Review

Analytical methodologies for the determination of cisplatin

M. Espinosa Bosch [a], A.J. Ruiz Sánchez [b],
F. Sánchez Rojas [c,*], C. Bosch Ojeda [c]

[a] *Department of Pharmacy, General Hospital, University Hospital "Virgen del Roc´ıo", Manuel Siurot s/n, 41013 Sevilla, Spain*
[b] *Department of Organic Chemistry, Faculty of Sciences, University of Ma´laga, Campus Teatinos s/n, 29071 Ma´laga, Spain*
[c] *Department of Analytical Chemistry, Faculty of Sciences, University of Ma´laga, Campus Teatinos s/n, 29071 Ma´laga, Spain*

Abstract

cis-Diamminedichloroplatinum(II) (cisplatin) is a important chemotherapeutic drug for cancer treatment since 1978. Unfortunately, because of the severe side-effects like nephrotoxicity, ototoxicity, etc., they are administered in small doses at low concentration establishing the maximum limit dosage to 100 mg/m^2 (21 days).

A variety of analytical methods have been proposed for the determination of cisplatin in biological fluids and tissues that permit the accurate determination at or below the part per billion level in the native sample. The purpose of the current review is to provide a systematic survey of the latest analytical techniques for the determination of cisplatin in biological samples.

Keywords: Cisplatin; Pharmaceutical analysis; Review; Analytical methodologies

Contents

1. Introduction

Cisplatin is for a long time in clinical use as efficient anti- tumour drug [1]. Cisplatin is one of the complexes responsible for the cell division inhibition phenomenon [2]. The success in cisplatin-based chemotherapy, however, strongly depends on how careful the drug's dosages are monitored in order to reduce severe side-effects which include nausea, vomiting, kidney dam- age and deafness and overcome cellular resistance. Despite causing severe side-effects, it is the preferred treatment for a variety of solid tumours, such as testicular and ovarian cancers, and is also used for treating bladder, cervical, head and neck, esophagean, and small cell lung cancer. However, the modes of action and toxicity of cisplatin are not well understood. The damage produced in the affected organs is probably due to the association of Pt or the parent drug metabolites to important pro- teins of the impacted organ. For this reason, the determination of cisplatin in biological fluids and tissues presents a particularly interesting challenge to the analytical chemist. Also, cisplatin is highly reactive in the body, and its biodegradation products may have activity and toxicity behaviour different from that of the parent drug. It has been suggested that cisplatin and its hydrated species may be responsible for both its anti-tumour and toxic effects [3–5].

In order to evaluate the drug in clinical situations and to optimize therapeutic regimens, analytical methods capable of separating the drug and its individual biotransformation prod- ucts and detecting these species at therapeutically relevant levels are required.

In this way, Riley [6] reviewed current methodologies until 1988 emphasizing their advantages and disadvantages. In the paper, these methods are divided into non-selective methods which detect only the platinum metal and selective meth- ods which are capable of detecting the intact compound. This author concludes that the selective methods generally use a fractionation step using high-performance liquid chro- matography (HPLC) followed by either on-line or off-line detection. Off-line detection by flameless atomic absorp- tion spectrometry (FAAS) requires the collection of fractions from the HPLC column and is somewhat tedious. On the other hand, sample preparation is minimal and biological flu- ids may be injected directly onto the column. Up to this moment, the most sensitive HPLC methods for the deter- mination of cisplatin and its analogues are using on-line electrochemical detection or post-column derivatization with bisulphite.

The aim of this paper is to review the more important analyt- ical procedures developed in the last years for the determination of cisplatin, the best studied inorganic anti-tumour drug by using selective methods.

2. Spectroscopic methods

2.1. UV–vis spectrophotometry

Cisplatin has a low molar absorptivity in the UV region and has no fluorescence. Therefore, a selective derivatizing reaction

is required for the detection of the drug in biological samples if the optical detection is sought.

A method for cisplatin determination in urine using derivative spectrophotometry has been developed and it has some disadvantages as the derivatizing reaction is completed in 24 h and the reagent cannot be found in the market [7].

Anilanmert et al. [8] present a method based on the mea- surement of the absorbance of the reaction product of the drug and *o*-phenylenediamine. The product was obtained at pH 6.2, in 30 min at 90 °C, giving a maximum absorbance at 705 nm, which is far beyond the wavelengths of the absorption of cisplatin, *o*-phenylenediamine, and the biomolecules in urine. The detection limit of cisplatin in spiked urine samples was 8.40 fl,g mL^{-1}.

2.2. Phosphorescence

Determination of cisplatin in urine and plasma in the range 5×10^{-7} to 5×10^{-5} M by quenched phosphorescence detec- tion was reported by Bauman et al. [9].

2.3. Atomic absorption spectrometry (AAS)

Wang and Shi established flameless AAS for determining the drug content and embedding rate of cisplatin chitosan microspheres. Microsphere sample was digested with 1:5 nitric acid. The standard curve of platinum was linear in the range from 0.10 to 0.60 fl,g mL^{-1} and the detection limit was 0.30 fl,g mL^{-1} [10].

A sensitive, accurate and precise method was developed by Verschraagen et al. [11] to simultaneously determine cis- platin and monohydrated cisplatin in plasma. The compounds were separated by high-performance liquid chromatography and quantified by off-line furnace atomic AAS. The linear ranges for cisplatin and monohydrated cisplatin in deproteinized plasma were 60–600 and 87.5–700 nM, respectively. The lower limits of quantification of cisplatin and monohydrated cisplatin in depro- teinized plasma were 60 and 87.5 nM, respectively. The utility of the assay is shown by the analysis of plasma samples from a patient who received 75 mg m^{-2} cisplatin as a 1-h intravenous infusion.

Bu et al. developed an assay for the determination of cis- platin in serum and tissue of cancer patients by AAS with coated graphite tube [12]. The tungsten-coated graphite tube could eliminate sample matrix effect and was sensitive and accurate.

SPI-77 is a relatively new dosage form of cisplatin encap- sulated in long-circulating Stealth® liposomes. This liposomal formulation of cisplatin, SPI-77, was developed in order to reduce systemic toxicity and to deliver cisplatin to tumours with improved specificity. Since it is yet unknown which compo- nents of cisplatin can be detected in blood after administration of SPI-77. Meerum Terwogt et al. [13] performed a concise in vitro experiment to investigate the distribution of liposomal encapsulated cisplatin in blood. The total platinum concentration (i.e. liposomal encapsulated platinum, protein-bound platinum released from the liposomes and free platinum) was measured in plasma after chemical destruction of the liposomes by addi- tion of the detergent Triton X-100 to the plasma sample. The

fraction of cisplatin released from the liposomal carrier and unbound to plasma proteins (free platinum) was measured in plasma ultrafiltrate by GF-AAS. The usefulness of the method was demonstrated in a phase I clinical and pharmacokinetic study. In addition, in vitro experiments were carried out to deter- mine the distribution of SPI-77 in blood. The results indicated that platinum from SPI-77 mainly concentrates in plasma and that binding to and/or endocytosis in red blood cells is negligi- ble.

In other way, Raghavan and Mulligan described atomic absorption method for the quantitation of cisplatin [14]. Cis- platin was reacted with diethyldithiocarbamic acid (DDTC), sodium salt, to yield a platinum–DDTC (Pt–DDTC) complex. The Pt–DDTC chelate was extracted into methylene chloride, the extract was mixed with acetonitrile, and the platinum content was then determined using a Zeeman atomic absorption (AA) spectrophotometer. The extraction conditions and AA experi- mental conditions were set up such that the detection level could
be extended to 0.5 ng mL^{-1}.

3. Electroanalytical methods

Initially, these methods had the disadvantages of accumula- tion of the drug on the electrode surface and matrix interferences, and also showed low recovery [15].

Petrlova et al. [16] investigate if a metallothionein (MT) modified hanging mercury drop electrode can be applied as a cisplatin electrochemical biosensor. The modification of the mercury electrode surface by MT and the determination of cis- platin were performed by adsorptive transfer stripping technique and differential pulse voltammetry. The detection limit (3 S/N) of cisplatin calculated from the decrease of CdT peak was about
2.5 pmol in 5 fl,l (0.5 fl,M) at the interaction time of 400 s. More- over, the authors tested the influence of human blood serum as a complex biological matrix on the way of determination of cisplatin.

4. High-performance liquid chromatography methods

El-Khateeb et al. [17] investigated the optimal conditions for HPLC separation of cisplatin and its two hydrolysis prod- ucts. With a C_{18} stationary phase at 37 °C, required an aqueous mobile phase with 3% (v/v) methanol, 0.05 mM sodium dodecyl sulphate, and pH 2.5 (adjusted with triflic acid). This procedure
was then used to measure levels of these compounds in ultrafil- tered serum after incubation for various times with cisplatin at 37 °C.

4.1. UV–vis detection

In the first years, two direct HPLC methods were reported. One necessitates a pre-treatment procedure and low tempera- tures; its detection wavelength of 210 nm is in the range of the components of biological fluids [18]. The other needs an automated column switching technique [19].

Farrish et al. described a cisplatin LC post-column derivatiza- tion assay in plasma [20]. Cisplatin plasma samples were treated

with acetonitrile and a citrate buffer solution to enhance cisplatin stability. Processed samples were analysed on a chemically generated anion exchange column using a customized post-column derivatization platform and refrigerated autosampler; detection at 290 nm and retention time 9 min. Assay was linear in the range 0.06–30.0 fl,g mL^{-1}.

The derivatization of cisplatin and its monoaqua form with diethyldithiocarbamate (DDTC) has been optimized and used in a post-column system to obtain a selective and sensitive method for the determination of the compounds [21]. Cisplatin was isolated on a strong anion-exchange column, while a strong cation-exchange column was used for the monohydrated com- plex. The reaction was quantitative using a packed-bed reactor with a surrounding temperature of 115 °C and a mobile phase consisting of 0.125 M succinic acid–sodium hydroxide buffer pH 5.2 and methanol (2:3, v/v). The resulting complex was mon- itored at 344 nm. This procedure was applied to the study of the pharmacokinetics of cisplatin in humans [22].

Because some Pt-based anti-cancer drugs are not easily detected spectrophotometrically, column derivatization meth- ods have been employed [20,22] to increase the sensitivity of the spectrophotometric detectors. Post-column derivatization of platinum compounds with sodium bisulphite [23] has been the most frequently utilized technique. The pharmacokinetic– toxicodynamic analysis of cisplatin and cisplatin-induced nephrotoxicity was conducted by determining the compounds in rat plasma, urine and kidney using a post-column HPLC method [24,25].

In a pharmacokinetic study, the concentration of free cis- platin was followed in plasma, scala tympani perilymph and cerebrospinal fluid (CSF) after an intravenous injection (12.5 mg kg^{-1}) in guinea pigs [26]. LC with post-column deriva- tization was used for quantitative determination of the drug. The distribution of cisplatin to CSF was fast; at 10 min after drug administration the concentration was 7 fl,g mL^{-1} and the CSF:plasma ratio was 0.37.

A quantitative analytical method for measuring unchanged cisplatin and high- and low-molecular-mass metabolites (fixed and mobile metabolites) in rat kidney and liver was devel- oped by Hanada et al. [27]. Unchanged cisplatin, separated from fixed and mobile metabolites in tissue homogenates by consecutive procedures of fractionation and ultrafiltration, was determined by HPLC with post-column derivatization. The detection limit for unchanged cisplatin in the cytosolic ultra- filtrate was 20 ng mL^{-1}(detection limit of 65 ng g^{-1} of Pt in kidney and liver).

Kizu et al. [28] proposed a post-column derivatization HPLC analysis of anti-tumour divalent (including cisplatin) and quadri- valent platinum complexes. It is based on the derivatization of platinum complexes by reaction with sodium bisulfite to corresponding products which has enhanced absorptivity at 280–300 nm. Detection limit at 290 nm was 20 nM for cisplatin. The method is applied in plasma and urine.

Many indirect HPLC methods in which Na-diethyl- dithiocarbamate [29] and sodium bisulphite/potassium dichro- mate [23] were used for derivatization were widely applied. These methods are quite expensive and the selectivity of both

indirect methods is not clear. Additionally, the maximum absorption wavelengths of reaction products are around the absorbance's of some macromolecules in biological fluids.

Hasson and Warshawsky [30] proposed a method based on the relative ease of formation of the complex between cisplatin and *o*-phenylenediamine and its fast elution form the fl,-Bondapak reversed-phase C_{18} column and detection at 703 nm.

Although a highly selective HPLC method was reported for determining the various species of platinum (including cisplatin and two toxic impurities) using 4-methyl-2-thiouracil in one run [31]. Separation is performed on a fl,-Bondapak C_{18} column with isocratic elution and detection at 315 nm also very close to the maximum absorption wavelength of some components in biological fluids.

Augey et al. [32] described a method for the quantitative anal- ysis of cisplatin in ultrafiltrate plasma in the presence of nickel chloride as internal standard. Cisplatin and the internal standard were chelated by exchange with diethyldithiocarbamate. After derivatization, the mixture was directly injected into the column. Chromatography was performed on an Ultrasphere column and detection at 260 nm. Limit of quantification was 0.03 fl,g mL^{-1} using only 0.5 mL of ultrafiltrate.

Khuhawar et al. developed a method for the determination of cisplatin based on pre-column derivatization of platinum(II) with bis(salicylaldehyde)tetramethylethylenediimine, extrac- tion in chloroform and elution from a 3 fl,m Hypersil ODS column with methanol–acetonitrile–water as mobile phase and detection at 254 nm [33]. The method was applied for the deter- mination of cisplatin as Pt(II) in a pharmaceutical preparation and in blood samples of cancer patients after infusion of cis- platin.

Khuhawar and Arain proposed spectrophotometric and HPLC methods for the determination of cisplatin and car- boplatin based on the pre-column derivatization of Pt(II) with 2-acetylpyridine-4-phenyl-3-thiosemicarbazone [34]. The complex was extracted in chloroform and detection at 380 nm. The complex eluted from a Phenomenex C_{18} column with methanol:water:acetonitrile:tetrabutyl ammonium bromide (1 mM) (44:30:25:1) with a flow rate of 1 mL min^{-1} and detec- tion at 260 nm (detection limit 10 ng mL^{-1} Pt). The method is applied to determination of cisplatin as Pt(II) in phar- maceutical preparation, serum and urine samples of cancer patients.

Lanjwani et al. [35] developed a procedure based on the pre-column derivation of Pt(II) with *N*,*N*t-bis(salicylidene)-1,2-propanediamine. The neutral platinum complex was extracted, concentrated in an organic solvent and then injected (5 fl,L) on a reverse-phase HPLC column, Varian Micro-Pak SP C_{18}.
The complex was eluted isocratically using a ternary mix- ture of methanol:acetonitrile:water (40:30:30) at a flow rate of 1.0 mL min^{-1} and detection at 254 nm (detection limit 4.0 ng per injection).

Cisplatin, transplatin, carboplatin and related neutral, anionic or cationic species of Pt(II) were separated with gradient elu- tion on a reversed-phase column dynamically modified with a mixture of cationic and amphiphilic modifiers [36,37].

A method was proposed by Lopez-Flores et al. [38] for deter- mination in plasma, cancer cell and tumour samples. Cisplatin separation was carried out on a reverse-phase column using methanol–acetonitrile–water as mobile phase, flow rate constant at 1.6 mL min^{-1} and analysis was performed at 23 °C and detection at 254 nm. The method was linear in the range of 0–2–10 ng L^{-1}.

A trace analytical procedure for the cytostatic drugs carmustine, chlorambucil, cisplatin, cyclophosphamide, cytarabine, etoposide, 5-fluorouracil, melphalan, methotrexate and vinblas- tine was developed by Kiffmeyer et al. [39] in order to evaluate the environmental hazards of these drugs in clinical waste water and sewage treatment plants. The analysis was performed using solid phase extraction with subsequent HPLC separation and quantitative determination by gradient elution techniques with DAD and fluorescence detection. Detection limits after the clean-up and enrichment procedure vary from 0.002 to 0.2 mg L^{-1}.

In other aspect, a method is described for the determination of residual levels of cisplatin from extracts of surfaces with very low surface area; from extracts of surfaces of coupons made of Teflon®, stainless steel, and glass; and in aqueous solution collected after rinsing equipment and parts [40]. Initially, the method was developed to determine cisplatin at concentrations ranging from 20 to 200 ng mL^{-1} by direct injection. Retain- ing the same method conditions, the scope of the method was expanded by the addition of a sample preconcentration step, allowing analyses at levels ranging from 0.5 to 20 ng mL^{-1}. Preconcentration is necessary for the determination of cisplatin in rinse waters at a quantifiable concentration of about 2 ppb. Under these conditions, the detection limit is about 0.2–0.3 ng mL^{-1}.

Residual cisplatin on different types of surfaces, including surfaces with very low surface area, can be determined by swabbing each test surface with a derivatizing solution. The cisplatin recovered in the swabbing solution can be analysed by HPLC using direct injection or preconcentration, depending on the expected level of cisplatin in the sample. Initial methods were developed to quantitate at a cisplatin concentration of about 100 ppb or higher in solution extracted from surfaces. How- ever, when surface areas are limited because of the size of the parts, solution concentration becomes very low as a result of the minimum volume required for extraction. To support the application of swabbing techniques to surface analysis, stain- less steel, Teflon, and glass surfaces were spiked with cisplatin at 2.5–20 ng cm^{-2}. Diethyldithiocarbamate (DDTC) was used as a derivatizing agent to increase sensitivity to UV absorption at 340 nm. DDTC also has application as a cleaning agent for cis- platin (e.g., for production equipment cleaning, spill clean-up). Destruction of cisplatin can be effected by the reaction of cis- platin with this cleaning agent. Derivatization of cisplatin will convert active cisplatin to platinum–DDTC on surfaces or in solution. Final cleaning can be accomplished using a water- for-injection rinse. After such a cleaning process, the rinse water, when collected and analysed, showed levels of free cis- platin less than the detection concentration of 0.2 ppb and a total platinum concentration less than 10 ppb as Pt–DDTC com- plex.

4.2. Electrochemical detection

A method has been developed consisting of solvent- generated anion exchange chromatography and on-line reductive electrochemical detection [41]. A hexadecyl- trimethylammonium-loaded reversed-phase HPLC column with a 5 mM citrate-buffered eluent (pH 6.5) is used. Exploiting adsorption of cisplatin at a dropping mercury electrode and NH_4^+-enhanced catalytic proton reduction, a detection limit of
10^{-8} M has been achieved. The method is applied to the quan- tification of cisplatin in plasma ultrafiltrate and urine.

4.3. Inductively coupled plasma atomic emission spectrometric (ICP–AES) detection

The applicability of reverse-phase ion-pair chromatography with on-line ICP–AES detection was investigated by Waal et al. for the analysis of cisplatin, its hydrolysis products and two methionine–platinum complexes in aqueous solutions [42]. The detector response for cisplatin was linear over three orders of magnitude. The detection limit was 3.5 ng, corresponding to a concentration detection limit of 35 ng mL^{-1}. The ion-pair sys- tem developed was applied to the analysis of biotransformation products originating from cisplatin in human and rat plasma in vitro and in vivo.

4.4. Inductively coupled plasma–mass spectrometry (ICP–MS)

Cairns et al. [43] reports the use of ICP–MS as a sensitive yet versatile detection system for HPLC to allow unequivocal identification and quantification of the platinum complexes including cisplatin. The same research group use HPLC–ICP–MS inter- face employing desolvation for speciation studies of platinum in anti-tumour drugs [44]. The eluent from the HPLC is nebulised into a heated cyclone spray-chamber and the solvent removed
using a Nafion membrane drier, held at 75 °C, and a cryogenic condenser. The condenser consists of 6 Peltier heat pumps connected to liquid cooled aluminium blocks. At a nebuliser gas flow rate of 0.6 L min^{-1}, the membrane drier removes 58% of the vapour and the Peltier condenser 75% of the remaining vapour,
i.e. a total desolvation efficiency of 89%. This enables the use of HPLC solvents which otherwise would destabilise the ICP, e.g. 100% acetonitrile or methanol, and permits the use of solvent gradients with minimal baseline drift. The limit of detection for platinum species has been 0.6 ng L^{-1}.

Falter and Wilken developed a procedure for the determination of cisplatin and carboplatin [45]. The compounds were separated by a HPLC column. Different reversed-phase C_{18} techniques were tested to obtain the best separation and detection results of the two platinum compounds. The solvent-generated anion exchanger method was selected as the method with the best handling and sensitivity. This technique allows a good separa- tion of the two compounds within 3 min. The eluent was on-line nebulised into the nebuliser gas flow using ultrasonic nebuli- sation. The resulting aerosol was driven by the nebuliser gas stream through a 30 cm heating path, a 60 cm cooling path for

desolvatisation and into the plasma of the ICP–MS. The detec- tion was carried out on platinum mass ^{195}Pt. The detection limit for cisplatin is 80 pg.

Hann et al. presented a method for analysis of cisplatin, monoaquacisplatin, diaquacisplatin, carboplatin and oxaliplatin in biological and environmental samples [46]. Chromato- graphic separation was achieved on pentafluorophenylpropyl-functionalized silica gel. Detection limit for cisplatin is 0.09 ng mL^{-1}, calculated at *m*/*z* 194, using aqueous stan- dard solutions (3 fl,L injection volume). Cisplatin, mono- and diaquacisplatin were measured in aquatic samples and in diluted urine of a cancer patient by HPLC–ICP–MS by the same research group [47], using 0.5 mM NaOH as eluent and a flow rate of 0.3 mL min^{-1}. Limits of detection of 0.74, 0.69 and 0.65 fl,g L^{-1} were calculated for cisplatin, monoaqua- and diaquacisplatin, respectively.

Bell et al. described a specific assay for cisplatin in human plasma ultrafiltrate and cell culture medium ultrafiltrate [48]. Separation of cisplatin (6 min) and monohydrated cisplatin (12 min) was achieved using a fl,-Bondapak C_{18} column and a mobile phase (0.075 mM sodium dodecyl sulphate and 3% methanol, adjusted to pH 2.5 with triflic acid) pumped at a flow rate of 0.5 mL min^{-1}. The analytes were detected with little background interference by ICP–MS monitoring of platinum masses (*m*/*z* 194/195). Calibration curves were linear over three orders of magnitude (0.05–8 fl,M) and the limit of quantitation was 0.1 fl,M.

Esteban-Ferna´ndez presented a work that is focused on the development of an analytical methodology for the separation and characterization of the different Pt-biomolecules present in kidney and inner ear cells cytosol from rats, which were previously treated with monodoses of, either cisplatin, carbo- platin or oxaliplatin equivalent to that used for human disease treatments [49]. Speciation studies in the samples from kidney and inner ear were performed coupling two-dimensional liquid chromatography (2D-LC) to ICP–MS. Size exclusion (SEC) and anion exchange fast protein liquid chromatography (FPLC) was employed for 2D orthogonal separation. After these separations, free drug peaks were not observed in any of the samples.

The binding of Pt to biomolecules was demonstrated by SEC and, independently of the drug used, Pt eluted as two main bands with molecular weights of 12 kDa and 25–65 kDa for inner ear samples, and as two different bands with 20 kDa and 50–60 kDa in the samples from kidney. However, the relative band intensity presented important differences for the three drugs. Using the same chromatographic conditions, it was shown that a metal- lothionein (MT) standard eluted in the same position as some of the cytosolic Pt-biomolecules.

High Pt-containing fractions eluting from the SEC column were analysed by anion exchange FPLC after a preconcentra- tion step. Among the different preconcentration methods tested, sample focusing on the head of the FPLC column shows main advantages. In this way, the separation by 2D chromatography of the high molecular Pt-species has been considerably improved. In other way, the efforts to speciate the transformation prod- ucts of platinum-containing drugs, including cisplatin, were fueled by interest in the action modes of these drugs at the molec-

ular level. Kinetic studies of the interaction of L-methionine [50], histidine containing peptides [51], DNA [52,53], and other hybrid molecules [54], with cisplatin and its relative plat- inum anti-tumour complexes in aqueous or saline solution have been investigated by HPLC–ICP–MS. An ion spray HPLC–MS interface achieved a detection limit of 2 pmol for cisplatin and DNA complexes [55] and a sensitive method (detection limit of 1 ng mL^{-1} cisplatin) combining ion pairing HPLC with ICP–MS was described for measuring cisplatin and some possible metabolites [56]. The separation conditions for cis- platin hydrolysis products and the reaction products of cisplatin with methionine, cysteine, and glutathione were investigated with sodium dodecylsulfate or heptanesulfonate as ion-pairing agents.

4.5. Electrospray ionization mass spectrometry (ESI-MS)

Mass spectrometry has great potential to provide rich struc- tural information on compounds. Because cisplatin has low solubility and is non-volatile, it is very difficult to determine platinum(II)-containing organometallic compounds. With the advent of ESI-MS, its role in the analysis of biomolecules and non-volatile compounds has increased, especially when advan- tage is taken of coupling with HPLC. In this sense, Cui and Mester examine the characteristics of cisplatin and its hydrated forms by ESI-MS–HPLC [57]. Three hydrolysis products were identified following incubation of cisplatin in aqueous solution. The technique was employed to investigate the time- and pH- dependent hydrolysis. It has been shown that the combination of full-scan, Zoomscan and tandem mass spectrometry coupled with LC provides a rapid and sensitive tool to identify platinum anti-cancer drugs and investigate their hydrolysis reactions.

5. Gas chromatography

A capillary gas chromatographic method was described by Khuhawar et al. [58] for the determination of cis- platin based on the complexation of platinum(II) with bis(isovalerylacetone)ethylenediimine and extraction in chloro- form. The chromatography was carried out on a BP1 or a BP5 column with an FID. The method was applied of the determi- nation of cisplatin in a pharmaceutical preparation and blood samples of cancer patients after infusion of cisplatin.

6. Capillary electrophoresis

The behaviour of cisplatin in aqueous chloride solutions at room temperature was studied by thin layer chromatography (TLC), thin layer electrophoresis and a combination of the two [59]. While solutions kept in the dark are relatively stable, they decompose if exposed to normal daylight. Up to six cationic, five uncharged and two anionic species could be separated in solutions aged for 7 days. The detection reagent used was 4- nitrosodimethylaniline.

Several capillary electrophoresis (CE) separations of the main hydrolysis products of cisplatin originated under simu- lated physiological conditions have been reported. Zenker et al.

[60] succeeded in separating simultaneously cisplatin and two hydrolysis products using a simple 1 mM phosphate buffer (pH 7.4) containing 4 mM NaCl.

Micellar electrokinetic capillary chromatography was used for the separation of platinum anti-tumour drugs (including cisplatin) in aqueous solutions with the use of sodium dodecyl sulphate in only one run [61]. The developed method was used for stability measurements of the complexes in solution.

The use of micellar electrokinetic chromatography with direct UV detection is described for the determination of intact cisplatin in human serum by Huang et al. [62]. The detection limits of platinum species studied were about 2–3 fl,mol L^{-1}.

Characterizing how platinum metallocomplexes bind to human serum albumin (HSA) is essential in evaluating anti- cancer drug candidates; using cisplatin as a reference complex, the application of CE to reliably assess drug/HSA interactions was validated [63]. Since this complex is small compared to the size of the protein, the binding response could only be recog- nized when applying CE coupled to a (platinum) metal-specific mode of detection, namely ICP–MS. This coupling allowed for confirmation of a specific affinity of cisplatin and novel Pt com- plexes to HSA, measurement of the kinetics of binding reactions, and determination of the number of drug molecules attached to the protein. As the cisplatin/HSA molar ratio increased, the reaction rate became faster with a maximum on the kinetic curve appearing at about 50 h of incubation at 20 times excess of cis- platin. When incubated with a 20-fold excess of cisplatin, HSA bound up to 10 mol of Pt per mol of the protein. This is indica- tive for a strong metal–protein coordination occurring at several results demonstrated the suitability of CE-ICP–MS as a rapid assay for high-throughput studying of drug/HSA interactions.

The interaction of cisplatin, carboplatin and analogues with nucleotides was systematically investigated [60,64–66], and the formed adducts were identified through a characteristic shift in their absorption bands compared to unmodified nucleotides.

Using conventional detectors, only limited structural infor- mation about DNA–Pt adducts can be revealed. CE coupled with a mass spectrometry detector has been used for the characteriza- tion of platinated DNA nucleotides, and the system was applied to investigate the kinetics of adduct interaction [67]. Also, gel electrophoresis coupled with ICP–MS was reported for the determination of proteins from cisplatin treated bacterial cells [68]. Proteins were partially separated by 1D polyacrylamide gel electrophoresis and analysed by laser ablation ICP–MS; using peptide fingerprinting methods central to proteomics, the band containing the highest levels of platinum was found to contain outer membrane protein A, which may be involved in cisplatin uptake.

7. Mass spectrometry

Currently, the combination of the resolving power of LC with the sensitivity and selectivity of ESI-MS is a promising analytical technique for the determination of platinum-based drugs. However, when selecting a mobile phase for LC–MS experiments, the reaction chemistry of cisplatin and its hydrol- ysis products must be considered in order to avoid ambiguities

in interpretation of the results. These platinum species react strongly with any nucleophilic compound, such as phosphate, acetonitrile, acetate, etc., which are commonly used in LC mobile phases. Furthermore, this technique is still compromised by the intense matrix-related chemical noise usually associated with ESI from LC.

An alternative approach is the separation of ions in the gas phase using a technique known as high-field asymmetric wave- form ion mobility spectrometry (FAIMS). In this way, cisplatin and its mono- and dihydrated complexes have been separated using a FAIMS analyzer interfaced with ESI and ion trap mass spectrometry (ITMS) [69]. The addition of helium to the nitro- gen curtain/carrier gas in the FAIMS device improved both the sensitivity and selectivity of the electrospray analysis. Intro- duction of a three-component mixture as curtain/carrier gas, nitrogen, helium and carbon dioxide, resulted in further improve- ments to sensitivity. Analytical results were linear over the concentration range 10–200 ng mL^{-1} for intact cisplatin with a corresponding detection limit of 0.7 ng mL^{-1} with no derivatization or chromatographic separation prior to analysis.

Most of cisplatin's cytotoxic properties are due to the interaction of the drug with DNA. However, other biological molecules present in the cell cytosol, such as MT (metal- lothionein) and GSH (glutathione) are potential targets for cisplatin and have been related to its side-effects or with the cellular resistance mechanisms to the drug. For these rea- sons, experiments simulating physiological conditions have been performed to study the specific cisplatin metabolites which interact with GSH and MT and to characterize the differ- ent drug–biomolecule adducts over time. A combination of size exclusion chromatography–ICP–MS (SEC–ICP–MS) and ESI-MS techniques has been used to provide sensible multi- elemental detection and structural information of the species of interest [70]. Time-dependent transformation of 10 fl,M cis- platin at neutral pH (7.4) produces different concentrations of the mono-aquo and oligomeric derivatives, as could be confirmed by ESI-MS. No di-aquo derivative was seen to be produced under these conditions at any of the incubation times used. Cisplatin and the oligomeric derivative were incubated with GSH and MT at different drug:biomolecule ratios. Adducts from cisplatin–GSH (1:500) and from cisplatin–MT (1:10) incubations were characterized by SEC–ICP–MS. While both GSH and MT reacted with cisplatin producing different compounds, only GSH reacted with the oligomeric derivative of cisplatin.

Some pioneering ESI-MS studies of platinum–protein inter- actions were reported a few years ago by Peleg-Shulman and Gibson, who used either ubiquitin or myoglobin as model pro- teins [71]. A number of platinum–protein adducts were identified and characterised in detail. Afterwards, a few additional ESI-MS studies of various metallodrug–protein adducts were reported by other research groups.

ESI-MS has been used to explore the binding of cisplatin to transferring by Allardyce et al. [72]. Binding with loss of HCl is observed. In addition, the threonine 457 amino acid residue, and hence the platinum(II) binding site, is located. The same research group expands this work by proposing a mechanism for the binding of cisplatin to transferrin, a mechanism which may

have implications concerning the effectiveness of this unique metallodrug in chemotherapy [73].

On the other hand, cytochrome *c* is a small electron-carrier heme protein, localised in the mitochondria, that plays a crucial role in apoptotic pathways. Cytochrome *c* is also known to be an excellent ESI-MS probe and has been the subject of a num- ber of investigations. For these reasons, Casini et al. [74] chose cytochrome *c* as the model protein for their study.

Recently, Hartinger et al. [75] present the application of elec- trospray ionization Fourier transform ion cyclotron resonance mass spectrometry (ESI-FT-ICR-MS) for characterizing directly the binding sites of platinum complexes to ubiquitin using a top-down approach employing FT-ICR-MS/MS.

8. Other detection methods

Cisplatin-induced ototoxicity is correlated with functional and morphological changes in the organ of Corti, the stria vas- cularis and the spiral ganglion. However, the cochlear sites of cisplatin uptake and accumulation have not been prop- erly identified. Therefore, VanRuijven et al. [76] developed an immunohistochemical method to, indirectly detect cisplatin in semithin cryosections of the guinea pig cochlea using an anti- serum containing antibodies against cisplatin–DNA adducts. Platinated DNA was present in the nuclei of most cells in the organ of Corti and the lateral wall after cisplatin administra- tion. Nuclear immunostaining was most pronounced in the outer hair cells, the marginal cells and the spiral ligament fibrocytes. This study is the first to demonstrate the presence of cisplatin in histological sections of the cochlea.

9. Conclusions

Cisplatin is a platinum-based drug that is widely used in the clinical treatment of various cancers, including testicular, ovarian, lung, and head and neck cancer. Cisplatin and monohy- drated cisplatin react with nitrogen, sulphur and oxygen residues on other biomolecules such as plasma proteins. As a result of these reactions, a variety of platinum species may be present in the body after treatment with cisplatin. There has been con- siderable interest in determining the concentration of cisplatin and its hydrolysis products in the body and under experimen- tal conditions in matrices such as plasma ultra-filtrate and cell culture medium ultra-filtrate because of these important clinical applications.

A common approach for determining the concentration of cisplatin has been to measure the total platinum con- tent of blood fractions and other biological fluids by flame and graphite furnace AAS, ICP–AES and ICP–MS. To avoid detection of platinum that may have become deactivated by reactions with plasma proteins, plasma is often depro- teinized by solvent protein precipitation or ultra-filtration before analysis. However, ultra-filtrates and solvent extractions of plasma may still contain different platinum species includ- ing the intact cisplatin, hydrated cisplatin and inactive forms of platinum. To further distinguish the species present in the systemic circulation after cisplatin treatment, there has been

increasing interest in the use of species-specific analytical tech- niques.

HPLC–ICP–MS is a technique that has gained popularity for detecting different chemical species of trace elements in environmental, biological and clinical samples. By directly coupling the HPLC column to an ICP–MS, which is set to detect the specific mass of interest (*m*/*z* 194/195 for platinum), specific and sensitive detection can be achieved with little background interference from complex biological matrices.

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