A novel method for speciation of Pt in human serum incubated with cisplatin, oxaliplatin and carboplatin by conjoint liquid chromatography on monolithic disks with UV and ICP-MS detection

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Abstract. The aim of this work was to develop a new method for rapid two-dimensional chromatographic separation of unbound Pt-based drugs and their complexes with proteins in human serum. For this purpose conjoined liquid chromatography (CLC) monolithic column was constructed by placing one CIM Protein G and one CIM diethylamino (DEAE) disk in a single housing. This enabled two-dimensional separation in a single chromatographic run. Speciation analysis of cisplatin, carboplatin and oxaliplatin interaction with synthetic mixture of serum proteins and human serum was performed. Separated Pt-species were monitored on line by UV and ICP-MS detection.

Keywords: Pt-based drugs, conjoined liquid chromatography (CLC), ICP-MS

1 Introduction

For cancer treatment, three Pt-based chemotherapeutic drugs are applied worldwide; cisplatin (*cis*-diaminedichloro-platinum(II)), carboplatin (*cis*-diammine(1,1-cyclo-butanedicarboxylato)platinum(II)) and oxaliplatin, ([(1R,2R)-cyclohexane-1,2-diamine](ethanedioato-O,O')platinum(II)) [1]. A major limitation of chemotherapy with platins is their unwanted toxicity towards healthy tissues producing severe side effects, like nephrotoxicity, ototoxicity, emetogenesis, neurotoxicity, and drug resistance in the targeted tumour cells [2]. Serum proteins are the first biological

ligands to interact with the administrated drugs. Knowledge of drug interactions in blood serum is very important in order to avoid severe side effects and to optimise or individualise dosage adjustment in clinical chemotherapy [3].

In speciation analysis of platinum in human serum, ion exchange (IE) chromatography is frequently used. As an alternative to classic – particle packed IE columns, monolithic supports have been successfully applied. They have several advantages over classical columns (e.g. greater robustness) but both are unable to separate unbound drug from immunoglobulins (IgG), which co-elute at the same retention time t_0 (Figure 1) [4]. To overcome this problem a conjoint liquid chromatography (CLC) column, containing one CIM Protein G and one CIM DEAE monolithic disk in a single housing, was constructed. Such set up allows two dimensional separation in one chromatographic run. CIM Protein G disk (affinity chromatography) binds Fc region of IgG thus separating free Pt-based drug from portion that is bound to IgG, while on a weak anion exchange CIM DEAE disk serum transferrin and albumin were separated. For separation, isocratic elution with Tris-HCl - $NaHCO_3$ buffer (pH 7.4) in the first min, followed by gradient elution with 1 mol L^{-1} NH_4Cl (pH 7.4) in the next 9 min, and acetic acid (AcOH) in the last 4 min was applied. To quantify separated Pt species and to compensate for different nebulisation and ionisation of solvents, which impair inductively coupled plasma mass spectrometry (ICP-MS) detection, post column isotope dilution (ID) was used.

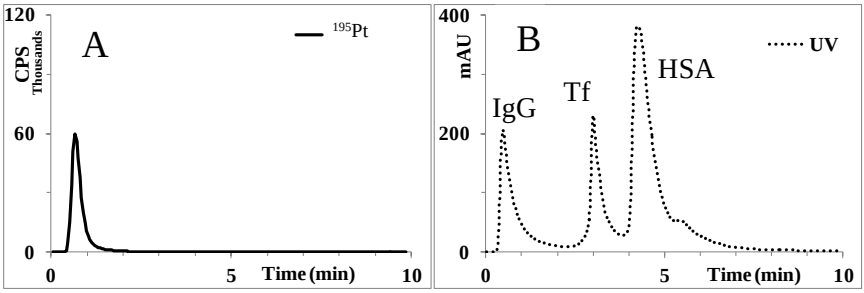

Figure 1: Co-elution of unbound drug and IgG. A) Chromatogram of cisplatin diluted in buffer A. B) Chromatogram of the mixture of standard serum proteins.

2 Materials and methods

Separations were performed by using Agilent (Tokyo, Japan) series 1200 HPLC system on a CLC column constructed with CIM DEAE-1 and CIM Protein G monolithic disks (Bia Separations, Ajdovščina, Slovenia). Protein signals were followed online with UV-Vis detector at 278 nm and ^{194}Pt, ^{195}Pt signals with Agilent 7700x ICP-MS. All reagents were from Merck (Darmstadt, Germany)

Buffer A: 0.05 mol L^{-1} Tris-HCl + 0.03 mol L^{-1} $NaHCO_3$, pH 7.4.

Buffer B: Buffer A + 2 mol L^{-1} NH_4Cl, pH 7.4.

Eluent C: 0.5 mol L^{-1} AcOH.

Buffer D: 0.2 mol L^{-1} Tris-HCl, pH 7.4.

Human serum apo-transferrin (Tf), human serum albumin (HSA) and γ-globulins (IgG) were purchased from Sigma-Aldrich (Steinheim, Germany). Cisplatin was obtained from Medoc (Hamburg, Germany), carboplatin from Actavis (Nerviano, Italy) and oxaliplatin from Sanofi Winthrop (France). Platinum enriched in ^{194}Pt isotope (Pt metallic plate, 15 mg) obtained from Oak Ridge National Laboratory (Oak Ridge, TN, USA) was used as post column spike.

Speciation was carried out at a flow rate of 1 mL min^{-1} and injection volume of 0.1 ml. To improve the resolution of unbound Pt species and Tf, isocratic elution with 100 % buffer A was applied in the first min, followed by linear gradient elution from buffer A to 50 % buffer B in the next 9 min, in order to separate Tf from HSA. IgG was then eluted from the column by isocratic elution with 100 % eluent C for 3 min. The eluate from the CLC column was passed through the UV and ICP-MS detection systems. Regeneration and equilibration steps were done at a flow rate of 6 mL min^{-1}; CLC column was first rinsed for 3 min with 100 % buffer D, than for 7 min with buffer 100 % B and 4 min with 100 % buffer A. In the final step, the column was equilibrated for 0.5 min with buffer A at a flow rate of 1 mL min^{-1}. The eluate from the regeneration and equilibration steps was directed to waste through a software controlled six-port valve. By applying the procedure described above at least 30 serum samples could be analysed without additional cleaning.

3 Results and discussion

The behaviour of Pt-based chemotherapeutic drugs on the CLC monolithic column was examined by two-dimensional chromatographic separation of 5-times diluted

samples of mixtures of standard serum proteins (Fig 2) and serum samples (Fig 3). The Pt mass flow was calculated using Pt^{195}/Pt^{194} isotope ratio.

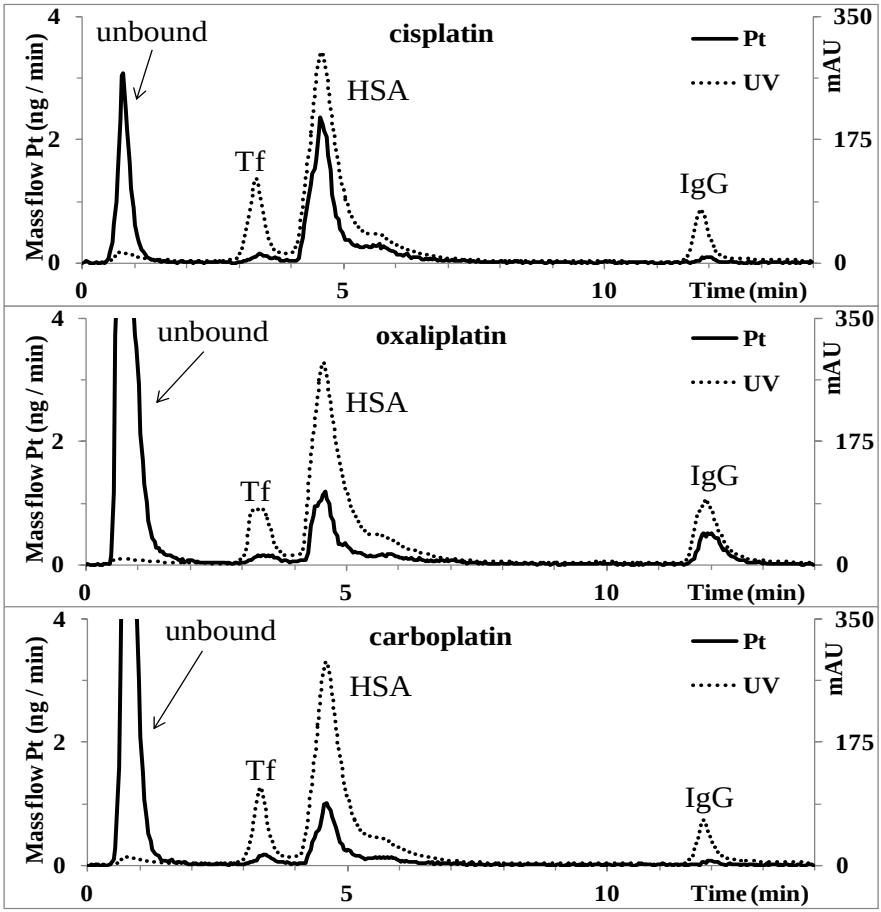

Figure 2: Chromatographic separation of a synthetic mixture of serum proteins spiked with a single Pt-based drug (100 to 200 ng Pt mL^{-1}, incubation time 24 h).

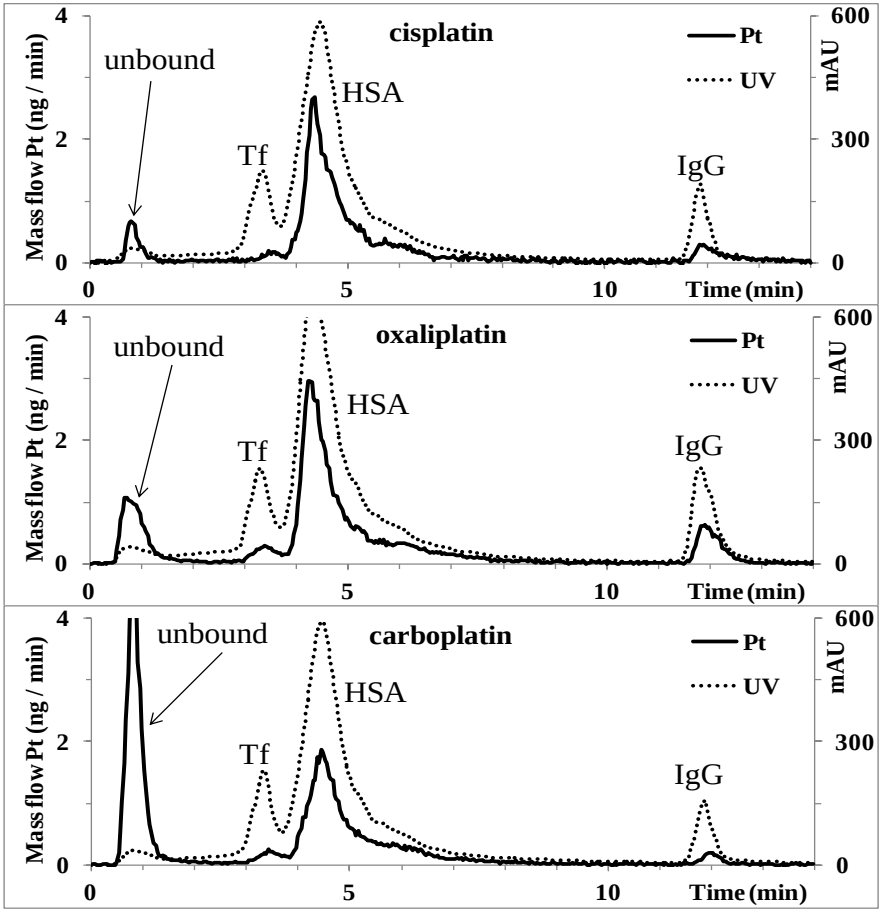

Figure 3: Chromatographic separation of human serum spiked with a single Pt-based drug (100 to 200 ng Pt mL^{-1}, incubation time 24 h).

As can be seen from Figures 2 and 3, the developed method separates all the major Pt species found in human serum. The use of post-column ID adequately compensated the changes in eluent composition, while also providing the means for quantifying our results. Unbound chemotherapeutic drugs in spiked samples of the synthetic mixture of serum proteins and serum samples were eluted from 0.4 to 1.3 min, while those bound to serum proteins were eluted under the chromatographic peaks of Tf (2.9 to 3.6 min), HSA (3.6 to 6.6 min) and IgG (11.7 to 12.7 min).

Detailed speciation data (summarised in table 1) is in good agreement with the literature [4].

Table 1: Distribution of Pt in human serum.

Pt drug	Incubation time (h)	Pt spike added	Unbound Pt	Pt bound to Tf	Pt bound to HSA	Pt bound to IgG	Column recovery
		(ng mL^{-1})	(ng mL^{-1})	(ng mL^{-1})	(ng mL^{-1})	(ng mL^{-1})	(%)
Cisplatin	24 h	137.4±1.1	19.5±0.1	2.50±0.02	103.5±0.8	5.00±0.04	95
Oxaliplatin	24 h	153.7±1.2	27.0±0.2	4.01±0.03	102.1±0.8	16.0±0.1	97
Carboplatin	48 h	165.6±1.3	77.5±0.6	3.02±0.02	75.5±0.6	3.01±0.03	96

4 Conclusions

The developed method is uniquely able to separate unbound drug from drug bound to IgG while preserving all the advantages of monolithic chromatography, mainly speed and robustness.

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For wider interest

Our work is based on monolithic chromatography, which offers several advantages over standard (particle packed) chromatographic columns. Monolithic supports have high permeability and therefore allow thorough cleaning during regeneration after each separation run. This enables great robustness of such chromatographic columns which in turn enables higher throughput of samples. Monolithic supports are also cheaper and offer possibilities to be applied in numerous chromatographic separations of compounds in environmental and biological samples.

Combining affinity and anion exchange monolithic disks enables us to construct so called conjoined liquid chromatography columns (CLC) that can perform 2D chromatographic separations in a single run.