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Speciation of cisplatin in environmental water samples by hydrophilic interaction liquid chromatography coupled to inductively coupled plasma mass spectrometry

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ABSTRACT

Cisplatin is still widely used for treatment of numerous types of tumours. Different speciation methods have been applied to study behaviour of the intact drug and its individual biotransformation species in various clinical samples. These methods are mainly based on electrophoresis, size exclusion (SEC) or ion chromatography (IC) techniques coupled to inductively coupled plasma mass spectrometry (ICP–MS). Hydrophilic interaction liquid chromatography (HILIC), which is a common technique for separation of polar substances, was rarely applied for separation of cisplatin and its hydrolysed metabolites. There is also a lack of information available on the occurrence of cisplatin and its hydrolysed complexes in the environmental waters. In the present study the concentrations of Pt were determined in hospital wastewaters by ICP–MS. A procedure for separation of cisplatin and its aqueous hydrolysed complexes by the use of HILIC column was optimized. Quantification of separated Pt species was performed by isotope dilution (ID)–ICP–MS procedure. Low limits of detection (LODs) and quantification (LOQs) were obtained for cisplatin and its hydrolysed complexes ranging from 0.0273 to 0.1726 ng Pt/mL and from 0.0909 to 0.5753 ng Pt/mL, respectively. Good repeatability of the procedure with relative standard deviation (RSD) lower than ± 2.3% was obtained. The column recoveries, which ranged from 95 to 101%, indicated that the procedure developed enabled quantitative speciation analysis of aqueous cisplatin complexes. The ZIC-HILIC–ID–ICP–MS procedure was successfully applied in speciation of cisplatin in spiked hospital wastewater samples.

1. Introduction

Cancer is a disease characterised by uncontrolled growth, increased division, decreased cell death and many other abnormalities of cancer cells. For its treatment, various metal-based chemotherapeutics are used [1,2]. Cisplatin (*cis*-[$PtCl_2(NH_3)_2$]), the first chemotherapeutic which was introduced into clinical trials in the late 1970s [3], is nowadays still widely used for treatment of numerous types of tumours (lung, bladder, gastric, head and neck, cervical, testicular and ovarian) [4–6]. For better understanding of anticancer therapy with cisplatin the behaviour of the intact drug and its individual biotransformation species in clinical samples has been intensively investigated by applying speciation analysis methods [3,7,8]. To study interaction of cisplatin with serum proteins Szpunar et al. [9] applied size-exclusion chromatography (SEC) coupled to inductively coupled plasma mass spectrometry (ICP–MS). Huang et al. [10] determined cisplatin and its hydrolytic metabolite in human serum by capillary electrophoresis techniques coupled to ICP–MS. Esteban-Fernández et al. [11] performed speciation of Pt in kidney and inner ear tissue of rats treated with Pt-based chemotherapeutics. Pt binding to proteins was followed by two-dimensional (2D) liquid chromatography (SEC plus fast protein liquid chromatography column (FPLC)) coupled to ICP–MS. Later 2D chromatography, comprised of SEC in combination with ion-exchange chromatography, using strong anion-exchange Mono Q FPLC column or monolithic convective interaction media (CIM) weak anion-exchange diethylamino (DEAE) column, was applied. By hyphenation to ICP–MS a study was performed on the kinetics of binding of cisplatin to serum proteins. The same chromatographic set-up was used to investigate the distribution of Pt species in serum of cancer patients receiving cisplatin [12]. Conjoint liquid chromatography (CLC) on short monolithic disks (affinity CIM Protein G disk and anion-exchange CIM DEAE) coupled to ICP–MS was then used for simultaneous 2D separation of ionic forms of Pt-based

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chemotherapeutics from the portions bound to different serum proteins [13]. In this study, the quantification of separated Pt species was performed by post-column isotope dilution (ID)–ICP–MS technique. Since the formation of deoxyribonucleic acid (DNA) adducts with cisplatin is crucial pharmacokinetic parameter, which must be optimized in cancer therapy based on Pt drugs, Hann et al. [14] examined the time-resolved interaction of cisplatin with guanosine monophosphate (GMP) by high performance ion chromatography (HPIC)–ICP-sector field (SF)MS. Pt/P ratio was determined by simultaneously measuring ^{31}P and ^{195}Pt.

It is well know that in physiological solutions, cisplatin undergoes hydrolysis. The hydrolysed forms of cisplatin react in a different extent with blood components and cell biomolecules [3,15]. The formation of cisplatin hydrolysed complexes is time-dependent and also depends on the concentration of chloride ion [16]. For investigation of cisplatin and its hydrolysed complexes, hydrophilic interaction liquid chromatography (HILIC) coupled to ICP–MS was found to be a promising technique [17,18]. However, HILIC, which is a routine technique for separation of polar drugs [19,20], was rarely used for separation of cisplatin and its hydrolysed complexes [17,18,21]. When HILIC is applied, organic solvents which are used in the separation procedure are not favourable for ICP–MS detection, due to carbon deposition on the sampler and skimmer cones. Hemström et al. [18] reported the choice of possible organic solvents for HILIC separation of cisplatin and highlighted that commonly used acetonitrile (ACN), reacts with cisplatin and upset speciation equilibria. To avoid the latter problems the use of dimethylformamide (DMF) or 20% 1-propanol in 25 mM ammonium formate buffer (pH 6.5) was recommended. Nygren et al. [17] applied HILIC–ICP–MS procedure to study the distribution of Pt species in whole cell lysate from in-vitro grown T289 malignant melanoma cells exposed to cisplatin. Separation of cisplatin and monoaquacisplatin was achieved by using DMF as an eluent. The LODs for cisplatin and monoaquacisplatin were 0.2 ng Pt/mL. Other group of researchers [21] used ACN to separate cisplatin, oxaliplatin and carboplatin in spiked human plasma samples by HILIC–ICP–MS. For cisplatin, the LOD was found to be 9.8 ng Pt/mL.

Other techniques, like reversed-phase ion-pairing chromatography were also implemented for speciation of cisplatin and enabled separation of neutral complexes from 1+ and 2+ charged Pt species. The LODs obtained for cisplatin were 1 ng Pt/mL [22] and 19.5 ng Pt/mL [23]. ESI–MS–HPLC was an appropriate technique for identification of Pt-based anticancer drugs and their hydrolysis products when Pt concentrations were in μg/mL range [24,25].

Table 1
Optimised ICP–MS operating parameters for the determination of the total Pt concentrations and quantification of Pt species after their separation on ZIC-HILIC column.

ICP–MS parameters	Total Pt concentrations	Pt species after chromatographic separation
Forward power	1550 W	1600 W
Plasma gas flow (Ar)	15.0 L/min	15.0 L/min
Carrier gas flow (Ar)	1.10 L/min	0.57 L/min
Dilution gas flow (Ar)	/	0.10 L/min
Optional gas flow (20% v/v O_2 in Ar)	/	9%
Nebuliser type	Micromist	Micromist
Isotopes monitored	^{193}Ir, ^{194}Pt, ^{195}Pt	^{194}Pt, ^{195}Pt
Integration time	0.3 s	0.4 s
Total acquisition time	17.1 s	2100 s
Spray chamber temperature	−5 °C	−5 °C

Due to the widespread use of Pt-based chemotherapeutics, Pt metabolites that are excreted with urine end-up in hospital or municipal wastewaters. Despite the potential emerging of cisplatin in the environmental waters there is a lack of literature data on occurrence of cisplatin and its hydrolysed complexes in surface waters. Therefore, the aim of our work was first to determine the content of Pt in hospital wastewaters and in wastewaters from inflow and outflow of sewage treatment plants by ICP–MS. The second aim was to optimize the HILIC procedure for separation of cisplatin and its hydrolysed complexes and to perform the speciation analysis of cisplatin in environmental waters by HILIC coupled to ICP–MS, using post-column ID–ICP–MS technique for the quantification of separated cisplatin species.

2. Materials and methods

2.1. Instrumentation

Total Pt concentrations were determined by inductively coupled plasma mass spectrometer (ICP–MS), model 7700 ×, from Agilent Technologies (Tokyo, Japan). HPLC separations were performed on an Agilent (Tokyo, Japan) series 1200 HPLC system with a quaternary pump equipped with a sample injection valve, Rheodyne model 7725i (Cotati, Ca, USA) fitted with a 5 μL injection loop. The column made from poly(etherether ketone) (PEEK) with bonded zwitterionic silica-based HILIC stationary phase (ZIC-HILIC PEEK HPLC column 2.1 ID, Merck, Darmstadt Germany) with a 20 × 2.1 mm guard column was used for separation of Pt species. The outlet of the chromatographic column was directly connected to the Micromist nebuliser and Scott-type spray chamber of the ICP–MS instrument. Quantification of separated Pt species was performed by post-column isotope dilution ICP–MS. ICP–MS operating parameters for determination of total Pt concentrations and Pt species after the chromatographic separation are given in Table 1.

2.2. Reagents and materials

Ultrapure water (18.2 MΩ cm) was obtained from a Direct-Q 5 Ultrapure water system (Millipore Watertown, A, USA). All chemicals were of analytical reagent grade. Ammonium formate, *n*-propanol, formic acid and sodium chloride were obtained from Merck (Darmstadt, Germany). Eluent A consisted of aqueous solution of 95% *n*-propanol +5% of 20 mM ammonium formate (pH 4). Eluent B was composed of aqueous solution of 50% *n*-propanol +50% 20 mM ammonium formate (pH 4), while buffer C was aqueous solution of 100 mM ammonium formate (pH 4). Cisplatin was obtained from Medoc (Hamburg, Germany). Merck stock Pt solution (1000 μg Pt/mL in 8% HCl) was diluted daily with water for the preparation of fresh calibration standard solutions that were used for the determination of the total concentration of Pt in the samples analysed. Platinum enriched in ^{194}Pt isotope (Pt metallic plate, 15 mg) obtained from Oak Ridge National Laboratory (Oak Ridge, TN, USA) was dissolved in 1 mL of aqua regia and diluted to 10 mL with an appropriate amount of HCl, so that the final concentration of HCl was 8%. The declared composition of the enriched Pt plate was 96.45 ± 0.05%, 2.46 ± 0.05%, 0.87 ± 0.02%, 0.18 ± 0.01%, 0.03 ± 0.00% and 0.01 ± 0.00% for the isotopes 194, 195, 196, 198, 192 and 190, respectively.

Sartorius (Goetingen, Germany) 0.45 μm cellulose nitrate membrane filters of 25 mm diameter were used in the filtration procedure.