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Bioanalysis of cisplatin analogues — a selective review*

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Abstract: A variety of analytical procedures have been described for the determination of cisplatin and its analogues in biological fluids (plasma, plasma ultrafiltrate and urine), as well as in solid tissues. This paper attempts to review those methods which have been most commonly used in practice. These analytical methods may be conveniently divided into non-selective methods which detect only the platinum metal and selective methods which are capable of detecting the intact compounds. The non-selective methods include X-ray fluorescence, proton induced X-ray emission, flameless atomic absorption (FAA) and high-performance liquid chromatography (HPLC). The latter method requires pre-column derivatization with diethyldithiocarbamate. The selective methods generally employ a fractionation step using HPLC followed by either on-line or off-line detection. Off-line detection by FAA requires the collection of fractions from the HPLC column and is somewhat tedious. On the other hand, sample preparation is minimal and biological fluids may be injected directly onto the column. The most sensitive HPLC methods for the determination of cisplatin and its analogues in biological fluids employ on-line electrochemical detection or post column derivatization with bisulphite.

Keywords: *Cisplatin analogues; high-performance liquid chromatography; flameless atomic absorption; X-ray fluorescence, electrochemistry, UV absorption; post-column derivatization; pre-column derivatization; column switching; plasma; urine; tissues; review.*

Introduction

Dichlorodiammineplatinum(II) was first described by Peyronne [1] in 1845 and was separated into its *cis* and *trans* isomers by Werner in 1898 [2]. However, the cytotoxic properties of cisplatin were not described until 1967 by Rosenberg *et al.* [3, 4]. Cisplatin entered clinical trials in the early 1970s and is now used widely for the treatment of various solid tumors, particularly those of the head, neck, bladder, ovaries, testes, lung and bone [5]. Over 2000 analogues of cisplatin have been evaluated as potential antineoplastic agents, in attempts to improve the therapeutic index and to reduce the severe side effects of cisplatin which include nausea, vomiting, kidney damage and deafness. Figure 1 shows the structures of some platinum complexes with good

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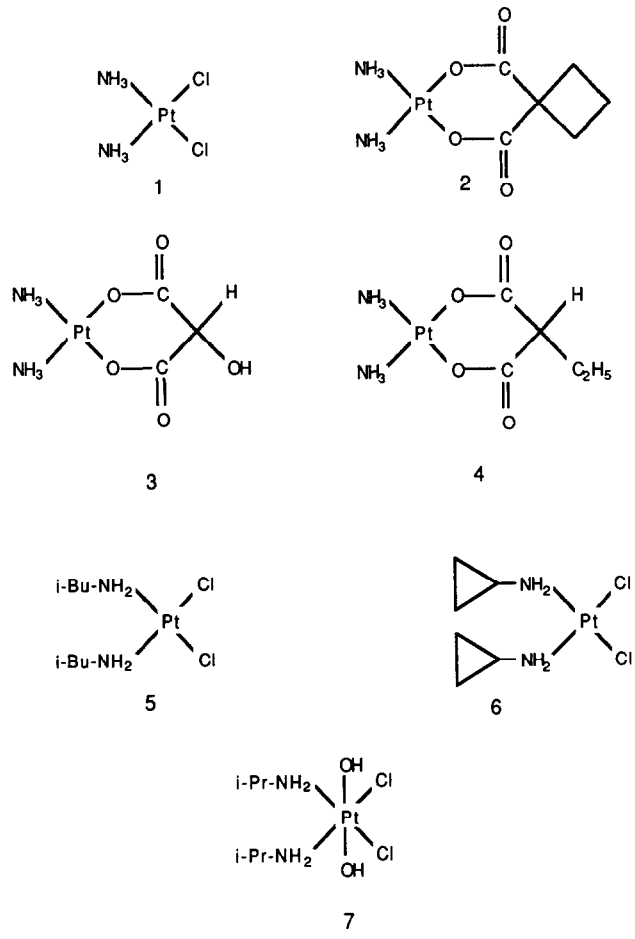


Figure 1
The structures of cisplatin and some of its analogues with known antineoplastic activity. 1. *cis*-dichlorodiammineplatinum(II) (cisplatin, CDDP); 2. diammine(1,1-cyclobutane-dicarboxylato)platinum(II) (carboplatin, CBDCA); 3. diammine(2-hydroxymalonato)-platinum(II); 4. diammine(2-ethylmatonato) platinum(II); 5. *cis*-dichlorobis(isobutyl-amine)platinum(II); 6. *cis*-dichlorobis(cyclopropylamine) platinum(II); 7. *cis*-dichloro-*trans*-dihydroxy-*cis*-bis(isopropylamine)platinum(IV) (iproplatin, CHIP).

antineoplastic activity, *in vitro* and *in vivo*. Of these analogues (Fig. 1), iproplatin (CHIP) and carboplatin (CBDCA) are presently undergoing clinical trials.

The determination of cisplatin and its analogues in biological fluids and tissues presents a particularly interesting challenge to the analytical chemist. The methods which have been described may be conveniently divided into those which rely on non-specific determination of platinum and those which permit specific determination of the intact molecules. This paper attempts to review current methodologies and to highlight their advantages and disadvantages, paying particular attention to ease of application and analytical sensitivity.

Non-specific methods

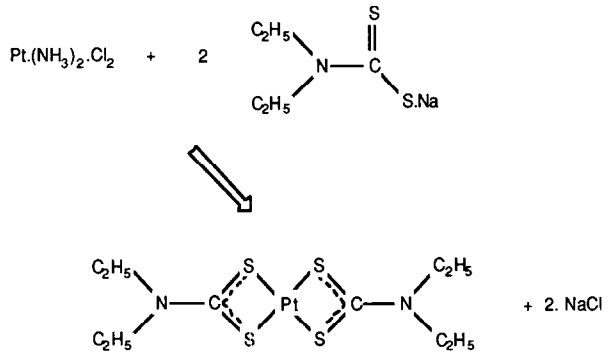
These methods rely on the non-specific determination of platinum and include X-ray

fluorescence (XRF), proton induced X-ray emission (PIXE), inductively coupled plasma (ICP), flameless atomic absorption (FAA) and high-performance liquid chromatography (HPLC) with pre-column derivatization. Although Pt(II) can be oxidized or reduced at various electrodes, these reactions are dependent on the nature of the coordinated ligands. In addition, a number of analytically undesirable reactions can occur at the electrode surface precluding the frequent use of electrochemical methods for the determination of platinum complexes in biological samples [6–8]. Alexander *et al.* [6] have used differential pulse and the catalytic hydrogen current resulting from platinum deposition at mercury for the sensitive determination of platinum in biological fluids. More recently, Wang *et al.* [8] have evaluated the interfacial behavior of cisplatin using cyclic voltametry and have also used this technique in an indirect method for the analysis of platinum in untreated urine samples. However, the most useful application of electrochemistry for the bioanalysis of cisplatin analogues is as a detection technique in liquid chromatography [9–11].

Both atomic emission and atomic absorption techniques have been used successfully in the bioanalysis of platinum. Proton induced X-ray emission (PIXE) spectroscopy has been used by Dikhoff *et al.* [12] for the characterisation of platinum in human kidneys and the tissues of the dog following intravenous administration of cisplatin. Wavelengths dispersive X-ray fluorescence (XRF) has been used [13] with success for the determination of low molecular weight (<25 kDa) platinum complexes in plasma ultrafiltrate. In this method the low molecular weight platinum complexes are separated from the plasma proteins by centrifugal ultrafiltration and derivatized with ethylenediamine. The resultant cationic complexes are then retained on a cellulose disc which has been impregnated with a cation exchange resin. The platinum is determined by XRF directly on the cellulose disc which is supported between two sheets of polypropylene film. Although PIXE and XRF provide good sensitivity for pharmacological and pharmacokinetic investigations, the complex sample pretreatment and availability of instrumentation have limited their usefulness as routine methods.

The most commonly used technique for the determination of platinum in biological fluids following administration of platinum containing drugs is flameless atomic absorption (FAA) [e.g. ref. 14]. The advantage of this technique is that relatively little sample pretreatment is required and urine, plasma and plasma ultrafiltrate can be introduced directly into the graphite furnace. The major disadvantage of this technique is the relatively high temperatures (2300°–2700°C) that are required for atomization. Such high temperatures can cause rapid decomposition of the pyrolytic graphite surface leading to analytical inaccuracy. Depending on the matrix, detection limits of 1–10 ng of platinum per ml of biological fluid can be achieved by FAA compared with 200 ng ml^{-1} by XRF. More recently, Dominici *et al.* [15] have used inductively coupled plasma (ICP) for the determination of platinum in plasma following IV infusion of cisplatin to pediatric patients suffering from neuroblastoma. This group [15] claimed detection limits of 2–4 ng of platinum per ml of plasma.

One of the most useful methods for the determination of platinum in biological samples involves derivatization with diethyldithiocarbamate (DDTC) to yield a single chromatophore (Scheme 1) which can be extracted into chloroform and then assayed by normal phase [16] or reversed phase [17] HPLC. This reaction was originally adapted simultaneously by Bannister *et al.* [16] and Borch *et al.* [17] for the determination of platinum excreted into urine (Fig. 2). This method, which has a detection limit of about 3 ng ml^{-1}, has been adapted subsequently for the analysis of plasma ultrafiltrate [18].



Scheme 1
Derivatization of cisplatin with diethyldithiocarbamate (DDTC).

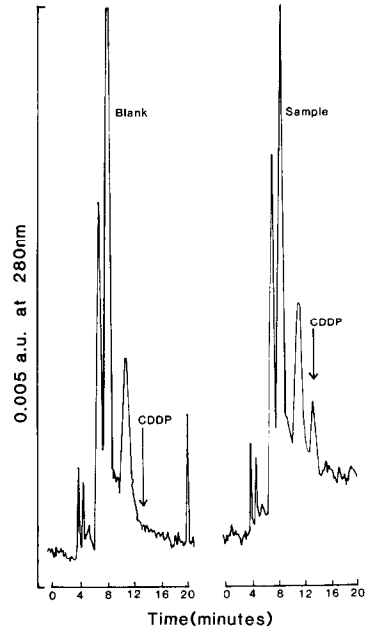


Figure 2
Chromatograms of urine containing cisplatin (3 ng ml^{-1}) after derivatization with DDTC (Scheme 1). (Reproduced from reference [16]).

Each of the non-specific methods described here has its own advantages and disadvantages and a choice should be made on the basis of the sensitivity required and the availability of instrumentation. In addition, when large numbers of routine samples are to be analyzed, then methods such as XRF or PIXE which have complex pre-treatment steps should be avoided. For clinical analysis and routine therapeutic drug monitoring, there is a good argument for the use of HPLC with pre-column derivatization with DDTC. This is based on evidence [18] that this method responds only to reactive (*sic*) platinum in plasma and urine and thus provides a better measure of biological activity than those methods which allow determination of total platinum. It can also be argued that this method is preferable to HPLC methods which permit determination of the intact drug, since the latter may not respond to biologically active biotransformation products.

Specific methods

A great deal of effort has been directed towards the development of specific methods for the determination of cisplatin and its analogues in biological fluids [19–29]. Most of the reports to date have dealt with the analysis of cisplatin, and the methods of choice have been based on HPLC. However, the HPLC of cisplatin is not particularly straightforward due to its lack of detectability and poor chromatographic properties. Although cisplatin is a neutral molecule, it may be retained on cationic stationary phases such as Partisil SAX [19, 20] or μBondapak C_{18} [20] using hydro-organic mobile phases. An alternative to the chemically bonded stationary phases [19–21] is the solvent generated phases [22–25] in which the cationic surfactant, hexadecyltrimethylammonium bromide, is physically adsorbed onto the surface of silica gel or alkylsilylsilicas. The advantage of the latter type of stationary phase is that they may be operated with purely aqueous mobile phases which are compatible with the electrochemical detectors described by Bannister *et al.* [9] and the post-column reaction detectors described by Marsh *et al.* [25]. In addition to cisplatin, solvent generated systems have also been used [22–25] for the chromatography of other platinum(II) and platinum(IV) complexes. In all cases retention is believed to occur via ion-dipole interactions between the cationic stationary phase and the neutral platinum complexes. It is interesting to note that Parsons *et al.* [26] have used a C_8 column modified by alkylsulphonates for the chromatography of cisplatin, iproplatin and carboplatin (Fig. 1). Finally, Hincal *et al.* [20] have coupled strong anion and strong cation exchange columns to permit retention of both cisplatin and the positively charged degradation products of cisplatin, $Pt.(NH_3).Cl.2H_2O^+$ and $Pt.(NH_3)2.(H_2O)_2^{2+}$, (Fig. 3). However, it should be noted that this method [20] was used as a stability-indicating assay for cisplatin in parenteral formulations and not as a bioassay.

Cisplatin and its analogues have low solubilities in organic solvents and are not readily extracted from biological fluids. Consequently, bioanalysis of intact cisplatin or its analogues generally involves direct injection of the biological fluid (plasma ultrafiltrate or urine). This can place considerable strain on the ability of the detection system to discriminate between the analyte and potentially interfering endogenous compounds.

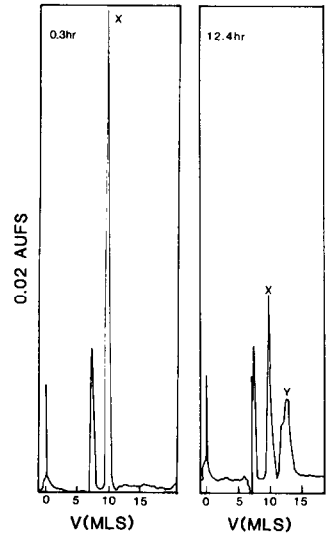


Figure 3
Chromatograms of aqueous solutions of cisplatin at various times after preparation. These chromatograms were obtained using Partisil SCX and Partisil SAX columns coupled in series and a mobile phase of 70% methanol in an acetate buffer (0.1 M, pH 5.6). Detection was by UV absorption at 280 nm. Peak X is cisplatin and peak Y represents the aqueous degradation products of cisplatin [$PT.(NH_3).Cl.2H_2O^+$ and $Pt.(NH_3)2.(H_2O)_2^{2+}$]. (Reproduced from reference [20].)

Several detection systems have been used for the on-line detection of platinum containing drugs and these include direct UV absorption [24, 27, 28], UV absorption following post-column derivatization [25] and reductive electrochemistry [9–11]. In addition to these on-line techniques, fraction collection and determination of the fractions by FAA has also been used in combination with HPLC for the determination of cisplatin in plasma ultrafiltrate [27] and urine [24]. Although fraction collection is tedious, sample preparation prior to chromatography is minimal and plasma ultrafiltrate or urine may be analyzed by direct injection onto the HPLC column.

Direct UV detection [23, 28] provides detection limits of about 1 $\mu g\ ml^{-1}$ at 280 nm and about 20 $ng\ ml^{-1}$ at 210 nm. Plasma ultrafiltrate may be injected directly onto the Partisil SAX column, eluted with a mobile phase of 60–80% methanol in an acetate buffer (pH 5.6, 0.1 M) and the cisplatin is detected free of endogenous interferences. The detection in this case is limited by the low molecular absorbtivity of cisplatin ($\epsilon = 100$, $\lambda = 280$ nm). Untreated urine cannot be injected into a mobile phase of greater than 60% aqueous methanol due to precipitation of inorganic salts. Cisplatin can be analyzed in urine with direct UV detection at 280 nm using a solvent generated anion exchange system. However, in this case automated column switching is required to separate the drug from endogenous substances [24] (Fig. 4). Kizu *et al.* [28] have described a sensitive HPLC method with direct UV detection at 210 nm for the analysis of intact cisplatin in urine and plasma. Unfortunately, this method [28] requires extensive sample pre-treatment prior to analysis.

The methods of choice for the determination of intact cisplatin or its analogues appear to be HPLC with either electrochemical detection [9–11] or post-column derivatization [2]. Both methods require minimal pre-treatment of urine or plasma ultrafiltrate and provide detection limits of 25–50 $ng\ ml^{-1}$. The use of electrochemical detection of cisplatin in the reductive mode was first described by Bannister *et al.* [9] (Fig. 5) who used a dropping mercury electrode in the detection device. This was improved later by others [10, 11] who used the more convenient glassy carbon electrode. Since cisplatin and its analogues can be detected [9] at −0.0 V versus Ag/AgCl, interferences from plasma

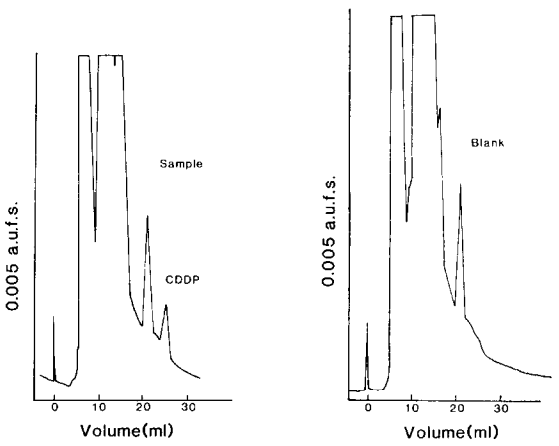


Figure 4
Chromatogram of cisplatin (100 $\mu g\ ml^{-1}$) in urine after HPLC with column switching, with UV detection at 280 nm. (For further details see text and reference [24] from which this figure was reproduced).

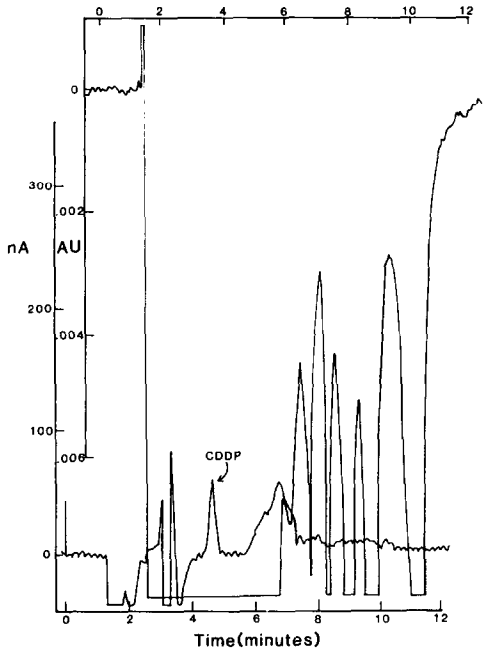


Figure 5
Chromatogram of cisplatin (2 μg ml^{-1}) in urine after HPLC with electrochemical (lower) and UV (upper) detection. (For further details see text and reference [9] from which this figure was reproduced.)

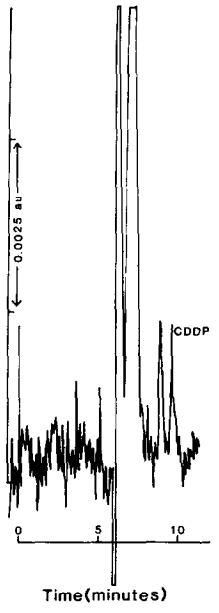


Figure 6
Chromatogram of cisplatin (5.6 ng injected) in plasma ultrafiltrate after HPLC with post-column derivatization and UV detection at 280 nm. (For further details see text and reference [25] from which this figure was reproduced.)

and urine constituents are minimal and the only major precaution that should be taken is the scrupulous removal of dissolved oxygen from the samples and from the mobile phase. A useful alternative to electrochemical detection is the post-column reaction detector described by Marsh *et al.* [25]. This detection system is based on the prior observation of Hussain *et al.* [29] that a strongly chromophoric species is produced when cisplatin is

reacted with bisulphite ions. In order for this reaction to be reproducible in a post-column reaction detection system, it was found [25] that the platinum complexes must first be reacted with potassium dichromate before derivatization with bisulphite. Therefore, this method of analysis requires three pumps: one for delivery of the solvent to the HPLC column and two for the post-column reactor. However, having been assembled, this system provides a reliable method for the analysis of a variety of cisplatin analogues in plasma ultrafiltrate and urine (Fig. 6), with detection limits comparable to those obtained by HPLC with reductive electrochemical detection.

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