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*Review*

# Analytical methodologies for the quantitation of platinum anti-cancer drugs and related compounds in biological media

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**Abstract**: The methods for analysis of platinum-based anti-cancer drugs in biological media are reviewed in this paper. Although emphasis is placed on cisplatin, attention is also given to several of its analogues (carboplatin, iproplatin) and their degradation and biotransformation products.

In an introductory section a short description is given of the historical background and the clinical applications of cisplatin. Reactions occurring in the body and in aqueous solutions are discussed because of their implications for the design of analytical procedures. After a detailed description of sample preparation and storage, attention is focussed on the analytical techniques used for the determination of either total platinum levels or individual platinum-containing compounds. The techniques discussed include atomic absorption and emission spectrometry, derivatization reactions and several chromatographic techniques with different detection methods. The specific advantages and disadvantages of these techniques are discussed.

**Keywords**: *Platinum drugs; bioanalysis; degradation products; biotransformation products; sample handling; chemical derivatization; atomic absorption and emission spectrometry; liquid chromatography.*

## Introduction

### *History*

The use of platinum compounds in cancer chemotherapy was initiated in the late 1960s by Rosenberg *et al.* [1]. While investigating the possible effects of an electric field on growth processes in bacteria, these authors discovered that certain compounds produced at the platinum electrodes caused an inhibition of the cell division process without markedly affecting the growth process [1], properties that were already known for anti-tumour compounds such as Actinomycin D [2]. This inihibition of cell division was eventually attributed to the presence of *cis*-diamminetetrachloroplatinum (IV) and *cis*-diamminedichloroplatinum(II) (cisplatin).

After promising animal studies, cisplatin entered clinical trials in 1971. The breakthrough for cisplatin came in 1977 when Cvitkovic and co-workers [3] at the Sloan Kettering Cancer Center in New York managed to considerably reduce the nephrotoxicity, associated with the use of cisplatin, by prehydration and diuresis. Cisplatin was registered by the US Food and Drug Administration in 1978.

### *Mechanisms of action*

The basic mechanisms of action of platinum-containing drugs still remain elusive. It is generally accepted that within the cell, the hydrated derivatives of cisplatin are the active species, and that the most likely target is DNA [4–6]. Among the different binding types, the intrastrand cross-link between adjacent guanine moieties is the major reaction product from a reaction of cisplatin with DNA [7]. However, the question which platinum–DNA adduct is responsible for anti-tumour activity still remains unanswered. In addition, several other mechanisms may prove to be important including, interaction with the membrane transport system, modification of the immune function and interference with critical amino acid transport systems [8].

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*Activity and toxicity*

Cisplatin has shown activity towards a variety of solid tumours. It is highly effective against testicular and ovarian cancers, cancers of the head and neck and osteosarcoma. Moderate activity is reported for lung and bladder cancer, cervical cancer and lymphomas [9–11].

The treatment with cisplatin is associated with several toxic side effects including nausea, vomiting, neurotoxicity, nephrotoxicity and ototoxicity [9]. At present the neurotoxicity is dose limiting [4].

*Analogues*

In an attempt to improve the anti-tumour activity and to reduce the severe side effects of cisplatin, more than 2000 different platinum compounds have been tested in the last 20 years [12, 13]. The structures and systematic names of cisplatin, its inactive *trans*-isomer and two of its most important second generation analogues, carboplatin and iproplatin, are listed in Table 1. Carboplatin and iproplatin are currently the most promising analogues in clinical trials. Carboplatin shows comparable activity towards ovarian cancers, together with a considerable reduction in toxicity. Bone marrow toxicity, however, is dose limiting for this drug [13].

Research is now directed towards the development of third generation analogues with a higher activity per unit dose, the development of compounds suitable for oral administration and compounds linked to immunoglobulins with natural products or biomolecules as carrier ligands [13].

*Scope of this review*

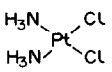
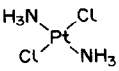
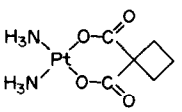
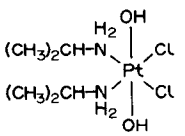
Notwithstanding the large number of analogues tested, cisplatin continues to be the most widely used drug in this class. The development of new analogues with a significantly improved effectiveness and a reduced toxicity will require more basic knowledge of the mode of action of cisplatin and its analogues. The availability of analytical techniques for the detection, the determination and identification of different platinum compounds in biological media is a prerequisite for following the fate of platinum compounds entering the body.

This review summarizes the analytical methodologies in use for the determination of several platinum anti-tumour drugs and their degradation and biotransformation products in blood, plasma and urine.

## General Analytical Considerations

A large number of analytical techniques have been used in the study of platinum anti-tumour agents and their transformation products in aqueous solutions and biological media. Early methods were often limited to the determination of total platinum concentrations. In the course of the investigations, however, it became clear that analytical techniques were needed capable of differentiating between the various platinum compounds, i.e.

**Table 1**
Structures and systematic names of selected platinum coordination compounds

| Line formula | Structural formula | Systematic name | Abbreviation |
|---|---|---|---|
| *cis*-$[PtCl_2(NH_3)_2]$ |  | *cis*-Diamminedichloro-platinum(II) | Cisplatin (CDDP) |
| *trans*-$[PtCl_2(NH3)_2]$ |  | *trans*-Diamminedichloro-platinum(II) | (*trans*-DDP) |
| $[Pt(C_6H_6O_4)(NH_3)_2]$ |  | Diammine(1,1-cyclobutane-dicarboxylato)platinum(II) | Carboplatin (CBDCA, JM-8) |
| $[PtCl_2(OH)_2(C_3H_9N)_2]$ |  | *cis*-Dichloro-*trans*-dihydroxo-*cis*-bis(isopropylamine) platinum(IV) | Iproplatin (CHIP, JM-9) |

speciation. In order to design proper analytical procedures, more knowledge about the reactions which may occur in the body is required. A detailed description of the physical properties of cisplatin can be found in ref. 12.

*Binding to proteins and red blood cells (RBCs)*

Cisplatin and several of its analogues are subject to protein binding [14–18], and the extent of binding varies for different platinum compounds (Fig. 1). Cisplatin shows an extensive protein binding, resulting in a rapid decrease in the free (ultrafilterable) platinum concentration following i.v. injections, whereas for iproplatin hardly any protein binding is observed. It should be noted that the term "protein-bound platinum" is not fully adequate because platinum may be bound to low molecular weight proteins which pass the commonly applied ultrafilters with molecular weight cut-offs of 25,000 or 50,000.

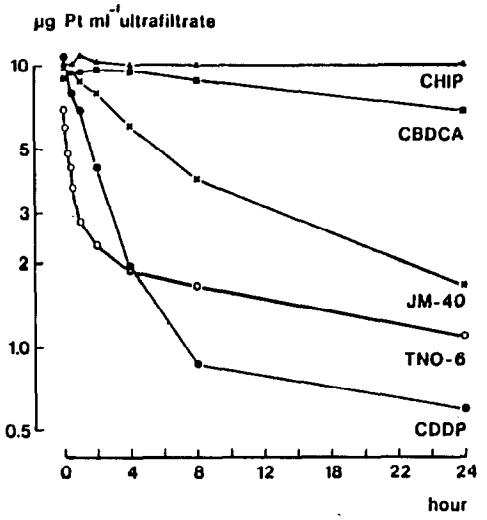



**Figure 1**
Binding of selected platinum compounds to human plasma proteins *in vitro*, as measured by the decline in ultrafilterable platinum. TNO-6 {aqua[1,1-bis(aminomethyl)cyclohexane sulphato platinum(II)]} and JM-40 [ethylenediammine malonato platinum(II)] are not considered in this review. (Reproduced from ref. 18 by permission.)

Plasma protein binding of cisplatin has been reported to be essentially irreversible [14, 15, 19]. However, Daley-Yates and McBrien [20] observed the plasma protein binding to be partly reversible. These authors found that 65, 26 and 7% of platinum was irreversibly protein bound for cisplatin, carboplatin and iproplatin, respectively. So far, the reason for these contradictory observations is not clear. In addition, Hegedus *et al.* [21] have reported that cisplatin bound to plasma proteins can still react with strong nucleophiles, such as diethyldithiocarbamate (DDTC). However, it is questionable whether their analytical technique provided sufficient selectivity to discriminate between the different forms of platinum.

Speciation of protein-bound platinum compounds can be accomplished by size-exclusion chromatography (SEC) [17] (Fig. 2). The protein-bound platinum is considered to be devoid of cytotoxic activity [22–24] and the protein-bound fraction is usually analysed only for its total platinum content.

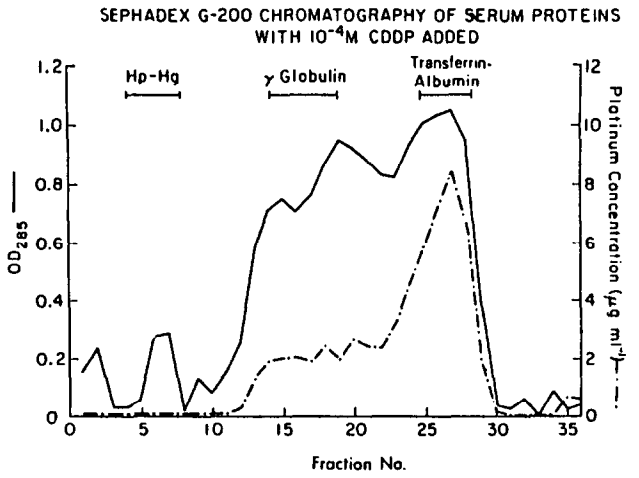



**Figure 2**
Chromatography on Sephadex G-200 of serum containing $10^{-4}$ M cisplatin. The chromatographic conditions are summarized in Table 6. The solid line shows protein elution, the broken line shows the platinum concentration in μg $ml^{-1}$ in each fraction. (Reproduced from ref. 17 by permission.)

Uptake of platinum into RBCs *in vivo* was shown to be very rapid [15], the mean maximum platinum level in RBCs being lower, by a factor of 3 to 4, than the level of platinum bound to proteins. The binding of platinum to RBCs *in vitro* proved irreversible [15]. As is the case with plasma proteins, most analyses are limited to total platinum contents.

*Intact drug*

As the platinum bound to RBCs and plasma proteins is not believed to contribute to anti-tumour activity, most analytical techniques deal with the ultrafiltrates of blood samples. So far, the majority of these analytical techniques have focussed on the determination of the platinum drugs in their intact form, as distinct from the determination of total filterable platinum. Because of the reactivity of the platinum compounds, the concentration of the intact drug is of more relevance than the total filterable platinum concentration, as the latter does not discriminate between the parent drug and transformation products possibly present in the ultrafiltrate.

*(Bio)transformation products*

Cisplatin and its analogues are subject to hydrolysis in aqueous solutions. The hydrolysis scheme for cisplatin and the calculated distribution of platinum species at equilibrium as a function of the chloride concentration are presented in Figs 3 and 4, respectively. In aqueous solutions, at slightly acidic pH and low chloride concentration, cisplatin and both of its hydrated derivatives will be present. In plasma, where the chloride concentration is approximately 0.1 M [26], cisplatin is expected to exist predominantly as the neutral dichloro complex. However, intracellular chloride concentrations in the order of 0.003 M [26] will promote the formation of the hydrated derivatives. It is generally assumed that these hydrated derivatives are important intermediates in the mechanism of action of cisplatin [27]. The hydroxo species are formed at higher pH and behave as inert metal complexes [28].

In solutions of the hydrated derivatives of cisplatin, hydroxo-bridged dimers and trimers may be present [29–31]. These species have been shown to be very toxic [32], but are not likely to be formed under biological conditions [11].

Carboplatin is less susceptible to nucleophilic substitution because of its dicarboxylato chelating ligand. Consequently, the extent of hydrolysis is smaller compared with that of cisplatin. Moreover, the rate of hydrolysis is slow [33]. For iproplatin no significant hydrolysis is observed in 24 h [33].

In aqueous solutions of cisplatin, the anions pyrophosphate, phosphate, acetate and sulphate can exchange with the chloride ligands of cisplatin [34, 35] (Fig. 5); nitrate and perchlorate are inactive in this respect. Half-lives for these reactions with 0.1 M anion concentrations are in the order of a few hours [34]. Intracellular concentrations of phosphate, pyrophosphate and carboxylate are sufficiently high to give rise to such exchange processes. Reactions of cisplatin within the cell will therefore depend not only on the chloride concentration, but also on the type and concentration of other anions and on the presence of charged phosphate groups, e.g. on DNA [34].

Cisplatin is susceptible to reactions with a large number of biological nucleophiles, in particular those containing sulphhydryl groups [14]. Several naturally occurring nucleophiles have been investigated: methionine [36–38], cysteine [33, 36, 38, 39], glutathione [33, 38, 39], histidine [40, 41], arginine [42], alanine [42, 43] and glycine [42–45]. In addition, the reactions with a number of non-biological sulphur containing nucleophiles, such as thiosulphate, diethyldithiocarbamate [38, 46], and bisulphite [47, 48], have been studied. A number of these reactions have also been investigated for carboplatin and iproplatin [4, 33, 38, 46].

Cisplatin may bind to metallothioneins, metal binding proteins of molecular weight 6000–7000 [4], and can show interactions with trace elements such as selenium [49].

Obviously, the reactions studied comprise only a selection of the transformations which cisplatin and its analogues might undergo in the body. More knowledge about the occurrence of biotransformation products is essential in order to obtain a better insight into the mechanisms of action and the toxic side effects of the platinum anti-tumour drugs.

*Demands on detection capabilities*

Figure 6 shows two typical decline curves for the ultrafilterable platinum concentrations following short-time injection (bolus injection) and 6-h infusion. For the 6-h infusion scheme,

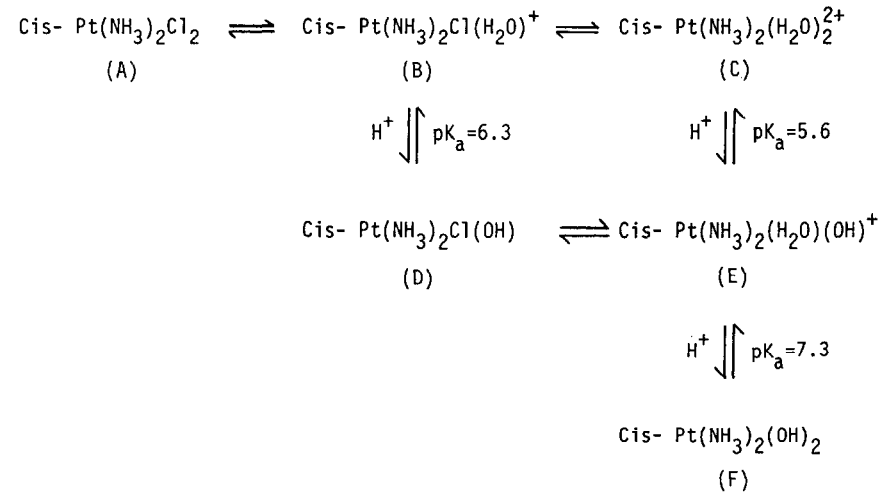



**Figure 3**
Hydrolysis scheme for cisplatin in aqueous solution. p$K_a$ values adopted from ref. 25.

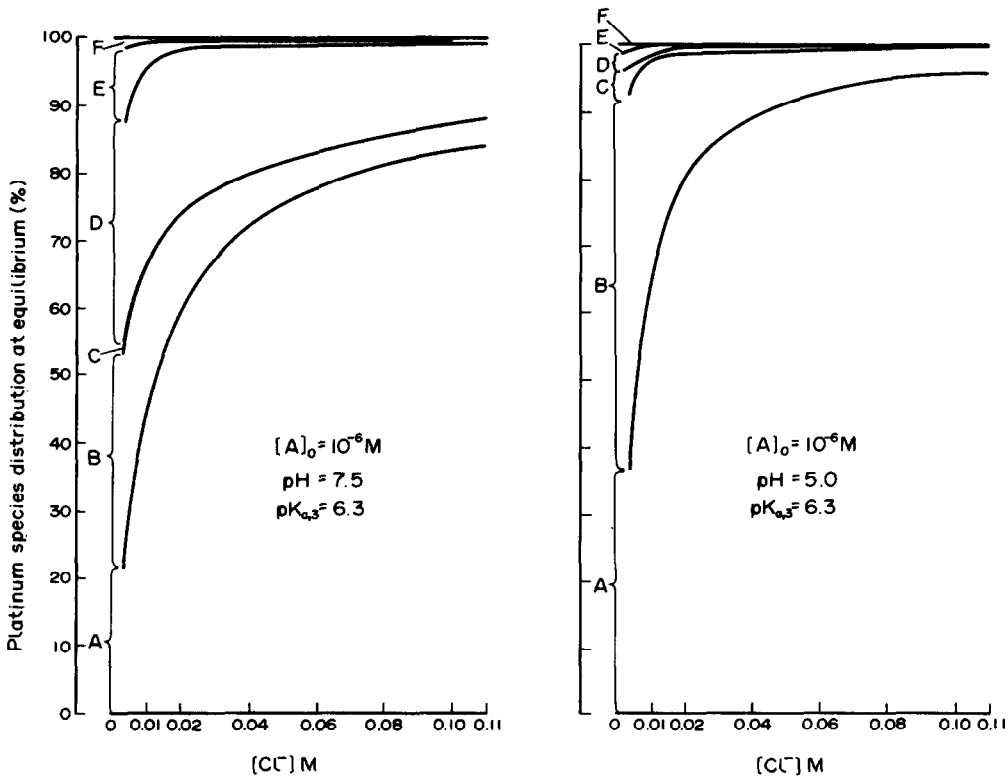



**Figure 4**
Computed cumulative platinum species distribution at equilibrium at 37°C and pH = 1.5 and 5.0 as a function of the chloride concentration. Compounds A to F: see Fig. 3. (Reproduced from ref. 25 by permission.)

ultrafilterable platinum concentrations are an order of magnitude lower to those for the short-time injection scheme. As a consequence, these concentrations fall below the detection limits usually encountered (approximately 10–50 ng Pt $ml^{-1}$) within 2 h after the end of the infusion. The lower the detection limit for an analytical technique, the longer the period is that platinum concentrations can be monitored. Detection demands will be even more stringent for techniques capable of speciating individual platinum compounds, as the

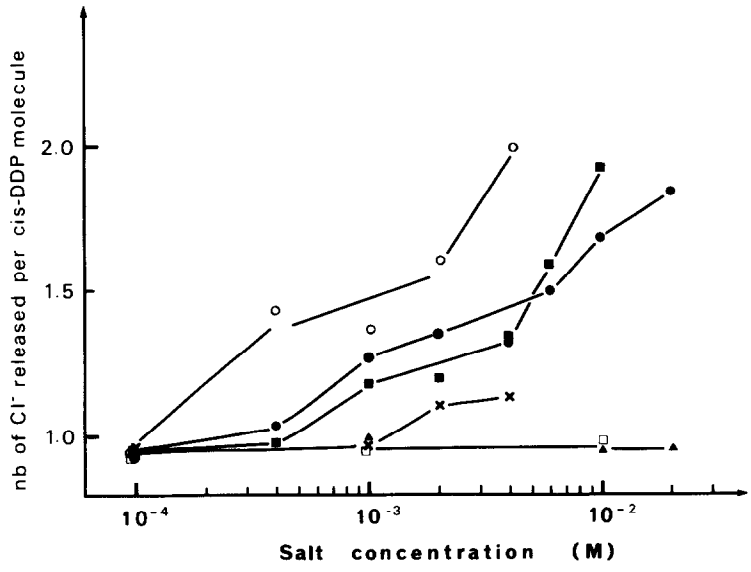



**Figure 5**
Effect of the concentration of various anions on the release of chloride ions from cisplatin at equilibrium as measured by potentiometry. ×, sodium sulphate; □, sodium nitrate; ▲, sodium perchlorate; ●, sodium acetate; ■, potassium phosphate; ○, sodium pyrophoshate; nb = number. (Reproduced from ref. 34 by permission.)

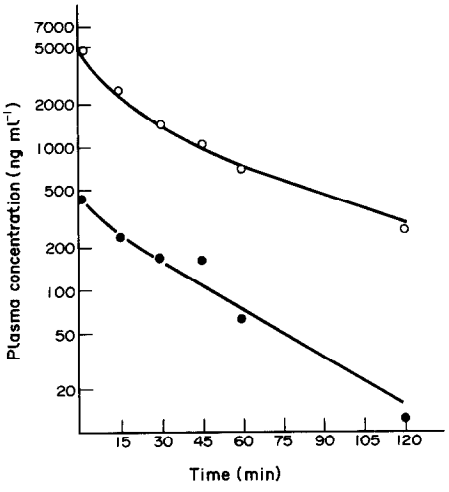



**Figure 6**
Typical plasma level decline of filterable platinum species following bolus injection (○) and 6-h infusion (●) of 100 mg $m^{-2}$ cisplatin. (Reproduced from ref. 22 by permission.)

concentrations of these species will inevitably be less than the total ultrafilterable platinum concentration. Liquid chromatographic separation techniques generally cause an additional dilution of the sample.

In general, analytical techniques with detection limits in the order of 10–50 ng $ml^{-1}$ are useful for the clinical monitoring of total platinum or intact drug concentrations. However, for the biotransformation studies there is a need for more sensitive analytical techniques.

## Sample Handling and Storage

The reactivity of the platinum anti-tumour drugs implies a limited stability in aqueous and biological media. Table 2 summarizes results for stability studies of cisplatin, carboplatin and iproplatin in different media. In view of the short half-life of cisplatin in water and blood plasma (2.3 and 1.5 h, respectively), great care has to be taken to obtain correct and reproducible results, especially in the analysis of "intact" cisplatin. Therefore, the use of an adequate sample handling procedure is mandatory.

*Aqueous solutions*

Cisplatin is stable in aqueous solutions with sodium chloride concentrations of 0.3% or higher [50–53] and it is usually formulated for i.v. injections as an aqueous solution containing 0.9% NaCl (isotonic saline). The presence of dextrose or mannitol does not appear to affect the stability of the drug [51, 52]. The stability has been found to be identical for storage in plastic bags and in glass bottles [52]. Garren and Repta [47] have shown reaction with bisulphite in drug formulations. Although exposure to normal laboratory light has been reported not to affect stability [51], changes in absorption spectra have been observed [53]. Therefore it is recommended to minimize exposure to light [53]. Refrigerated solutions of cisplatin should have a concentration

**Table 2**
Summary of stability studies for cisplatin, carboplatin and iproplatin in aqueous/plasma media.* (Reproduced from ref. 50 by permission of the authors and Preston Publications, a division of Preston Industries, Inc.)

| Platinum compound studied | Aqueous or plasma medium | Half-life ($t_{1/2}$) |
|---|---|---|
| CDDP (Strem) | Distilled water, HPLC-grade (20 ppm) | 2.33 h |
| | Saline infusion solution (0.9% NaCl) (100 ppm) | Stable at 6 days |
| CDDP (Bristol Labs or Johnson Matthey) | Saline infusion solution (0.018% NaCl) (20 ppm) | 18 h |
| | Saline infusion solution (0.9% NaCl) (100 ppm) | Stable at 6 days |
| | Saline infusion solution (0.09% NaCl) (100 ppm) | 22.6 h |
| | Blood plasma† (0.45% NaCl) (50 ppm) | 1.45 h |
| CBDCA (Johnson Matthey) | Saline infusion solution (0.9% NaCl) (100 ppm) | Stable at 7 days |
| CHIP (Johnson Matthey) | Saline infusion solution (0.9% NaCl) (40 ppm) | Stable at 6 days |
| | Distilled water, HPLC-grade (40 ppm) | Stable at 6 days |
| | Blood plasma (50 ppm)‡ | 30 h |

*All studies were done via constant-temperature (37°C) water bath incubation of initial solutions, with removal of aliquots and individual analyses done at regular, timed intervals. All methods of analysis involved reductive LCEC.
†The recovery at zero time of CDDP from blood plasma at this level was 95–100%.
‡The recovery at zero time of CHIP from blood plasma at this level was about 70–75%.

<0.6 mg $ml^{-1}$ to avoid precipitation [53]. In general, calibration solutions of cisplatin are discarded after 7 days [50, 54].

Carboplatin and iproplatin have been found to be stable for 24 h in infusion solutions containing sodium chloride and dextrose [52]. The stability of iproplatin has been reported not to be related to the chloride concentration [52]. Carboplatin seemed to be somewhat less stable in solutions with high chloride concentration (0.9%, w/v), and possibly carboplatin is converted to cisplatin in the presence of chloride ions [52]. This finding is in contradiction with the stability reported by Krull *et al.* (stable for 7 days, Table 2) [50]. Sewell *et al.* [55] have reported on the stability of carboplatin in infusion solutions containing mannitol. There was no loss of carboplatin from pre-filled polypropylene syringes stored at 4°C for 5 days. At 37°C, the loss of carboplatin was 3.1% over 24 h.

*Blood, plasma and plasma ultrafiltrate*

Blood samples are usually collected in test tubes containing EDTA, heparin [56] or citrate [57] as the anticoagulant. Care has to be taken when using heparin, because it might affect plasma protein binding, as has also been shown for zinc [58, 59]. Moreover, in spite of the presence of anticoagulants, clotting may occur upon thawing [60], resulting in inhomogeneous samples.

The following sample handling procedure has been suggested by Long *et al.* [61]:

(1) rapid centrifugation of blood samples and removal of erythrocytes;
(2) ultrafiltration of plasma to remove proteins;
(3) immediate analysis or flash freezing and storage of the ultrafiltrate at $\leq -10$°C.

Plasma ultrafiltrate samples prepared in this way are claimed to be stable for 72 h [61]. Ultrafiltrate samples containing carboplatin, stored at −25°C, are reported to be stable for several weeks [62].

Except for minor variations, the above procedure is generally accepted as the standard procedure for blood samples. Centrifugation is commonly performed for 5 to 15 min at 1000***g***. Ultrafiltration is usually carried out over Amicon Centriflo CF-50A filters ($M_W$ cut-off 50,000) by centrifugation for 10 min at 1000***g***. In this way, more than 98% of the plasma proteins are removed [63, 64]. Filters can be cleaned by sonication for 30 min in distilled water and can be stored in ethanol–water (10:90, v/v) [63]. Before use, excess water is removed by centrifugation. The ultrafiltration is sometimes performed under refrigeration at 4°C. In general, the entire ultrafiltrate is

collected and its platinum concentration is regarded equal to the concentration of "free", non-protein-bound platinum species in the original sample.

The ultrafiltration procedure has been studied in more detail by Van der Vijgh *et al.* [18]. Two different ultrafiltration systems (Amicon Centriflo CF-50A cones and Amicon MPS-1 micropartition system with YMT filters) were evaluated in terms of recoveries and the time course of the platinum concentration in the filtrate during ultrafiltration. Because of the higher recoveries and the possibility of processing smaller sample quantities (<1 ml), the MPS-1 filter system was shown to be a valuable alternative to the CF-50A cones. The observed time dependence of the platinum concentration in the filtrate during ultrafiltration, again stresses the need for careful evaluation and standardization of all individual steps in the analytical procedure.

Removal of proteins can also be accomplished by denaturation with acetonitrile or trichloroacetic acid [56]. However, this approach may lead to erroneous results. In the first place it can introduce a redistribution of the drug and secondly, both acetonitrile and acetate may react with cisplatin or with its transformation products [34, 35, 65]. RBCs are usually analysed after digestion with nitric acid [15].

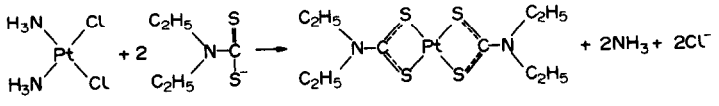
*Urine*

For urine samples, flash freezing and storage over solid carbon dioxide (−60°C) is recommended [66]. Samples prepared in this way are stable for 48 h; when stored at −11°C, there is a 30% loss of intact cisplatin after 24 h [66]. Carboplatin is relatively stable and urine samples stored at −25°C can be analysed within a few weeks after collection [62]. After thawing, urine samples are generally sonicated and filtered.

## Determination of Total Platinum

*Derivatization procedures*

Total platinum concentrations in urine have been determined using sodium diethyldithiocarbamate (NaDDTC) as the derivatizing agent [67] (Scheme 1):



**Scheme 1**

The platinum–DDTC complex was extracted in chloroform and determined by HPLC, using a CN-modified silica packing with heptane–isopropanol (82:18, v/v) as the mobile phase. The high molar absorptivity of the platinum–DDTC complex (43,000 $M^{-1}$ $cm^{-1}$) allowed the determination of platinum down to levels of 25 ng $ml^{-1}$ with 9 ml urine samples [67].

Similar derivatization procedures, using DDTC as the complexing agent, have been reported by Borch *et al.* [68], Reece *et al.* [69], Drummer *et al.* [70] and Andrews *et al.* [71]. The procedure reported by Reece *et al.* [69] for plasma ultrafiltrate is rather laborious, but offers a lower detection limit (2.5 ng $ml^{-1}$). Drummer *et al.* [70] applied the DDTC derivatization to urine as well as plasma ultrafiltrate samples, using nickel(II) as the internal standard. The same procedure was used by Andrews *et al.* [71] for plasma ultrafiltrate. Figure 7 shows typical chromatograms for the platinum and nickel DDTC complexes in saline and plasma ultrafiltrate on an ODS column with methanol–water (4:1, v/v) as the mobile phase. However, the authors have shown that about 25% of the platinum present in the ultrafiltrate is resistant to DDTC derivatization [71]. As these components were not considered cytotoxic, it was suggested that the DDTC-derivatized platinum represents the "active" platinum species. Complexes of cisplatin with glutathione, cysteine and thiosulphate were relatively unreactive towards DDTC, while complexes of cisplatin with methionine and cisplatin itself were nearly completely converted into their DDTC adducts [71]. This difference in reactivity towards DDTC for different platinum compounds, however, makes the derivatization procedure unsuitable for total platinum determinations. When this procedure is still selected for platinum determinations, great care has to be taken in the interpretation of the results.

Besides the HPLC determination of the platinum–DDTC complex, a voltammetric procedure has been also applied [72].

Inagaki [73] has reported a total platinum determination in urine using *N,N'*-bis(3-mercapto-2-quinoxalinyl)-1,3-propanediammine as the complexing agent, followed by derivative spectrometric analysis.

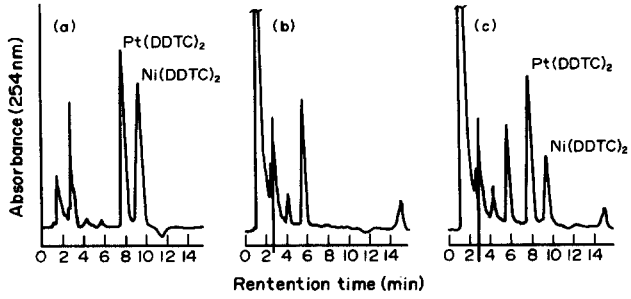



**Figure 7**
Chromatograms of extracts of DDTC derivatized samples (Scheme 1): (a) cisplatin and nickel chloride internal standard in saline; (b) blank plasma ultrafiltrate; and (c) plasma ultrafiltrate with internal standard of a patient receiving 100 mg $m^{-2}$ cisplatin by i.v. infusion. (Reproduced from ref. 71 by permission.)

*Atomic absorption spectrometry*

The various spectrometric techniques reported in literature for the determination of total platinum are surveyed in Table 3. Electrothermal atomization atomic absorption spectrometry (ETA–AAS) is the most frequently used technique. From Table 3 several general features of platinum determinations by ETA–AAS become apparent. Whole blood and tissues are usually digested by wet ashing procedures involving mixtures of nitric acid, perchloric acid and hydrochloric acid. However, the resulting nitrate and perchlorate in the sample have been shown to cause interferences in the platinum determination [74, 60, 80, 83, 84]. The nitrate interference could be overcome by the addition of hydrochloric acid or perchloric acid followed by heating [74, 60] or by the addition of an ammonia solution to the digested sample in the graphite furnace [84]. Alternatively, tissues can be digested by dry ashing procedures [81, 78] or analysed directly after homogenization [79]. McGahan *et al.* [86] have compared dry and wet ashing procedures for tissue samples. In contrast to the findings of Denniston *et al.* [81], dry ashing was shown to result in a significant but unexplained loss of analyte, while a complete recovery was obtained for a specific wet ashing procedure. In the procedure reported by Priesner *et al.* [79] sample digestion could even be avoided. Sample preparation involved only tissue homogenization using all-glass homogenizers. The Triton X-100 detergent aided in tissue homogenization and was reported to be essential in producing uniform and consistent drying of the sample in the graphite furnace. Complete recoveries were obtained, while only minimal interferences from the matrix were observed [79].

For urine, plasma, serum and ultrafiltrate samples, various procedures have been proposed, which require minimum sample pretreatment. In general, the biological matrices appear to interfere with the platinum determination and therefore, in most cases, standard addition techniques or matrix-matched standards are used (Table 3, columns "Interferences" and "Sample treatment"). More specific data on interfering compounds present in these matrices are reported for nitrate (see above), chloride [83, 79] and iron [83].

The analytical ETA–AAS methods in the various references are similar. Specific attention has been paid to the importance of ashing by ramp-wise raising of the temperature [75, 80] in order to remove the organic material. Also step-wise ashing procedures have been applied. Upper ashing temperatures were reported ranging between 1200–1500°C [79, 80, 86]. Above 1600°C loss of analyte occurred [86]. With ashing below 1100°C multiple atomization peaks were observed in the analysis of urine samples [80]. Atomization was preferably performed at 2600–2750°C [80, 83, 86], although Priesner *et al.* [79] reported a temperature of 2350°C to be adequate. Deuterium or Zeeman-effect background corrections are often necessary. Good agreement between these two methods was obtained [86], although the Zeeman system gave only about half the sensitivity of the deuterium-based system. Detection limits for ETA–AAS are in the order of 10–40 ng $ml^{-1}$ for plasma, ultrafiltrate and urine.

*Atomic emission spectrometry*

Atomic emission spectrometric techniques have only recently been introduced for the determination of total platinum concentrations

**Table 3**
Spectrometric techniques for the determination of total platinum

| First author | Ref. | Sample | Technique | Detection limit | Interferences | Sample treatment |
|---|---|---|---|---|---|---|
| Miller | 74 | Tissues | EGA–AAS, D-bg.corr. | 200 ng $g^{-1}$ | Nitrate | Wet digestion |
| Jones | 60 | Whole blood, urine | ETA–AAS, D-bg.corr. | 30 ng $g^{-1}$ blood (sample vol. 5 g), 3 ng $g^{-1}$ urine, (sample vol. 50 g) | Nitrate | Wet digestion; urine digests centrifugated to remove insoluble potassium perchlorate; volatilization of perchloric acid at 1900°C in the graphite furnace; matrix-matched standards |
| LeRoy | 75 | Tissues, plasma, urine | ETA–AAS, D-bg.corr., 265.9 nm | 30 ng $ml^{-1}$, 100–200 ng $g^{-1}$ tissue | Plasma components | Urine, plasma: no pretreatment; matrix-matched standards for plasma. Tissues: wet digestion |
| Pera | 76 | Tissues, blood | ETA–AAS, D-bg.corr., 265.9 nm | 100 ng $g^{-1}$ tissue, 20 ng $g^{-1}$ plasma | No nitrate interference | Tissues, blood: wet digestion (24 h); separation of hydrolysate; addition of nitric acid; separation of acid phase. Plasma: direct analysis |
| Bannister | 63 | UF | X-ray fluorescence | 240 ng $ml^{-1}$ | — | Reaction with ethylenediammine at room temperature for 24 h; collection on cation-exchange disc |
| Bannister | 77 | UF | ETA–AAS, 265.95 nm | 35 ng $ml^{-1}$ | — | Reaction with ethylenediammine at room temperature for 24 h; collection on cation-exchange disc; elution with hydrochloric acid |
| Hull | 78 | Serum, UF | ETA–AAS, 265.9 nm | 25 ng $ml^{-1}$ | Plasma and urine components | Wet digestion; complexation with ammonium 1-pyrrolidinedithio-carbamate; extraction into MIBK; matrix-matched standards |
| Priesner | 79 | Plasma, urine, tissues | ETA–AAS, 265.19 nm | 20 ng $g^{-1}$ (ng $ml^{-1}$) | Chloride when ashing at 700°C | Homogenization after dilution with a Triton X-100 solution |

| | | | | | | |
|---|---|---|---|---|---|---|
| Smeyers-Verbeke | 80 | Plasma, serum, urine, digested blood | ETA–AAS, D-bg.corr. | 2.5 ng $ml^{-1}$ | Nitrate, perchlorate | Plasma, urine and serum: no pre-treatment; blood: wet digestion |
| Denniston | 81 | Tissues | ETA–AAS, 265.95 nm | 400 ng $g^{-1}$ | None observed | Dry digestion |
| Cano | 82 | Plasma, urine | ETA–AAS, tantalized furnaces | 10 ng $ml^{-1}$ | — | Plasma: dilution with nitric acid solution; urine: dilution with water. Matrix matched standards. Nebulizer type sample introduction |
| Sharma | Tissue, blood, urine | ETA–AAS, D-bg.corr. | — | Nitrate, iron, chloride, biological compounds | | Wet digestion; after cooling addition of hydrogen peroxide. Standard addition technique |
| Matsumoto | 84 | Tissues | ETA–AAS | 8 ng $ml^{-1}$ | Nitrate | Wet digestion; interfering nitrate removed by addition of ammonia solution |
| Queralto | 85 | Plasma, UF | ETA–AAS, Z-bg.corr., 266.2 nm | 45 ng $ml^{-1}$ | — | Plasma diluted with a Triton X-100 solution |
| McGahan | 86 | Serum, urine, tissues | ETA–AAS, L'vov platforms, D- or Z-bg.corr., 265.9 nm | 60 ng $ml^{-1}$ | — | Serum and urine: no pretreatment; tissues: dry digestion and dissolution in different acids or wet digestion including treatment with hydrogen peroxide |
| Shearan | 87 | Urine | ETA–AAS, 265.9 nm | 400 ng $ml^{-1}$ | Biological compounds | Dilution with nitric acid solution |
| Vermorken | 15 | Plasma, UF, urine, RBCs | ETA–AAS | — | — | Plasma and urine: dilution with solutions containing sodium chloride and hydrochloric acid; RBCs: wet digestion |
| Reece | 69 | Plasma, UF, RBCs | ETA–AAS, Z-bg.corr., 269.95 nm | 10–25 ng $ml^{-1}$ | — | Dilution with a Triton X-100 solution |

**Table 3**
Continued

| First author | Ref. | Sample | Technique | Detection limit | Interferences | Sample treatment |
|---|---|---|---|---|---|---|
| El-Yazigi | 88 | Plasma | ETA–AAS, bg.corr., 265.95 nm | 50 ng $ml^{-1}$ | — | Dilution with a Triton X-100 solution; matrix-matched standards |
| Dominici | 89 | Serum, urine | ICP–AES, 214.423 nm | 2–4 ng $ml^{-1}$ | Biological compounds | Serum and urine: dilution with water. Urine: aqueous calibration standards. Plasma: standard addition technique |
| Lo | 90 | Urine | ICP–AES, bg.corr., 214.423 nm | 50 ng $ml^{-1}$ | — | No pretreatment; matrix matched standards or background correction |
| Belliveau | 91 | Blood, urine, plasma, tissues | DCP–AES, 299.8 nm | 100 ng $ml^{-1}$ or 100 ng $g^{-1}$ tissue | Biological compounds | Body fluids: no pretreatment; tissues: homogenization after mixing with a Triton X-100 solution; matrix-matched standards |
| McLoughlin | 92 | Plasma, UF, urine | DCP–AES, 265.95 nm | 40 ng $ml^{-1}$ urine, 20 ng $ml^{-1}$ UF, 200 ng $ml^{-1}$ plasma | None observed | Samples diluted with solutions of nitric acid and Triton X-100 |
| Alimonti | 93 | Serum, urine, tissues, UF | ETV–ICP–AES, 214.423 nm | 0.25–0.5 ng $ml^{-1}$ | Biological compounds | Serum and urine: dilution with water; tissues: dry ashing. Matrix-matched standards |
| Charney | 94 | Biological samples | ETV–ICP–MS | ca. 5 pg $ml^{-1}$ | — | — |

Abbreviations: D-bg.corr. = deuterium background correction; Z-bg.corr. = Zeeman-effect background correction; UF = ultrafiltrate.

in biological samples (Table 3). Dominici *et al.* [89] have used inductively coupled plasma atomic emission spectrometry (ICP–AES) for the determination of total platinum levels in serum and urine. The term plasma refers in this context to a hot, partially ionized, gas. Detection limits of 2–4 ng $ml^{-1}$ compare favourably with those obtained using ETA–AAS. Although interferences due to differences in viscosity and surface tension of the sample solutions have been observed, these effects are generally less dramatic than for ETA–AAS. The wide linear-dynamic range (four orders of magnitude for ICP–AES and only two for ETA–AAS) and the high precision and accuracy [89] make this technique competitive with the established ETA–AAS technique. A procedure similar to that described by Dominici *et al.* [89] has been reported by Lo *et al.* [90]. The detection limit of 50 ng Pt $ml^{-1}$ urine, however, is an order of magnitude poorer than that reported by Dominici *et al.* [89]. This is probably caused by the inadequate spectrometer arrangement available to the authors.

Introduction of the sample into the ICP by electrothermal vaporization (ETV) can improve detection limits by an order of magnitude to 0.25–0.5 ng $ml^{-1}$, with the additional advantage that only small quantities of sample are required (100 µl per analysis) [93]. Matrix effects, however, cannot be neglected and specific calibration curves have to be used for each type of sample [93].

Apart from ICP, direct current plasmas (DCP) have also been used [91, 92]. Since background or spectral interferences were virtually absent with DCP, calibration could be performed with aqueous solutions [92]. Total platinum determinations in body fluids and brain tissues have been described using a graphite filament for sample introduction into the DCP [91]. In this technique, only 10 ≃ 50-µl samples of brain tissue homogenates or body fluids are deposited on a carbonized nylon filament. After desolvation and partial ashing of the sample, the filament is passed through the DCP. The technique is susceptible to matrix effects and consequently standard addition or matrix matching have to be employed [91].

Very promising results have been reported for inductively coupled plasma–mass spectrometry (ICP–MS), combined with electrothermal vaporization [94]. Detection limits for a 10-µl biological sample are reported to be at the 5 pg $ml^{-1}$ level [94]. This means an improvement of two to three orders of magnitude compared with conventional ICP–AES and ETA–AAS techniques, and two orders compared with ETV–ICP–AES.

*Miscellaneous techniques*

Although several electrochemical techniques are suitable for the determination of platinum in aqueous solutions, their applicability for biological samples is limited. In general, the detector response depends strongly on the nature of the ligands coordinated to platinum and is affected by electrode surface phenomena and reaction-medium effects [12, 87]. Especially for biological media, these limitations make it hard to produce meaningful results [87].

X-ray fluorescence has been applied by Bannister *et al.* [63] (Table 3). The platinum in the ultrafiltrate was converted to a cationic species by reaction with ethylenediamine and collected on paper impregnated with cation-exchange resin. The cation-exchange discs could be analysed directly.

Platinum determination by neutron activation analysis has a low detection limit (0.3 ng Pt) [95, 96], but is associated by long and complex sample handling. The same holds for techniques using radioisotopes [95, 97]. In addition, these latter techniques are difficult to use for *in vivo* clinical studies.

## Determination of Intact Drug and (Bio)transformation Products

Apart from the determination of total platinum concentrations, there is a considerable interest in the determination of individual platinum compounds. Thus far, the majority of speciating analytical techniques in use in platinum drug research, have dealt with the determination of drugs in their intact form. Nearly all these techniques are based on liquid-chromatographic separation of the drugs from other platinum compounds and interfering species. Different types of chromatography have been used (ion-exchange, ion-pair, normal-phase, reversed-phase, thin-layer) in combination with various detection techniques.

Although the concentration of the intact drug provides more adequate information than the total concentration of free platinum species, determined as "total ultrafilterable

platinum", information about the distribution of other platinum species is most often lost. This is primarily due to the differences in retention behaviour between the parent cisplatin and possible biotransformation products, such as the hydrated derivatives and the platinum–amino acid complexes. Thus, while cisplatin is easily retained on anion-exchangers and ion-pair systems with cationic detergents, the positively charged transformation products are unretained in these systems. The reverse holds for chromatographic systems suitable for the speciation of the positively charged species.

In this respect, $^{195}Pt$ nuclear magnetic resonance (NMR) spectroscopy [98] shows attractive features for speciation studies, at least for the analysis of aqueous solutions of these compounds. Unfortunately, the different species are not physically separated and are therefore not available for further characterization.

*Metal surfaces and mobile phases*

Especially in speciation studies, care has to be taken to preserve the identity of the species under investigation. For these highly reactive platinum compounds, the possibility of reactions with metal surfaces and mobile phases has to be considered. Cisplatin is reported to react with aluminium [12]. As there are no indications of its incompatibility with stainless steel [12], no special precautions are required with respect to the HPLC apparatus used. Unfortunately, this does not hold for buffers and organic modifiers. As described in the section "General analytical considerations", acetate and phosphate can take part in exchange reactions in solutions of platinum drugs. Consequently, acetate and phosphate buffered mobile phases should be used with great care. This most probably holds for other buffer types as well. In addition, the organic modifiers acetonitrile and dimethylsulphoxide (DMSO) have shown reactivity towards platinum compounds [6, 65, 99]. Therefore, alcoholic modifiers should be preferred [65].

Special attention should always be given to such undesirable effects, not only in the development of new analytical procedures, but also in the application of established techniques.

*Anion and cation exchangers*

The earlier methods for the determination of intact cisplatin are based on strong ion exchangers. Essential data on these systems are given in Table 4. The anion exchange system described by Chang *et al.* [64], and later on adapted by Repta *et al.* [14], is capable of separating cisplatin from its hydrolysis products and from some of its analogues. A typical chromatogram obtained for plasma ultrafiltrate is shown in Fig. 8. Since the absorptivity of cisplatin above 220 nm is low ($\epsilon_{max(301\,nm)} = 130\ M^{-1}\ cm^{-1}$), the direct UV detection at 280 or 300 nm is very insensitive. In addition, peak shapes and resolution are poor, while about 80% of the platinum present in the sample was eluted in the solvent front [14]. The latter effect is probably caused by the presence of positively charged species, which are unretained in an anion exchange system. In order to separate these species, cation exchange chromatography was explored. The chromatographic system reported by Safirstein *et al.* [100] was found suitable for separating cisplatin and its hydrated derivatives (Fig. 9). It has been applied to the analysis of plasma and urine samples of rats treated with cisplatin. From these experiments it was concluded that the predominant species present in urine and ultrafiltrate was the unchanged cisplatin [100]. Aqueous solutions of cisplatin have also been analysed by Hincal *et al.* [51] using an anion exchanger and a cation exchanger in series. In contrast to the system described by Safirstein *et al.* [100], their chromatographic system does not discriminate between the hydrated derivatives.

Strong cation exchangers have also been applied to the determination of *trans*-Pt $(NH_3)_2Cl_2$ in the presence of cisplatin (Woollins *et al.* [101]) and to the separation of platinum drugs containing the 1,2-diaminocyclohexane (DACH) ligand and their possible biotransformation products (Mauldin *et al.* [102]).

*Ion-pair chromatography*

Ion-pair chromatography was introduced for the determination of intact platinum drugs by Riley *et al.* [66, 103]. A reversed-phase ($C_{18}$) column was used, with a mobile phase containing hexadecyltrimethylammonium bromide (HTAB). With clinical samples it was found that between 75–95% of cisplatin was excreted unchanged in the urine [66]. Cisplatin was supposed to be retained on the column primarily as a result of ion–dipole interaction between cisplatin and the adsorbed cationic

**Table 4**
Ion-exchange systems for the analysis of platinum drugs

| First author | Ref. | Fig. | Column | Mobile phase | Detection | Sample | Compounds | Detection limit |
|---|---|---|---|---|---|---|---|---|
| Chang, Repta | 64, 14 | 2.8 | Partisil 10 SAX, 250 × 4.6 mm i.d., 50 µl loop | Methanol–acetate buffer (0.1 M; pH = 3.8) 1:1 and 4:1 | UV 280 nm, ETA–AAS after derivatization | UF | Cisplatin | 1000 ng $ml^{-1}$ (UV); 40 ng $ml^{-1}$ (AAS) |
| Safirstein | 100 | 2.9 | Partisil 10 SCX, 250 × 4 mm i.d., 10 µl loop (urine), 500 µl loop (UF) | Ammonium formate buffer (0.012 or 0.03 M) or acetate buffer (0.03 M), pH = 3.5 | Radioactivity, ETA–AAS | UF/urine | Cisplatin, hydrolysis and biotransformation products | — |
| Woollins | 101 | — | Partisil 10/25 SCX, 250 × 4.6 mm i.d. | Ammonium phosphate buffer (0.25 M) | UV 254 nm | Aqueous solutions | Cisplatin and its *trans*-isomer after treatment with thiourea | 0.01% *trans* in *cis* |
| Mauldin | 102 | — | Partisil 10 SCX, 250 × 4.5 mm i.d., 100 and 500 µl loop | Phosphate buffer (0.05 M, pH = 4), 15% acetonitrile, (a) without $KNO_3$ and (b) with 1.0 M $KNO_3$; gradient elution | Radioactivity | Aqueous solutions | DACH–platinum complexes, Pt–amino acid complexes | — |
| Hincal | 51 | — | Partisil 10 SAX and SCX in series, 50 µl loop | Acetate buffer (0.1 M; pH = 5.6)–methanol 3:7 | UV 280 nm | Aqueous solutions | Cisplatin, hydrolysis products | 20,000 ng $ml^{-1}$ |

Notes: (1) Detection limits usually apply to the drug under investigation in aqueous solutions, unless stated otherwise. (2) If, besides cisplatin, more drugs are listed under "Compounds", detection limits apply to cisplatin, unless stated otherwise. (3) Abbreviations in Table 2.4–2.7: UF = ultrafiltrate; DACH = diamminocyclohexane; DME = dropping mercury electrode; HMDE = hanging mercury-drop electrode.

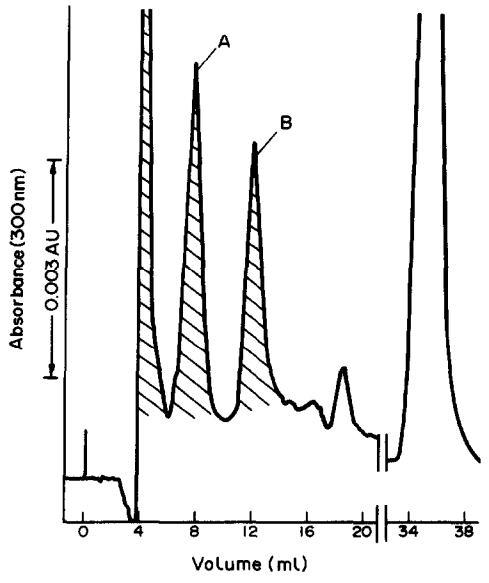



**Figure 8**
Strong anion-exchange chromatogram of plasma ultrafiltrate incubated with cisplatin. Only the cross-hatched peaks were found to contain platinum. The retention volume of cisplatin was 6.6 ml. (Reproduced from ref. 14 by permission.)

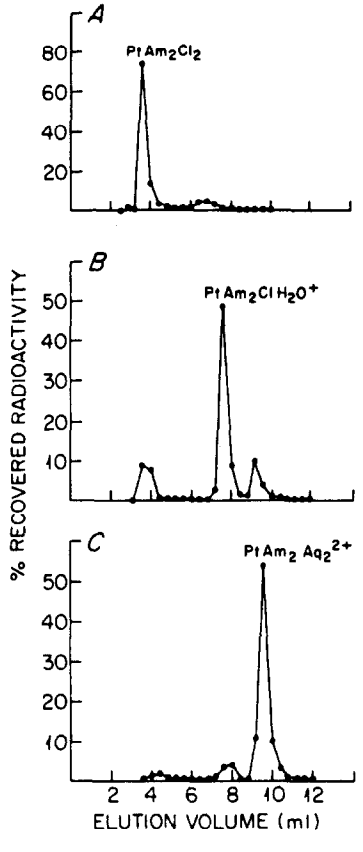



**Figure 9**
Chromatograms of [$^{195}$Pt]cisplatin (A) and hydrated derivatives of cisplatin (B and C), obtained using a strong cation-exchanger (Am = ammine). (Reproduced from ref. 100 by permission.)

surfactant [54, 104]. The ion-pair system developed by Riley *et al.* [66, 103] constituted the basis of many other chromatographic procedures for the determination of cisplatin and its analogues (Table 5).

A number of these studies deal with the application of different detection techniques for liquid chromatography. Figure 10 shows some typical chromatograms for cisplatin in plasma and urine, obtained with different detection techniques. Next to UV detection, ETA–AAS detection is most frequently used. However, its off-line nature makes this technique unattractive for routine applications. For elecrochemical (EC) detection both solid [50, 56, 107] and mercury-drop electrodes [103] have been applied. In general, the detection limits are two to three orders of magnitude better than those obtained with conventional UV detection. An important advantage of the solid electrodes is that by combination of two or more sets of electrodes selectivity can be improved [56]. A disadvantage, which holds for nearly all EC detection techniques, is the strong dependence of the signal response on the nature of the ligands coordinated to platinum. Thus, while detection limits of 10–100 ng $ml^{-1}$ could be obtained for cisplatin, carboplatin could be detected only down to 5 μg $ml^{-1}$ [50]. The practical utility of EC detection therefore strongly depends on the EC response of the species in question.

The LCEC method reported by Richmond *et al.* [107] involves chloride assisted oxidation at platinum electrodes. However, the addition of chloride to the mobile phase should be discouraged because of the ligand-exchange potential of chloride ions and the deleterious effects of chloride on the HPLC apparatus.

Marsh *et al.* [108] have developed a post-column reaction detector for cisplatin and several analogues. It is based on the reaction of cisplatin with sodium bisulphite in the presence of potassium dichromate [48, 108]. A disadvantage of this detection technique is its incompatibility with organic modifiers. Quenched phosphorescence detection [109, 110] has been applied to the determination of cisplatin and carboplatin, but suffers from interference by phosphorescence-quenching compounds present in the matrix. However, for the determination of cisplatin in plasma and urine, this problem has been overcome by the use of an off-line clean-up column [110]. The post-column reaction detection and the quenched

**Table 5**
Ion-pair systems using cationic detergents for the analysis of platinum drugs

| First author | Ref. | Fig. | Column | Mobile phase | Detection | Sample | Compounds | Detection limit |
|---|---|---|---|---|---|---|---|---|
| Riley | 66, 103 | 2.10 | Hypersil and ODS Ultrapak (switching system), 20 μl loop | Citrate buffer (0.01 M; pH = 7), 0.1 mM HTAB | UV 280 nm, ETA–AAS | Spiked urine | Cisplatin, carboplatin iproplatin, DACH–Pt complex | $10^5$ ng ml$^{-1}$ (UV), 2000 ng ml$^{-1}$ (AAS) |
| Earhart | 105 | — | Varian MCH 10 (ODS), 300 × 4 mm i.d. | 0.005 M tetrabutyl ammonium chloride in water | UV 300 nm, ETA–AAS | Urine | Intact versus not-intact cisplatin | — |
| Krull, Ding | 50, 56 | 2.10 | Alltech C18, Biophase C18, 250 × 4.6 mm i.d., 20 μl loop | Acetate buffer (0.01 M; pH = 4.6), 0.15 mM HTAB, methanol, NaCl | Amperometry: working electrode Au/Hg or glassy carbon, reference Ag/AgCl | UF | Cisplatin, carboplatin iproplatin | 10 ng ml$^{-1}$ (red); 160 ng ml$^{-1}$ (ox); 100–200 ng ml$^{-1}$ for spiked plasma; 5000 ng ml$^{-1}$ carboplatin; 100 ng ml$^{-1}$ iproplatin |
| Bannister | 106 | 2.10 | Technicon C8, 150 × 4.6 mm i.d., column temperature 62°C, 50 μl loop | Citrate buffer (0.005 M; pH = 6.5), 0.1 mM HTAB | Polarography: DME 0.5 and 1 s, HMDE | Urine | Cisplatin, iproplatin | 64 ng ml$^{-1}$ 0.5 s DME; 36 ng ml$^{-1}$ 1.0 s DME; 1.4 ng ml$^{-1}$ HMDE; <100 ng ml$^{-1}$ in urine |
| Richmond | 107 | — | ODS, 20 μl loop | 1 mM HTAC1, made up to 0.1 M with KCl | Differential pulse voltammetry | Serum | Cisplatin | 50 ng ml$^{-1}$ |
| Marsh | 108 | 2.10 | Hypersil ODS, 100 × 4.6 mm i.d. or 150 × 4.6 mm i.d., 20 μl loop | Citrate buffer (0.01 M; pH = 5.25), 0.1 mM HTAB | Post-column reaction with sodium bisulphite, UV 290 nm | UF | Cisplatin, carboplatin malonato–Pt, DACH–Pt and etheendiammine–Pt complexes | 40 ng ml$^{-1}$; <150 ng ml$^{-1}$ in UF; 1200 ng ml$^{-1}$ Carboplatin; 40–1200 ng ml$^{-1}$ for others |

**Table 5**
Continued

| First author | Ref. | Fig. | Column | Mobile phase | Detection | Sample | Compounds | Detection limit |
|---|---|---|---|---|---|---|---|---|
| Gooyer | 109 | — | Hypersil ODS, 100 × 4.6 mm i.d., 20 µl loop | Citrate buffer (0.001 to 0.01 M, pH 5.0 to 6.2), methanol (0–10%), 0.01 M biacetyl, 0.02 mM HTAB | Quenched biacetyl phosphorescence | UF, urine | Cisplatin, carboplatin | 90 ng $ml^{-1}$; 120 ng $ml^{-1}$ Carboplatin |
| Baumann | 110 | 2.10 | Spherisorb ODS, 250 × 4.6 mm i.d., 100 µl loop | Citrate buffer (0.001 M, pH = 5.8)–methanol 98:2, 0.005 M biacetyl, 0.02 mM HTAB | Quenched biacetyl phosphorescence | UF, urine | Cisplatin | 90 ng $ml^{-1}$ (urine); 45 ng $ml^{-1}$ (plasma) |
| Elferink | 46 | — | Spherisorb ODS2, 150 × 4.6 mm i.d., 10 µl loop | 0.1 mM HTAOH, pH = 3 ($H_2SO_4$), 0.1 M $Na_2SO_4$ | UV 214 nm, ETA–AAS | UF | Cisplatin, carboplatin and reaction products with thiosulphate | — |
| Dedon | 38 | — | Econosphere C18, 250 × 4.6 mm i.d. | 0.01 M Tetrabutyl ammonium hydrogen sulphate (pH = 3.5 with phosphoric acid), 1–20% acetonitrile | UV 225 nm | Aqueous solutions | Cisplatin, iproplatin Pt complexed with amino acids, thiosulphate and diethyldithio-carbamate | — |

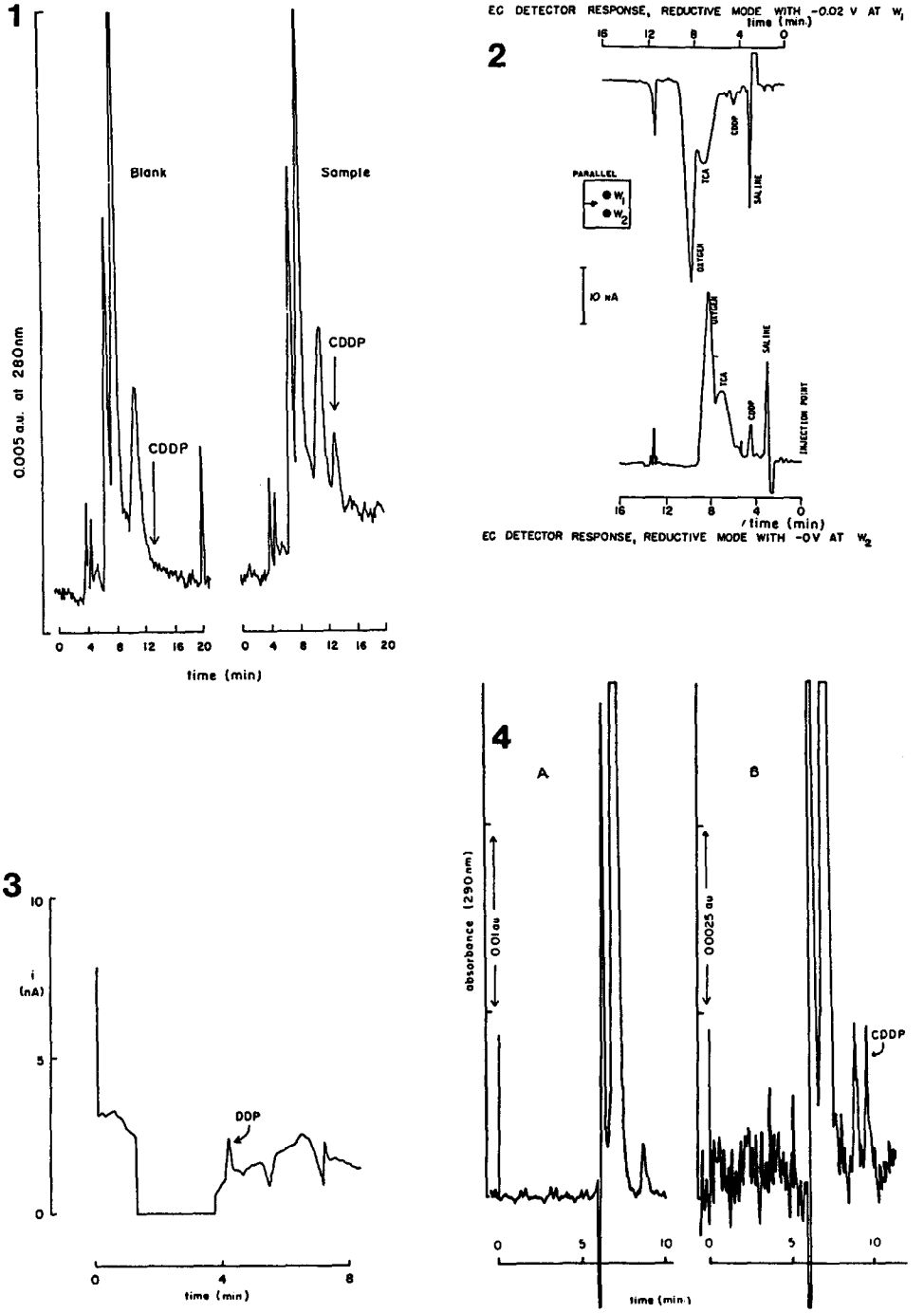



**Figure 10**
Representative chromatogram of HTAB based ion-pair systems using different detection techniques: (1) urine spiked with 100 μg $ml^{-1}$ cisplatin; UV 280 nm detection; (reproduced from ref. 66); (2) ultrafiltrate of patient plasma; dual Au/Hg electrode EC-detection; (reproduced from ref. 56, by courtesy of Marcel Dekker, Inc.); (3) patient urine; hanging mercury-drop electrode (HMDE) EC-detection; (reproduced from ref. 106); (4) plasma ultrafiltrate blank (A) and plasma ultrafiltrate containing cisplatin (B); post-column reaction detection; (reproduced from ref. 108, copyright 1984, American Chemical Society); (5) blank plasma (A) and plasma spiked with $5 \times 10^{-6}$ M cisplatin (B), after a pre-column clean-up procedure; quenched phosphorescence detection; (3 = cisplatin); (reproduced from ref. 110, copyright 1987, Pergamon Journals Ltd). (All figures reproduced by permission.)

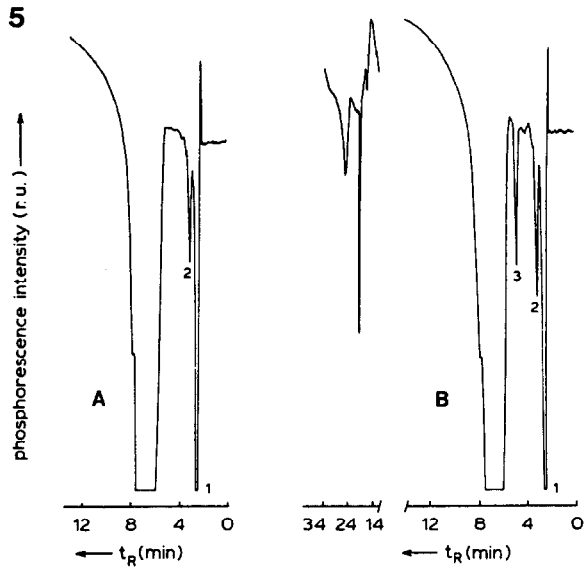



**Figure 10 (continued)**

phosphorescence detection provide acceptable detection limits for cisplatin (40–150 ng $ml^{-1}$). The quenched phosphorescence detection also provides a comparable detection limit for carboplatin (120 ng $ml^{-1}$) [109].

Systems based on HTAB and tetrabutylammonium sulphate have also been used for the speciation of reaction products of cisplatin and its analogues with different kinds of nucleophiles [38, 46].

Although the ion-pair systems mentioned thus far allow the determination of the platinum drugs in their intact form, they do not discriminate between the potentially important positively charged species. For this purpose, ion-pair systems with anionic detergents are more suitable. Ion-pair systems based on sodium dodecylsulphate (SDS) and alkylsulphonates are listed in Table 6. The presence of SDS in the mobile phase results in excessive differences in retention of the solutes of interest, i.e. primarily the platinum–methionine complexes and the hydrated derivatives. Consequently, gradient elution has to be applied to obtain convenient analysis times [36, 111]. Figure 11 shows a separation of platinum species originating from cisplatin in rat plasma *in vivo*. Several of these species were tentatively identified as the unchanged cisplatin (B), two platinum–methionine complexes (C and D) and a hydrolysis product of cisplatin (F) [111]. These results demonstrate the potential of this type of ion-pair chromatography for the separation of metabolic platinum species. However, two remarks should be made with regard to this chromatographic system. In the first place, it has been questioned whether the acetonitrile, present in the mobile phase, might have reacted with the hydrated derivatives during the analysis [65]. Secondly, gradient elution with acetonitrile–water 9:1 (v/v) severely disturbs the adsorption equilibrium of SDS on the stationary phase. This will result in long equilibration times between successive analyses.

The use of detergents with smaller chain length, such as the $C_6$–$C_8$ alkylsulphonates, offers a more attractive compromise in terms of retention and resolution [65]. Figure 12 shows a chromatogram, obtained with ICP–AES detection, for human plasma ultrafiltrate incubated with cisplatin. This approach offers unique possibilities for on-line element-specific detection. Moreover, as the ICP-detector response is nearly independent of the molecular form of the element, detection limits for other platinum compounds will be in the same range and may approach those of off-line ETA–AAS. A number of metabolites could be detected, two of which were identified on basis of co-elution as the mono and dihydrated derivatives of cisplatin. However, no metabolite species could be detected in a plasma sample of a patient receiving cisplatin in chemotherapy [65].

Parsons *et al.* [112, 113] have evaluated EC

**Table 6**
Ion-pair systems using anionic detergents for the analysis of platinum drugs

| First author | Ref. | Fig. | Column | Mobile phase | Detection | Sample | Compounds | Detection limit |
|---|---|---|---|---|---|---|---|---|
| Daley-Yates | 36,111 | 2.11 | Spherisorb ODS, 250 × 5 mm i.d., 10 µl loop (urine), 90 µl loop (UF) | Gradient from 0.005 M SDS to acetonitrile–water 9:1 | Radioactivity, ET–AAS | UF, urine | Cisplatin, biotransformation products | <200 ng $ml^{-1}$ |
| Riley | 37 | — | Hypersil ODS, 200 × 4.6 mm i.d., 20 µl loop | Phosphate buffer (0.1 M; pH = 2.1), 50 mM hexane sulphonate, 10–12% acetonitrile | UV 254 nm | Aqueous solutions | Cisplatin, Pt–methionine complexes | — |
| de Waal | 65 | 2.12 | Hypersil ODS, 150 × 4.6 mm i.d., 100 µl loop | Phosphate buffer (0.01 M; pH = 2.8), 1 mM octane sulphonate (a); phosphate buffer (0.006 M; pH = 2.8), 1 mM octane sulphonate, 5% 2-propanol (b); gradient elution | ICP–AES | UF | Cisplatin, biotransformation products | 35 ng $ml^{-1}$ |
| Parsons | 112 | — | Bondapak C18, 150 × 3.9 mm i.d., 25 µl loop | Acetate buffer (0.01 M; pH = 4.6), 5 or 10 mM heptane sulphonic acid | Amperometry: working electrode Au/Hg or HMDE, reference Ag/AgCl | UF | Cisplatin, biotransformation products | 62 ng $ml^{-1}$ (red) |

**Table 6**
Continued

| First author | Ref. | Fig. | Column | Mobile phase | Detection | Sample | Compounds | Detection limit |
|---|---|---|---|---|---|---|---|---|
| Parsons | 113 | 2.13 | Zorbax C8, Zorbax ODS, 150 × 4.6 mm i.d. (C8), 80 × 6.2 mm i.d. (C18), 25 μl loop | Acetate buffer (0.01 M; pH = 4.6), 5 mM heptane sulphonic acid (10% methanol) | Polarography: HMDE | Aqueous solutions | Cisplatin, carboplatin iproplatin, DACH–Pt complex | 62 ng $ml^{-1}$ |
| Kristjansson | 114 | — | Hypersil ODS, 80 × 4.6 mm i.d., 20 μl loop | Phosphate buffer (0.04 M; pH = 2.65), 1.5 mM hexane sulphonate; Phosphate buffer (0.04 M; pH = 2.65), 6–10 mM hexane sulphonate, 8% methanol | UV 214 nm | Aqueous solutions | Cisplatin, hydrolysis products, dimer, trimer | 1000 ng $ml^{-1}$ (oligomers) |
| Dedon | 38 | — | Econosphere C18, 250 × 4.6 mm i.d. | Phosphate buffer (0.05 M; pH = 2.5), 2.5 mM heptane sulphonate | UV 225 nm | Aqueous solutions | Cisplatin and its *trans*-isomer, carboplatin, iproplatin Pt complexed with amino acids, thiosulphate and diethyldithio-carbamate | — |

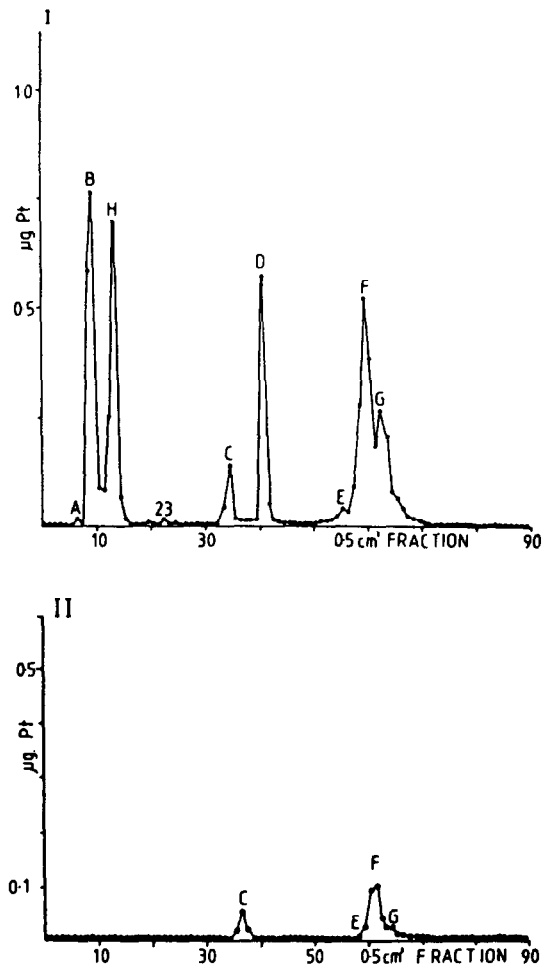



**Figure 11**
Ion-pair chromatography using SDS as the detergent of platinum species in rat plasma ultrafiltrate, obtained 2 h (I) and 3 h (II) after injection of 5 mg $kg^{-1}$ cisplatin. Ultrafiltrate samples were 20-fold concentrated by lyophilization. Vertical axis shows µg Pt per 0.5 ml fraction, as measured by ETA–AAS. (Reproduced from ref. 111 by permission, copyright 1984, Pergamon Journals Ltd.)

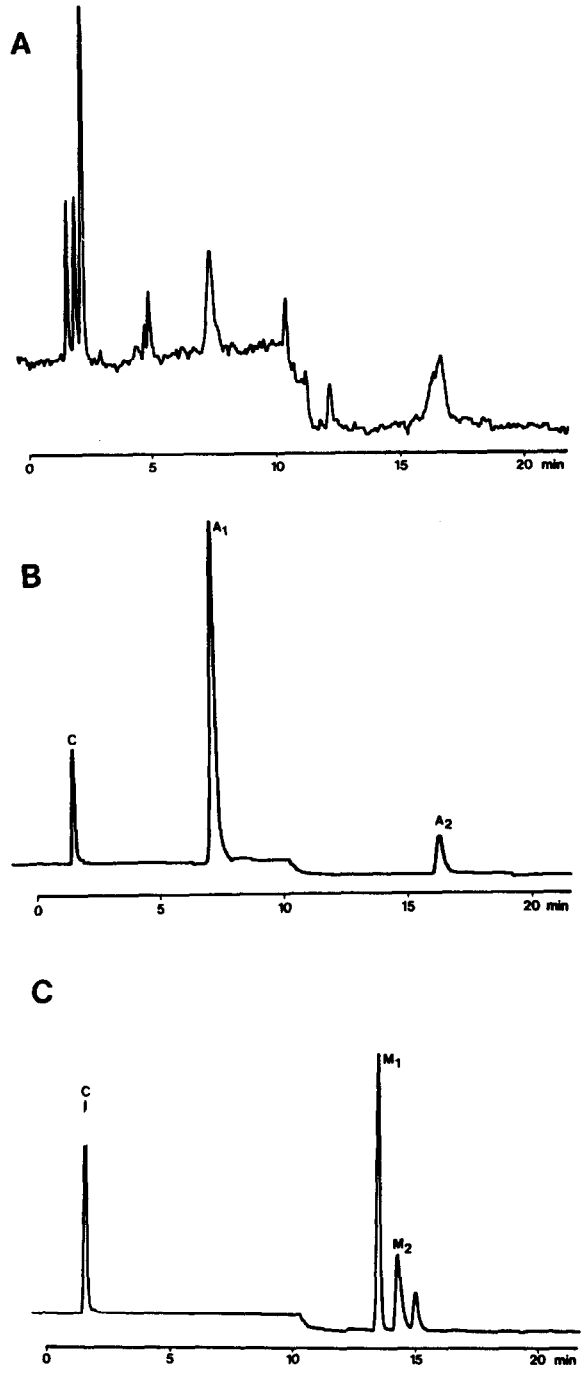



**Figure 12**
Gradient elution chromatograms of human plasma ultrafiltrate incubated with cisplatin (200 µg $ml^{-1}$) at 37°C for 24 h (A), the hydrolysis products of cisplatin (B) and methionine platinum complexes (C). Ion-pair chromatography with sodium octanesulphonate was used in combination with on-line, element specific ICP–AES detection. (Reproduced from ref. 65 by permission.)

detection for cisplatin, its analogues and several degradation products, using an ion-pair system with heptanesulphonate (Fig. 13). The monohydrated-derivative of cisplatin could be detected <1 h after incubating cisplatin in human plasma ultrafiltrate, even in the presence of 100 mM sodium chloride [113]. Alkylsulphonate-based ion-pair systems have also been reported to be suitable for the determination of the hydroxo-bridged oligomers of cisplatin [114]. However, a different mobile phase was required for each species to be determined. The dihydrated derivative and the hydroxo-bridged dimer could not be separated. This may be caused by on-column degradation of the dimer to the dihydrated

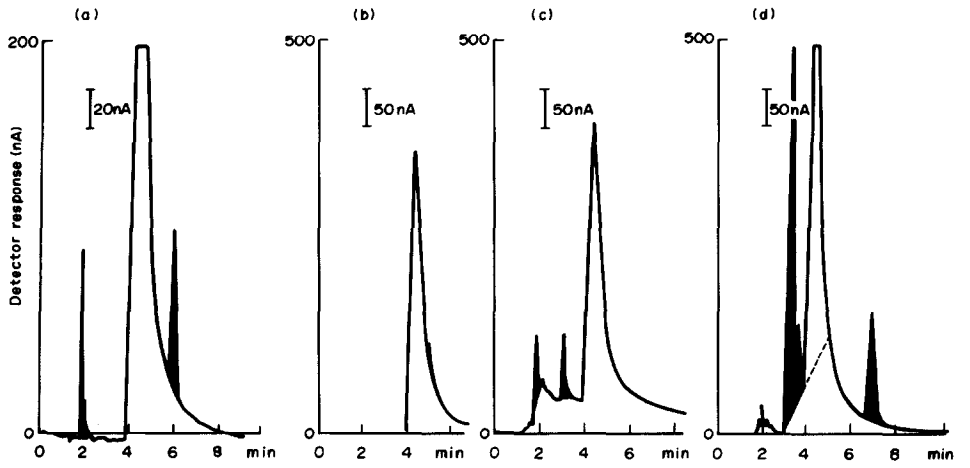



**Figure 13**
Ion-pair chromatography of platinum complexes dissolved in water and subsequently stored for 3 months at 4°C using EC detection. (a) 0.333 M cisplatin (recovery 105%), (b) 1 mM iproplatin (recovery 19%), (c) 1 mM carboplatin (recovery 110%) and (d) 1 mM of a DACH–Pt complex (recovery 92%). (Reproduced from ref. 113 by permission.)

derivative, as the dimer is unstable at pH <3.5 [30].

Ion-pair systems with alkylsulphonate detergents have also been used to monitor the reactions of cisplatin with methionine [37] and reactions of cisplatin, its *trans*-isomer, carboplatin and iproplatin with cysteine, methionine, glutathione, DDTC and STS [38].

*Normal-phase, reversed-phase and gel permeation chromatography*

Chromatographic systems for the determination of platinum drugs have also been based on stationary phases of different polarity and on gel-permeation packings (Table 7). Stationary phases ranging from highly polar (silica) to relatively non-polar (octadecyl-bound silica), have been applied primarily to the determination of drugs in their intact form. Pendyala *et al.* [117] has separated a number of metabolites of iproplatin in plasma and urine on a phenyl-type column. The chromatographic method reported by Arpalahti *et al.* [121] is the reversed-phase equivalent of the method described by Woollins *et al.* [101] for the determination of *trans*-$Pt(NH_3)_2Cl_2$ in the presence of cisplatin (see section "Ion exchange chromatography"). In general, low-wavelength UV detection is used, because of its improved sensitivity as compared to conventional UV detection for the platinum drugs at 301 nm ($\epsilon_{301}$ = 130 $M^{-1}$ $cm^{-1}$; $\epsilon_{203}$ = 5200 $M^{-1}$ $cm^{-1}$ [12]). Elferink *et al.* [118–120] have described differential pulse polarographic detection extensively.

Gel permeation chromatography has been used both for intact drug determinations [52, 122] and for speciation studies [14, 17, 123–126]. Gullo *et al.* [17] studied the protein binding of cisplatin (see also Fig. 2). The separation described by Noji *et al.* [123] enables discrimination between cisplatin and its hydrated products. However, the chromatogram was complicated and the retention times were more than 1 h. Mason *et al.* [126] obtained several platinum-containing fractions from urine and kidney cytosol, viz. two high molecular weight protein bound fractions ($M_W$ > 250,000 and $M_W$ = 20,000, respectively), a heterogeneous low molecular weight fraction, and a low molecular weight fraction ($M_W$ < 1500). It was suggested that the heterogeneous metallothionein-like fraction may play an important rôle in the accumulation and retention of platinum in the kidney [125]. In addition, urine and kidney cytosol have been shown to contain low molecular weight compounds ($M_W$ < 1500) with similar chromatographic properties [126]. Repta and Long [14] have obtained more information on low molecular weight metabolites in plasma ultrafiltrate incubated *in vitro* and in a urine sample of a patient receiving cisplatin (Fig. 14). In plasma ultrafiltrate *in vitro* a number of biotransformation products were found; in human urine, *in vivo*, one major metabolite with an estimated molecular weight of 440 was found [14]. However, the extensive biotransformation reported in this study has been questioned by Riley *et al.* [103] and Safirstein *et*

**Table 7**
Normal-phase, reversed-phase and gel permeation systems for the analysis of platinum drugs

| First author | Ref. | Fig. | Column | Mobile phase | Detection | Sample | Compounds | Detection limit |
|---|---|---|---|---|---|---|---|---|
| Van der Vijgh | 115 | — | Porasil silica, 300 × 3.9 mm i.d., 10 μl loop | Acetonitrile–water 9:1 | UV 214 nm | UF, urine | Ethylenediammine–Pt(II) malonate | 350 ng $ml^{-1}$ |
| Gaver | 116 | — | Lichrosorb diol, 250 × 4.1 mm i.d. | Phosphoric acid (0.015%)–acetonitrile 8:92 (UF) or 11:89 (urine) | UV 229 nm | UF, urine | Carboplatin | 1000 ng $ml^{-1}$ (UF), 5000 ng $ml^{-1}$ (urine) |
| Pendyala | 116 | — | Bondapak phenyl | Methanol–water 1:9 or gradient | UV 214 nm, ETA–AAS | UF, urine | Iproplatin, biotransformation products | 500 ng $ml^{-1}$ (iproplatin) |
| Cheung | 52 | — | Lichrosorb amino, 250 × 4.6 mm i.d., 10 μl loop | Acetonitrile–water 9:1 (cisplatin) or 85:15 (iproplatin) | UV 210 nm | Aqueous solutions | Cisplatin, iproplatin | — |
| Cheung | 52 | — | Spheri-5 RP8, 100 × 4.6 mm i.d., 10 μl loop | Water | UV 210 nm | Aqueous solutions | Carboplatin | — |
| Elferink | 118 | — | Spherisorb ODS2, 110 × 4.6 mm i.d. or 40 × 4.6 mm i.d. 20 μl loop | Sodium sulphate solution (0.05 M; pH = 3 with sulphuric acid), 10–30% methanol | Differential pulse amperometry HMDE | UF, urine | Aqua[1,1-bis(amino methyl)cyclo-hexane] sulphato Pt(II) and derived species | 20 ng $ml^{-1}$ |
| Elferink | 119 | — | Spherisorb ODS2, 150 × 4.5 mm i.d., 50 μl loop | 0.05 M sodium perchlorate in water | Differential pulse polarography; DME | UF, urine | Carboplatin | 20 ng $ml^{-1}$ (water), 40 ng $ml^{-1}$ (UF), 400 ng $ml^{-1}$ (urine) |

**Table 7**
Continued

| First author | Ref. | Fig. | Column | Mobile phase | Detection | Sample | Compounds | Detection limit |
|---|---|---|---|---|---|---|---|---|
| Arpalahti | 121 | — | Technopak C18, 300 × 3.9 mm i.d. | Ammonium acetate (0.1 M) in water–methanol 95:5 | UV 260 nm | Aqueous solutions | Cisplatin and its *trans*-isomer after treatment with thiourea | 0.5% *trans* in *cis* |
| Kizu | 122 | — | Hitachi gel no. 3013-N, 150 × 4.6 mm i.d., 100 μl loop | Methanol–water 15:85, 10 mM sodium chloride | UV 210 nm | UF, urine | Cisplatin | 150 ng $ml^{-1}$ |
| Cheung | 52 | — | Hamilton PRP-1, 250 × 4.1 mm i.d., 10 μl loop | Acetonitrile–water 1:9 | UV 220 nm | Aqueous solutions | DACH–Pt complex | — |
| Noji | 123 | — | Toyo Soda G1000PW, 600 × 7.5 mm i.d. | Sodium sulphate (0.1 M) | UV | Aqueous solutions | Cisplatin and its *trans*-isomer, DACH–Pt complexes, hydrolysis products | — |
| Gullo | 17 | 2.2 | Sephadex G-200 | Tris–HCl (0.1 M; pH = 8), 1 M sodium chloride | UV 285 nm, ETA–AAS | Serum | Cisplatin, protein-bound Pt | — |
| Mason | 124–126 | — | Sephadex G-15, G-50, DE-52 cellulose, CM sepharose, sephacryl S-200 | Tris–HCl (0.01 M; pH = 7.4) (cytosol); Tris–HCl (0.1 M; pH = 5.6) (urine) | ETA–AAS | Urine, cytosol, serum | Cisplatin, biotransformation products | — |
| Repta | 14 | 2.14 | Biogel P2, 1100 × 13 mm i.d., loop <400 μl | Water | ETA–AAS | UF, urine | Cisplatin, biotransformation products | — |

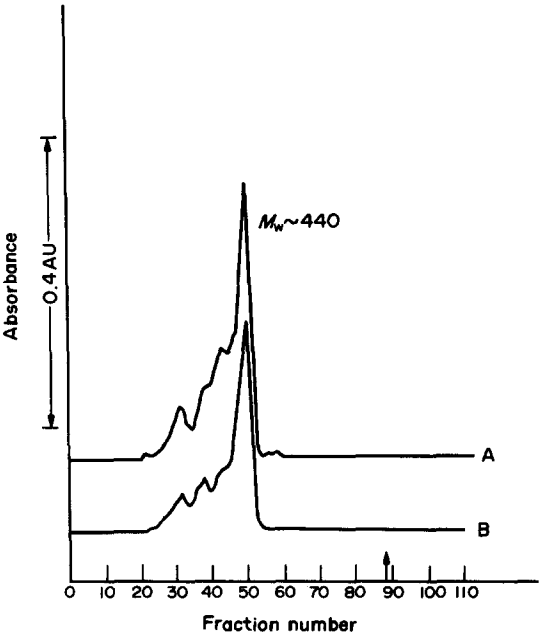



**Figure 14**
Gel permeation chromatograms of platinum containing species in ultrafiltrate of plasma obtained from a patient 45 min (curve A) and 2 h (curve B) after dosing with 50 mg $m^{-2}$ cisplatin. The curves are offset for purposes of clarity; the arrow on the abscissa represents the location where cisplatin would be eluted if present. (Reproduced from ref. 14 by permission.)

*al.* [100], who found that cisplatin is excreted predominantly unchanged in human and rat urine.

*Miscellaneous techniques*

Thin-layer chromatography has been used for the analysis of platinum compounds in urine and for aqueous cisplatin–amino acid mixtures [42, 71]. High-performance thin-layer chromatography (HPTLC) on Silicagel 60 plates has been reported by De Spiegeleer *et al.* [127]. According to that technique, cisplatin is separated from its *trans*-isomer and its hydrolysis products. The plates are sprayed with *p*-nitrosodimethylaniline in diethylether and, after drying, scanned at 515 nm. Preliminary experiments indicated that this technique could also be applicable to the determination of cisplatin in urine.

An approach related to ion-exchange chromatography was applied by LeRoy *et al.* [128]. Platinum species in human urine were fractionated by successive elution from a cation-exchange resin. A water-elutable fraction and a HCl-elutable fraction were collected. The authors found that after cisplatin infusion, the proportion of the HCl-elutable

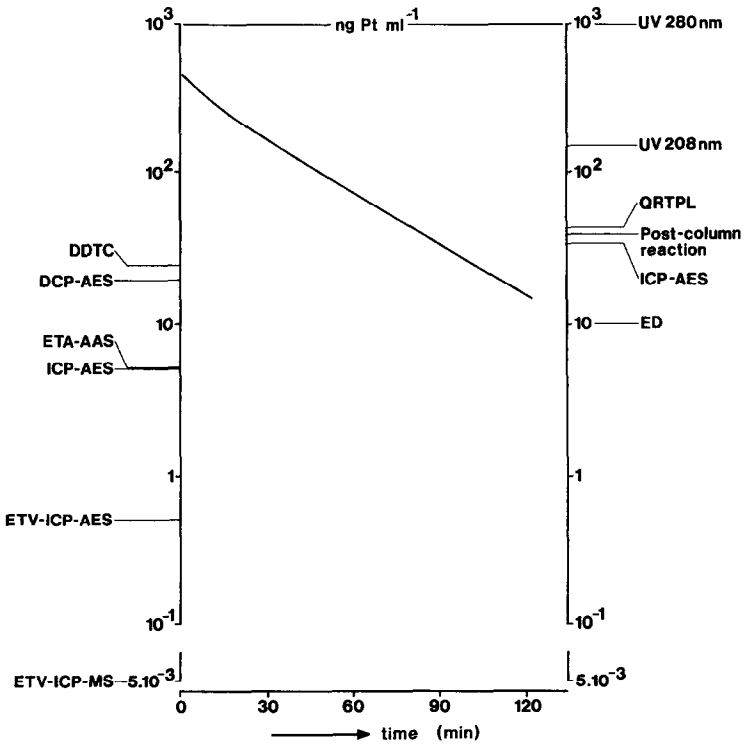



**Figure 15**
Comparison of detection limits for the determination of total platinum (left side) and for the determination of cisplatin (right side) in relation to a typical plasma level decline of ultrafilterable platinum species after 6-h infusion of 100 mg $m^{-2}$ cisplatin (adopted from Fig. 6). DDTC = derivatization with diethyldithiocarbamate; QRTPL = quenched room temperature phosphorescence in liquids; ED = electrochemical detection.

fraction increased at the expense of the water elutable fraction. However, Riley *et al.* [103] and Safirstein *et al.* [100] have questioned these findings.

## Discussion

For a correct interpretation of analytical data, it is of paramount importance that the obtained results, as well as the experimental conditions, are well documented. It is essential to distinguish between the various forms in which platinum may be present in the sample, e.g. as protein-bound platinum, free ("ultrafilterable") platinum, intact drug or biotransformation products. In particular, in platinum speciation studies, special attention should be given to the preservation of the chemical identity of the species originally present in the sample.

Although there are indications for the *in vivo* transformation of cisplatin, neither the absence nor the existence of biotransformation products in plasma or urine of patients treated with cisplatin has been proved unequivocally. This is primarily because of the lack of analytical techniques capable of determining these species at sufficiently low concentration levels (Fig. 15). Therefore, parallel with the developments in the determination of total platinum, detection capabilities should be increased at least an order of magnitude, either by the development of new detection techniques or by the application of sample enrichment strategies. The combination HPLC–ICP–MS is especially worth investigation.

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