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Chemical degradation of platinum oncolytics in urine and speciation of the inorganic contaminants cisplatin and carboplatin relevant in waste water

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ABSTRACT

Understanding the prevalent form of platinum (Pt) based antineoplastic agents, used in chemotherapy, is of importance to develop a remediation strategy that restricts aquatic exposure. The speciation of Pt-based compounds was measured in actual patients' urine using hydrophobic interaction liquid chromatography (HILIC) equipped with an ICP-MS detector. Carboplatin showed poor metabolization and intact excretion 11 h after administration, whereas cisplatin underwent a rapid aquation in the first 7 h. To compare, the *in vitro* degradation rate of cisplatin, carboplatin and oxaliplatin was determined in synthetic human urine, mimicking true environmental conditions. The fraction of intact molecules was measured at regular intervals following incubation at 37 °C, resulting in degradation rate constants. The chemical stability was highest for carboplatin ($k = 0.0143 \pm 0.0012$ min^{-1}), versus faster degradation of oxaliplatin and cisplatin by exponential decay with k_1 of 0.0026 ± 0.0001 min^{-1} and $k_2 = 5.59 \pm 0.46 \times 10^{-6}$ min^{-1}, respectively. These kinetic parameters can serve as input to further expand modelling databases and improve the predictive power of speciation software to estimate eco-toxicity risks. Considering the strong residual cytotoxicity of the platinum antineoplastic molecules following renal clearance and human excretion, the contaminants are of high environmental concern and offer potential for metal recovery using advanced treatment steps. In such water treatment processes, especially carboplatin, above all cancerostatic platinum compounds, should be addressed since it is more persistent in the aquatic environment.

1. Introduction

Platinum-based oncolytics are frequently applied antineoplastic pharmaceuticals in cancer treatment worldwide. Cisplatin is the first-line chemotherapeutic agent to suppress tumor growth and metastases in several cancer types, despite its adverse side-effects [1]. Together with carboplatin and oxaliplatin, these three drugs make up for most of the used cancerostatic platinum compounds (CPCs) and supply shortages have been reported owing to their absolute medical necessity [2]. The mode of action is universal: after dissociation of the pro-drug, a bidentate coordinative bond is formed between the Pt atom and N^7 of DNA base pairs, hence inhibiting further DNA replication. Their active role in suppressing DNA replication is supported by co-pharmaceuticals, such as paclitaxel (Taxol ®) or pemetrexed (Alimta ®). Usually, also $Na_2S_2O_3$ is intravenously dosed along CPC to counter free Pt^{2+} concentrations in the serum that can be as high as 12 mg L^{-1} [3]. The risk of oto- and nephrotoxicity is further decreased by a rapid renal clearance, induced by mannitol, so most residual cisplatin is ultimately cleared from the human body through urinary excretion within the first 24 h [4]. Other excretion pathways, such as faecal excretion, sweat or saliva are only minor [5].

At this point, residual fractions of CPC that are present in the waste water enter the sewage system. Their fate, however, remains

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mostly unexplored [6]. Bio-accumulation of Pt from medicinal origin can occur in the aquatic environment through the discharge of sewage, while the use of sludge in agriculture forms a terrestrial threat [7]. CPC can impact non-target organisms and possess a far more toxic character compared to endocrine therapy agents or antibiotics [8]. The physicochemical characteristics of Pt and the processes that lead to the occurrence of soluble and bioavailable species in the environment are still poorly understood [9], but exposure to 14 μg L^{-1} already induced a 16% increase in reproductive impairment in *Daphnia magna*, while loss in organism weight was observed even below this exposure level [10]. Platinum occurrence usually falls within the nanogram per liter range [11], albeit higher concentrations of more than 750 μg L^{-1} have been retrieved in hospital waste water [8].

Environmental concerns may arise from a high stability of the compounds, in combination with low immobilization by adsorption on sediments, given the low K_{ow} [12]. CPC are recalcitrant and have a low removal in traditional wastewater treatment plants (WWTP), leading to potentially high remaining toxicity and cancerogenicity of the pharmaceuticals in downstream water bodies [13]. Global awareness has risen on the necessity of addressing Environmentally Persistent Pharmaceutical Pollutants (EPPPs) [14]. Based on a precaution principle, residues of mutagenic pharmaceutical should be prevented from spreading in the environment. Especially aquatic pollution is troublesome given it is a major route for exposure of CPC.

The European Commission has undertaken actions to reduce the environmental exposure to pharmaceuticals as part of the Water Framework Directive. It imposes a threshold of 0.010 μg L^{-1} to distinguish individual potentially harmful pharmaceuticals, including cisplatin, carboplatin and oxaliplatin. Currently, no exceedance of the levels of individual Pt-based antineoplastics in wastewater that enters municipal treatment has been reported [15]. Below 0.010 μg L^{-1}, the detection has been analytically challenging since distinguishing each compound requires the use of contemporary analytical techniques, such as LC-MS or ESI-MS/MS. It also explains the current lack of studies that focus on the environmental effects, in contrast to a plethora of clinical reports.

A variety of treatment technologies has been proposed towards individual CPCs, including biological techniques by means of axenic microbial cultures of *C. metallidurans* and *S. oneidensis* [16], and physico-chemical techniques such as ozonation [17].

Of key interest to estimate the residual toxicity of CPC and associated environmental risks associated to their release is the detailed understanding of prevalence and stability of pharmaceutical CPC in wastewater. Surveying the element speciation or complexation of Pt is critical to the consequent immobilization of the metal complex [18] in its parent form and associated by-products [19]. It is a first step towards establishing an effective treatment strategy that avoids noxious CPC release into the environment [20], while providing opportunities to simultaneously recover Pt.

Waste water bearing precious metals can be considered as a secondary source for recovery. However, examples of actual platinum recovery from liquid streams are still rare. It is expected that the recycled fraction of Pt amounts to 25.4% of the virgin platinum production rate in 2023 [21], with a major share coming from automotive sector. A more sustainable management of the raw material, supported by its high commodity price, can anticipate on an increased scarcity of the precious metal. But developing an effective strategy for resource recovery requires to first elucidate the Pt speciation in sewage systems [11]. In this way, CPC species that are of prime ecotoxicological interest can be identified. The aim of this work is to determine the fraction of original CPC compounds in patient's urine, once the cytostatics are excreted and compare the degradation rate with *in vitro* solutions of cisplatin, carboplatin and oxaliplatin in synthetic human urine, mimicking true environmental conditions. Hence, by the use of a hyphenated analytical technique, the degradation profile of three primary platinum based antineoplastic agents will be derived.

2. Materials and methods

2.1. Clinical study and urine collection

A prospective study aimed to monitor the degradation of platinum-containing antineoplastic drugs in the urine of two patients undergoing chemotherapy. At regular intervals, urine samples were collected from cancer patients receiving treatment with cisplatin or carboplatin antineoplastics. Intravenous CPC administration was done at the Department of Medical Oncology. From the urine excreted within the following 24 h, 50 mL was filled in labelled polyethylene tubes and frozen immediately to −20 °C. Further analysis was done in the analytical laboratory to determine the platinum speciation. The clinical study was approved by the ethical committee of University Hospital Ghent (registration number B670201422304) and an informed consent was given by all participants in the study. Circumstantial data of the patients can be found in Table 1.

2.2. In vitro degradation of CPCs

The *in vitro* degradation was examined by dissolving cisplatin, carboplatin and oxaliplatin in ultrapure water at the concentration of 0.25 mM Pt. To the cisplatin solution, 150 mM NaCl was added, similar to the actual infuse, to prevent the unintended, early dissociation of chloride ligands [22]. The leaving of chloride or carboxylic chelating groups according S_N1 reaction is in all cases the rate determining step in the process. For example, the half-life of pristine cisplatin in ultrapure water prior to hydrolysis of chloride (Equation (1)) is merely 2.1 h and 3.1 h for its second transformation into diaquacisplatin (Equation (2)) [23].

$$Pt(NH_3)_2Cl_2 + H_2O \Leftrightarrow [Pt(NH_3)_2(H_2O)Cl]^+ + Cl^- \quad \text{Equation 1}$$

$$[Pt(NH_3)_2(H_2O)Cl]^+ + H_2O \Leftrightarrow [Pt(NH_3)_2(H_2O)_2]^{2+} + Cl^- \quad \text{Equation 2}$$

The compounds were spiked to simulated human urine, of which characteristics are displayed in Table 2, to obtain a final Pt concentration of 500 μg L^{-1}. Each solution was kept in a thermostatic bath at 37 °C and sub-samples taken out immediately and after 1, 2, 4, 6, 8, 24 and 69 h for filtration over 0.45 μm pore size filters. A volume of 1 mL of the filtrate was stored in HPLC vials at −20 °C.

2.3. Determination of total Pt concentrations

An aliquot of 2.5 mL urine was diluted with 2 mL of 65% HNO_3 (Chem-Lab, Zedelgem, Belgium) and Milli-Q water (Millipore, Billerica, MA) to a total volume of 6 mL, preceding the digestion step that was assisted by microwave heating in open vessels (Mars 6, CEM, Matthews, NC). A power of 1,200 W was exerted to reach a temperature of 120 °C in 10 min, which was then hold constant for 30 min. Afterwards, the solutions were cooled down to room temperature and diluted, first, to a total volume of 50 mL by Milli-Q water and, secondly, a ten-fold dilution by a solution containing 10 μg L^{-1} indium, serving as internal standard. The measurement of Pt concentrations was performed by Inductively Coupled Plasma -

Table 1
Characteristics of the two patients that participated in the clinical study and received cisplatin ($n = 1$) and carboplatin ($n = 1$) antineoplastic agents during chemotherapy.

Antineoplastic	Sex	Body weight (kg)	Creatinine clearance (mg dL^{-1})	Administered Pt dose (mg)	Co-medication
Cisplatin	♀	54	0.65	78.5	Pemetrexed
Carboplatin	♀	70	1.80	124.7	Paclitaxel

Table 2
Characteristics of synthetic human urine used for the *in vitro* degradation of CPC.

Parameter	Value
pH	5.9
Albumin	<10 mg L^{-1}
Creatinine	0.5 g L^{-1}
Cl^-	26.6 mM
PO_4^{3-}	95.0 mM
SO_4^{2-}	96.1 mM

Quadrupole Mass Spectrometry (ICP-QMS, ELAN DRCe II, PerkinElmer SCIEX, Waltham, MA) with detection of Pt at *m/z* 195 in standard mode. An external standard series of Pt in the concentration range of 1 μg L^{-1} to 400 μg L^{-1}, including a blank, was used to construct a linear regression equation with $R^2 > 0.9990$. The instrument limit of detection for Pt, calculated as three times the standard deviation of a blank solution containing 1% HNO_3, is 5 ng L^{-1}.

2.4. Speciation-analysis of CPC

Speciation of Pt was determined by Hydrophilic Interaction Liquid Ion Chromatography (HILIC) on a HPLC system (PerkinElmer 200 Series, Waltham, MA) coupled to Inductively Coupled Plasma Mass Spectrometry (ICP-MS) according to the method described by Hann et al. [22]. A volume of 5 μL was injected in a packed Discovery® HS F5 (Supelco Analytical, Sigma-Aldrich, MO) UPLC column of 150 × 2.1 mm dimensions and eluted at 200–250 μL min^{-1} by an eluent solution consisting of 10 mM ammonium acetate in 2% MeOH. ICP-MS detected the $^{195}Pt^+$ intensity at a dwell time of 100 ms. The chromatogram was recorded in Chromera software (PerkinElmer, Waltham, MA) and plotted in Excel 2016 (Microsoft, Redmond, WA). Calibration was done by linear, least-squares regression of the surface area with external standards ranging from 0.10 μM Pt to 5.13 μM Pt concentration. The limit of detection for each species was derived according the method of Vial and Jardy [24], represented in Equation (3) in which $\hat{\sigma}_{x/y}$ is the regression residual standard deviation and a the sensitivity (Equation (4)). The theoretical number of plates N of the chromatographic separation method was calculated from the retention time t_r and the full width at half maximum height $w_{1/2}$ by Equation (5).

$$LoD = 3 \times \frac{\hat{\sigma}_{x/y}}{a} \quad \text{Equation 3}$$

$$\hat{\sigma}_{x/y} = \sqrt{\frac{\sum_i (y_i - \hat{y}_i)^2}{n-2}} \quad \text{Equation 4}$$

$$N = 5.54 \times \left(\frac{t_r}{w_{1/2}}\right)^2 \quad \text{Equation 5}$$

3. Results and discussion

3.1. Speciation-analysis of CPC by HPLC hyphenation to ICP-MS

With regard to consecutive treatment processes, the element speciation in urine was studied in function of time. The analytical method for detecting the individual CPCs was verified on beforehand. A chromatogram of external standards of CPC can be found in Fig. S1. From Fig. 1 in which the relative response (peak area/concentration) is plotted as function of the respective concentration on a logarithmic scale, it was found that the relative response is constant in the entire concentration range. The data showed independence in the peak area distribution and a linear relation to all four analyte quantities applies. Consequently, the limit of detection was established for cisplatin (1.1 μM Pt), monoaquacisplatin (0.7 μM Pt), carboplatin (0.4 μM Pt) and oxaliplatin (0.2 μM Pt). The theoretical number of plates of the chromatographic separation N was calculated to 337 for cisplatin, 910 for monoaquacisplatin, 2,167 for carboplatin and 3,312 for oxaliplatin.

3.2. Metabolization of CPC and stability detected through excreted urine

Fig. 2 shows the changes of Pt speciation in the urine of patient 1 at four different times after administration of 78.5 mg Pt under the form of cisplatin. A large shift from cisplatin ($t_r = 2.94$ min) to monoaquacisplatin ($t_r = 2.55$ min) is observed. The time-dependent aquation of cisplatin has been reported earlier and is expressed by a pseudo-first order rate constant k_1 of $(1.97 \pm 0.07) \times 10^{-3}$ min^{-1} by analytical detection using HILIC coupled to ICP-MS [22], $(1.07 \pm 0.05) \times 10^{-3}$ min^{-1} using HPLC-ICP-MS with isotope dilution [25] and 1.43×10^{-3} min^{-1} using ^1H–^{15}N NMR [26]. Results for k_1 are overall comparable. The signal

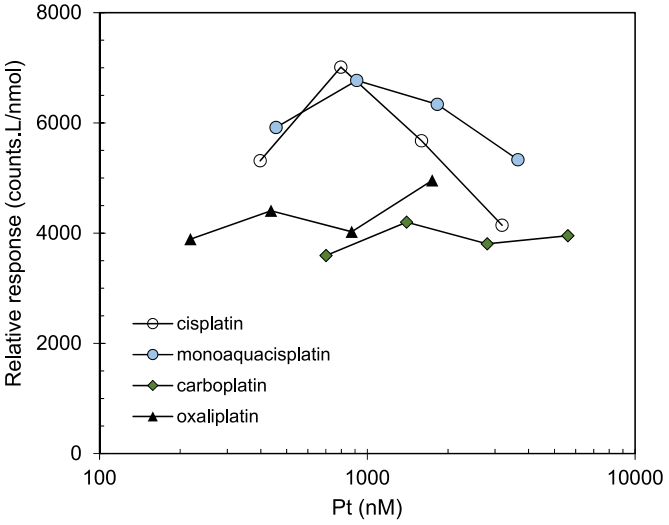

Fig. 1. Relative response (peak area/concentration) of Pt compounds as function of the concentration, confirming the independence of the peak area regardless the concentration level within the linear dynamic range.

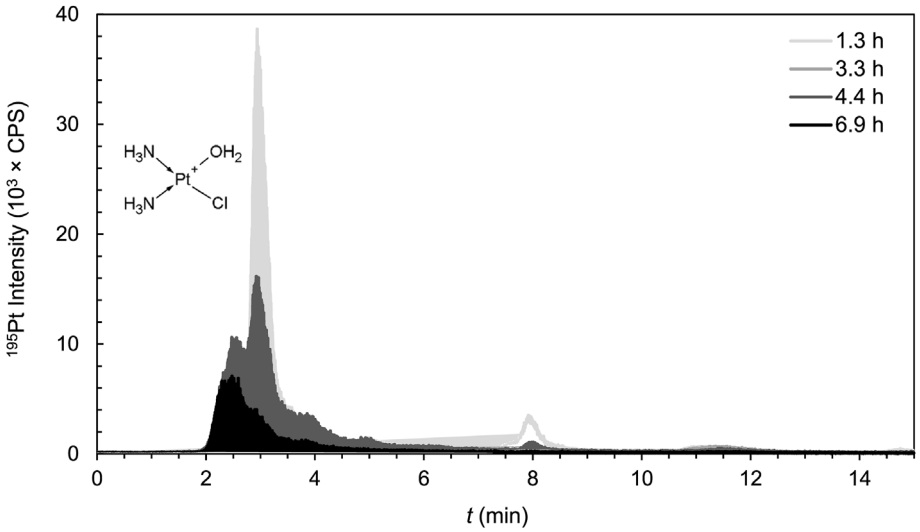

Fig. 2. Chromatogram of patient's urine collected at four different times following to the intravenous administration of cisplatin, showing the *in vivo* degradation of cisplatin to first monoaquacisplatin (structure shown).

attributed to cisplatin decreases until the original cisplatin peak is eventually fully resolved in the chromatogram baseline after 6.9 h. The aquation reaction takes place over the time span of renal clearance.

High chloride levels in the blood (98−106 mmol L^{-1}) [27] initially protect the parent cisplatin. The neutral character of the molecule allows for passive migration through cellular membranes. Only in the cytosol, where a lower chloride concentration prevails, exchange of chloride ligands with water molecules yields the active substance. Pt(II) subsequently forms both intra- and interstrand combinations of linked double-stranded DNA fragments through the sp^2-hybridised N^7 atoms of guanine or adenine. From the administered dose, a rapid clearing from the plasma occurs by renal elimination, while a small fraction of Pt is associated to plasma proteins in the blood [28].

In contrast, no substantial *in vivo* degradation was observed with carboplatin administered in 124.7 mg quantity to patient (Fig. 3). The peak at t_r 4.63 min shows the intact elution of carboplatin in the collected urine, even 11.0 h after chemotherapy. This observation indicates prime focus should be given towards carboplatin as a highly persistent antineoplastic agent in the aquatic environment.

Oxaliplatin, not tested in this study, was previously shown to degrade in chloride-containing media by substitution into the double chlorinated Pt compound, except when dextrose was kept at a 5 (*m/v*)% concentration [29].

3.3. *In vitro degradation rate of CPCs in urine*

Following excretion in toilets, the urine flow is typically diluted and mixed with other types of wastewater. The changes in aquatic composition can also cause further variations in the element speciation [30,31]. Chemical reactions involving CPC are the dissociation or exchange of ligands, association to (bio)molecules [32], oxidation to Pt(IV) and further agglomeration into colloids. To study these changes, the *in vitro* degradation of Pt compounds at 37 °C in urine is clarified by speciation analysis. Fig. 4 plots the fraction of the original CPC remaining unmodified in the urine as a function of time, up to 69 h. The data were successfully fitted to kinetic models (Table 3) with all parameters being significant

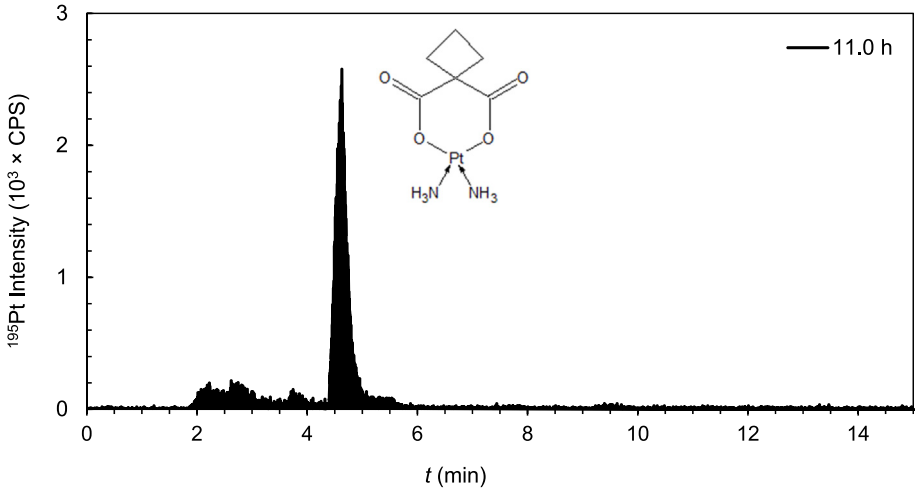

Fig. 3. Chromatogram obtained of urine from patient collected after 11.0 h following to the intravenous administration of carboplatin, showing the *in vivo* stability of the drug. The sole peak at 4.63 min corresponds to carboplatin.

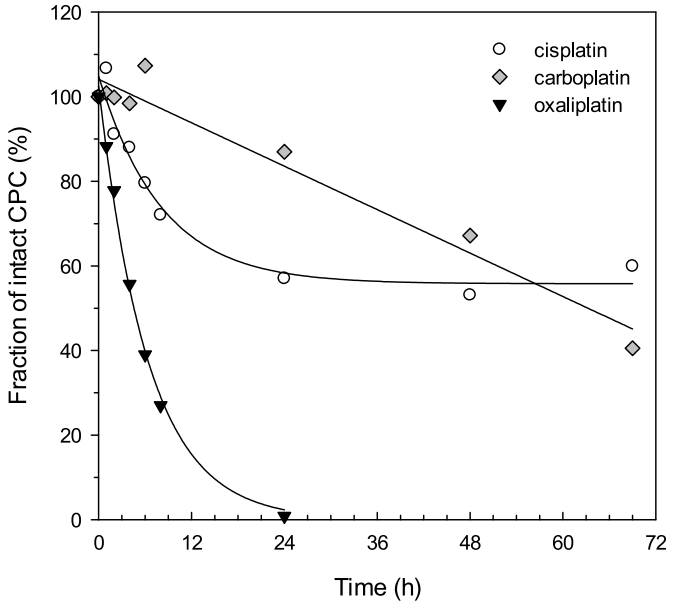

Fig. 4. Decrease of CPC in function of time monitored by HILIC-ICP-MS, showing the *in vitro* degradation at 37 °C in synthetic human urine. The resulting model parameters of each compound are shown in Table 3.

Table 3
Output parameters ($p < 0.01$) from the regressions shown in Fig. 4, mathematically describing the *in vitro* degradation of CPC in synthetic human urine at 37 °C in function of time (min).

Compound	Model	Kinetic equation	R^2	F
Carboplatin	Zero order	$[Pt] = 104.12 - 0.0143t$	0.958	135.1
Oxaliplatin	First order	$[Pt] = 102.51\ exp(-0.0026t)$	0.997	1540.4
Cisplatin	Second order	$\frac{1}{[Pt]} = \frac{1}{100} + 5.59 \times 10^{-6} t$	0.935	71.6

($p < 0.01$).

Three most prevailing platinum antineoplastics disintegrate following unique reaction orders, namely zero-, first- and second-order. It was derived that the decay of oxaliplatin is fastest ($k_1 = 0.0026 \pm 0.0001$ min^{-1}), followed by carboplatin ($k = 0.0143 \pm 0.0012$ min^{-1}) and cisplatin ($k_2 = 5.59 \pm 0.46 \times 10^{-6}$ min^{-1}). Carboplatin only started to show degradation after 24 h and followed a linear trend further along.

Extrapolation of the stability data of pristine CPC as a way to estimate ecotoxicity risks is hampered by the rendering of many active by-products that may exhibit even higher cytotoxic properties [29]. Nevertheless, the derived degradation rates can serve as kinetic input parameters to expand modelling databases and improve the predictive power of speciation software that are currently lacking the ability to include complex molecules, such as CPC, due to missing experimental data. Overall, the slow degradation confirms high stability of platinated antineoplastics in natural waters [33].

3.4. Comparison of platinum oncolytic stability and implications for waste water treatment

The larger stability of carboplatin compared to cisplatin, observed in both *in vivo* and *in vitro* experiments, can be related to their molecular structure. All three CPC are platinate complexes of a square planar tetra structure that differ only in chelating moieties [34]. Aside from the amino (-NH_2) groups, Pt(II) shares covalent bonds with two chloride (-Cl) atoms in cisplatin and two carboxyl (-COOH) groups in both oxaliplatin and carboplatin. These latter two exhibit a higher stability due to the higher bond enthalpy with oxygen versus chloride (Table 4). Though, the solution's ionic strength and temperature play a crucial role in the complex stability. The water chemistry and composition of the liquid stream can exert a large shift in the equilibrium. For instance, high electrolyte levels, *e.g.* in sewage systems, can favor the chemical equilibrium and protect Pt complexes from nucleophilic hydrolysis reactions through S_N1 ligand exchange [35].

Screening tests have shown that cisplatin is not bio-degradable [36] and while some microorganisms have evolved to tolerate heavy metal complexes, a distinct reduction in bacterial growth has been observed for hospital waste waters by means of OD_{600} measurement. The genotoxic potential of CPC has been further proven by the Ames test, showing ability of genetic alterations over time [37]. Next to direct ecotoxic effects, these drugs pose a (small) risk for humans upon bio-magnification through consumption of contaminated seafood [8]. Although doses of anticancer drugs which enter the aquatic environment are much lower than the therapeutic prescriptions in cancer treatment, they still form a danger at these (ultra)low levels.

Current WWTP have difficulties in eliminating CPC [38] and the use advanced treatment processes is recommended. Biological treatment using axenic cultures of *Shewanella oneidensis* and *Cupriavidus metallidurans* supplied with H_2 gas as electron donor could partially remove CPC at lab-scale [39]. In ecosystems, most degradation of metal-complexes is ascribed to photo-degradation [20]. Certain studies explored the use of UV or O_3 for enhanced oxidation of cisplatin [17]. A broader set of tertiary treatment technologies, besides ozonation, that additionally offer potential for Pt recovery is favored. Electro- and physico-chemical methods, consisting of electrowinning, membrane filtration [40] or (bio-)sorption [41], can notably concentrate Pt and are preferred to this extent. Whilst most attention in publications is dedicated to cisplatin, we propose the focus on carboplatin as the priority amongst CPC remediation in contaminated water bodies based on its high stability and absolute Pt quantities emitted. On average, dosing of carboplatin is about 4 times higher compared to cisplatin [4].

4. Conclusions

The analytical determination of Pt oncolytics in the saline matrix was successful using HPLC coupled to ICP-MS. It was demonstrated that the *in vivo* metabolization of carboplatin after 11 h is limited as the urine of a patient treated with 125 mg renders a single peak for intact compound. Cisplatin, meanwhile, undergoes a complete aquation after nearly 7 h, ascribed to the influence of lower chloride concentrations.

Following the urinary excretion, oxaliplatin and cisplatin exhibit a relatively fast degradation of the original compound into several by-products, while carboplatin remained more stable in urine. As a result, the chemical *in vitro* stability was highest for carboplatin ($k = 0.0143 \pm 0.0012$ min^{-1}), while oxaliplatin and cisplatin rapidly degraded following an exponential decay with k_1 of 0.0026 ± 0.0001 min^{-1} and $k_2 = 5.59 \pm 0.46 \times 10^{-6}$ min^{-1}, respectively.

Considering the strong cytotoxicity of residual platinum antineoplastics following renal clearance and human excretion, the contaminants are of environmental concern. We propose the remediation of CPC to reduce genotoxic effects, even when low concentrated due to severe dilution. Tertiary treatment technologies, allowing also for recovery of the precious metal, should focus on removing carboplatin, due to its highest stability among the platinum-based antineoplastics that were tested.

Table 4
Chemical properties of bonds in the complexes of platinum oncolytics.

Bond	Difference in electronegativity (Pauling)	Bond length (Å)	Bond enthalpy (kJ mol^{-1})
Pt − O	1.16	2.65	347
Pt − Cl	0.88	2.33	269
Pt − N	0.76	2.03	156

Declaration of competing interest

Upon this statement, authors declare to have submitted a manuscript entitled *"Chemical degradation of platinum oncolytics in urine and speciation of the inorganic contaminants cisplatin and carboplatin in waste water"* that is **free of conflict of interest**.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.emcon.2023.100262.

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