. C. Ellison, T. K. Hunt, S. Roy, *Am. J. Physiol. Heart C* **2002**, *282*, H1821–H1827.
22. (a) S. F. Swaim, D. M. Vaughn, S. A. Kincaid, N. E. Morrison, S. S. Murray, M. A. Woodhead, C. E. Hoffman, J. C. Wright, J. R. Kammerman, *Am. J. Vet. Res.* **1996**, *57*, 394–399; (b) F. X. Maquart, G. Bellon, B. Chaqour, J. Wegrowski, L. M. Patt, R. E. Trachy, J. C. Monboisse, F. Chastang, P. Birembaut, P. Gillery, J. P. Borel, *J. Clin. Invest.* **1993**, *92*, 2368–2376; (c) I. Tenaud, I. Sainte-Marie, O. Jumbou, P. Litoux, B. Dreno, *Br. J. Dermatol.* **1999**, *140*, 26–34.
23. (a) A. Gupte, R. J. Mumper, *Cancer Treat. Rev.* **2009**, *35*, 32–46; (b) V. L. Goodman, G. J. Brewer, S. D. Merajver, *Curr. Cancer Drug Targets* **2005**, *5*, 543–549; (c) F. Tisato, C. Marzano, M. Porchia, M. Pellei, C. Santini, *Med. Res. Rev.* **2010**, *30*, 708–749.
24. N. Cioffi, M. Rai, *SpringerLink (online Service)*, Springer, Berlin; Heidelberg, **2012**, p. xvi, 556 p.
25. Z. Aziz, S. F. Abu, N. J. Chong, *Burns* **2012**, *38*, 307–318.
26. A. J. Griffin, T. Gibson, G. Huston, A. Taylor, *Ann. Rheum. Dis.* **1981**, *40*, 250–253.
27. J Barber, C. Rostron, *Pharmaceutical chemistry*.
28. W. Kaim, B. Schwederski, *Bioinorganic chemistry: inorganic elements in the chemistry of life: an introduction and guide*, Wiley, Chichester, **1994**.
29. G. J. Fosmire, *Am. J. Clin. Nutr.* **1990**, *51*, 225–227.
30. *British pharmacopoeia*, Published for the General Medical Council by Constable & Co, London.

Further Reading

F. A. Cotton, G. Wilkinson, C. A. Murillo, M. Bochmann, *Advanced inorganic chemistry*, 6th ed., Wiley, New York; Chichester, **1999**.

H.-B. Kraatz, N. Metzler-Nolte, *Concepts and models in bioinorganic chemistry*, Wiley-VCH, [Chichester: John Wiley, distributor], Weinheim, **2006**.

J. D. Lee, *Concise inorganic chemistry*, 5th ed., Chapman & Hall, London, **1996**.

G. A. McKay, M. R. Walters, J. L. Reid, *Lecture notes. Clinical pharmacology and therapeutics*, 8th ed., Wiley-Blackwell, Chichester, **2010**.

R. M. Roat-Malone, *Bioinorganic chemistry: a short course*, Wiley, Hoboken, N.J. [Great Britain], **2002**.

G. J. Tortora, B. Derrickson, *Principles of anatomy and physiology*, 12th ed., international student/Gerard J. Tortora, Bryan Derrickson. ed., Wiley [Chichester: John Wiley, distributor], Hoboken, N.J., **2009**.