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An investigation on possible oligomer formation in pharmaceutical formulations of cisplatin

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Summary

Concern has been raised that the anti-neoplastic agent, cisplatin, when formulated in isotonic sodium chloride solution may degrade to di-μ-hydroxo-bis(*cis*-diammineplatinum(II)) (**4**) and tri-μ-hydroxo-tris(*cis*-diammineplatinum(II)) (**5**) which have been shown to be therapeutically inactive and highly toxic. The formation of **4** and **5** in pharmaceutical preparations of **1** is therefore of concern and was the subject of this study. Using HPLC procedures developed for the monitoring of *cis*-diamminemonochloromonoaquoplatinum(II)) (**2**), *cis*-diamminediaquoplatinum(II) (**3**) and oligomeric species **4** and **5**, the long-term (1 year) stability of cisplatin (1 g/l) was investigated in 0.9% sodium chloride solution. Oligomeric products **4** and **5** were not found at the detection limits of 1 mg/l. The degradation of **2**, **3**, **4**, and **5** in aqueous sodium chloride solutions were also studied; all of these species degraded to cisplatin. Results stongly suggest that **4** and **5** are not formed in formulations in which cisplatin is dissolved in isotonic sodium chloride solution.

Introduction

Cis-dichlorodiammineplatinum(II) (cisplatin **1**, Fig. 1) is an antineoplastic agent that has been established as first line therapy in the treatment of a number of solid tumors that have proven to be refractory to other therapeutic modalites. However, the compound is reactive toward nucleophiles, undergoing hydrolysis to form aquated products (**2** and **3**) (Reishus and Martin, 1961; Greene et al., 1979; Erhart, 1978; Hincal et al.,

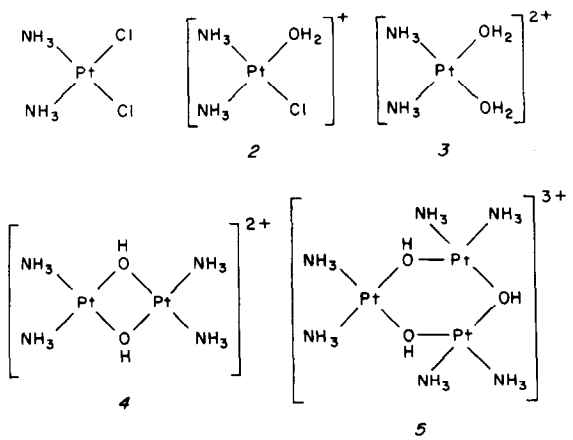

Fig. 1. Structures of **1**, **2**, **3**, **4** and **5**.

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1979). Preparations of cisplatin are stabilized by dissolution in isotonic sodium chloride solutions and under those conditions suffer only 3% loss through aquation in 24 h at room temperature (Erhart, 1978; Greene et al., 1979; Hincal et al., 1979). Formation of dihydroxo bridged dimer from diaquoplatinum(II) compounds (**2** and **3**) was postulated by Lim and Martin (1976); later Faggiani and co-workers isolated **4** and **5** from aqueous (salt-free) solutions of **2** and **3** (Faggiani et al., 1977a and b). The presence of such species in cisplatin formulations or in i.v. ad-mixture solution represents a potential liability to the patient, since these oligomers have been reported to be much more toxic than the parent drug (Aggarwal et al., 1980; Broomhead et al., 1980; Rosenberg, 1978) and displayed no anti-tumor activity at non-lethal doses (Rosenberg, 1978). To determine the proclivity of cisplatin to degrade to toxic oligomeric species (**4** and **5**), the long-term stability of cisplatin solutions as well as the stability and kinetic behavior of the aquated species (**2** and **3**) and oligomers **4** and **5** have been studied in isotonic sodium chloride solutions.

Materials and Methods

Instrumentation

The HPLC unit consisted of an Altex Pump Model 110A, and Omniscribe Recorder, an LDC UV III Detector (214 nm) except for the detection of **1** for which a Waters 440 Detector (280 nm) was used. Samples were applied to the system with an Altex 210 Injector fitted with a 20 μl loop. An 80 mm long column (4.6 mm internal diameter) packed with 5 μm ODS Hypersil was used for measuring **2, 3, 4** and **5**; for measurements of **1**, a 100 mm column with the same packing material loaded with hexadecyltrimethylammonium bromide as described by Riley et al. (1981) was used. A pH-stat (Metrohm) was configured with a 655 Dosimat, a 614 Impulsomat, a Model 632 pH meter and a titration vial that was thermostated at 30.0 °C with a Haake D2 constant temperature circulator. NMR spectra were recorded at 64.5 MHz with a Varian XL 300 spectrometer using a 10 mm probe (Hollins and Lippard, 1983). FT-IR spectra of **4** and **5** were recorded from 4000 to 400 cm^{-1} with an IBM Model 32 IR-Spectrophotometer.

Materials

Cisplatin (100% pure) was obtained from Eli Lilly Laboratories. Oligomers **4** and **5** were synthesized and recrystallized according to published procedures (Faggiani et al., 1977a, 1978) as their nitrate and sulfate salts, respectively, using the modifications described by Boreham et al. (1981). Their structural assignments were supported by X-ray crystallography; the structure of **4** was further supported by comparing its ^{195}Pt-NMR spectra with that reported in the literature (Hollins and Lippard, 1983). The nitrate salt of **4** and the hexahydro sulfate salt of **5** both showed the same unit crystal cell dimensions and same IR spectra as have been reported by Faggiani et al. (1977a, 1978). The IR spectra, consistent with structure **4**, were characterized by bands at 1044 cm^{-1} (Pt–OH bending) and 548, 525 and 498 cm^{-1} ($N_2PtO_2PtN_2$ skeletal stretching vibrations; Faggiani et al., 1977a). Identification of **5**, was supported by characteristic spectral bands at 1119, 520 and 467 cm^{-1}, as previously reported (Faggiani et al., 1978).

Monomers **2** and **3** were prepared by adding one or two equivalents of silver nitrate, respectively, to an acidic suspension of **1** (Boreham et al., 1981). The resulting solutions were filtered to remove the silver chloride precipitate and used as such in subsequent studies. Reagents were obtained from the following suppliers and used as received: citric acid monohydrate (Baker), sodium citrate dihydrate (tribasic) (Baker), sodium phosphate (monobasic) (MCB), phosphoric acid (Baker); nitric acid (Mallinckrodt), sodium chloride (Fisher), silver nitrate (Sigma), silver sulfate (Aldrich), 1-hexanesulphonic acid sodium salt (Eastman Kodak) and hexadecyltrimethyl-ammonium bromide (HTAB) (Aldrich). The phosphate buffer used in the mobile phases for elution of compounds **2, 3, 4** and **5** was prepared by mixing a 40 mM solution of sodium phosphate (monobasic) with a 40 mM solution of phosphoric acid to adjust the pH to 2.65. The citrate buffer used in the mobile phase for cisplatin elution was

TABLE 1

Mobile phase, flow rates and retention volumes

Compound	Mobile phases composition	Flow (ml/min)		V_R^a (ml)
		Kinetics study	Stability study	
1	0.1 mM HTAB in 10 mM citrate buffer pH 5.25	1.0	1.0	3.4
2	10 mM 1-hexanesulphonate in 40 mM phosphate buffer pH 2.65	0.6	–	1.8
3	6 mM 1-hexanesulphonate in 40 mM phosphate buffer pH 2.65: methanol (92 : 8)	2.0	–	4.6
4	6 mM 1-hexanesulphonate in 40 mM phosphate buffer pH 2.65: methanol (92 : 8)	1.0	2.0	4.6
5	1.5 mM 1-hexanesulphonate in 40 mM phosphate buffer pH 2.65: methanol (92 : 8)	1.0	1.2	2.2

[a] V_R = retention volume.

prepared by mixing 10 mM citric acid solution and 10 mM sodium citrate (tribasic) solution to adjust the pH to 5.25. The HPLC grade methanol was from Fisher. Deionized and distilled water was used to prepare all aqueous solutions. The mobile phases and flow rates used to elute individual components are given in Table 1. All chromatograms were run at ambient temperature. Component concentration was determined from peak height measurements. The linearity of peak height as a function of concentration was evaluated for **1, 2, 3, 4** and **5** over the concentration range indicated in Table 2 (for these calculations the intercepts were forced through zero). A linear relationship exists between response and concentration for all analytes.

TABLE 2

Standard curves [a] for **1, 2, 3, 4** and **5**

Compound	Slopes(S)	Range (mg/l)
1	0.60	4–1200
2	1.03	1– 100
3	0.99	1– 100
4	2.53	1– 100
5	2.26	1– 100

[a] $y = S \times C$ where y is peak height in mm, S is the slope (mm·l/mg) and C is concentration in mg/l. The correlation coefficient for the least-squares fit was above 0.999 for all 5 curves.

Degradation profile of aquated and oligomeric species

The degradation of **2, 3, 4** and **5** and the formation of cisplatin from these materials was followed in solutions in which chloride concentration was varied. All sample solutions were ca. 0.2 mM or lower in substrate concentration to ensure that at least a 20-fold excess of sodium chloride was maintained. All kinetic studies were performed at 30.0 °C. The pH was controlled with the pH-stat by addition of 0.01–0.50 M HNO_3 (concentration depending on the pH to be maintained). Samples were prepared by taking a fresh solution of the respective platinum compound and adding sufficient 4.0 M sodium chloride solution and water to give the targeted chloride concentration in a final volume of 10.0 ml. The kinetic experiment was initiated with the addition of chloride solution. All kinetic experiments were followed (by HPLC) for more than one half-life. The observed rate constants were evaluated from the slope of ln(peak height) versus time. In the kinetic experiments in which the degradation of **5** was followed, all samples were diluted 4-fold before injection onto the HPLC system to avoid the effects of high sodium chloride concentration on the chromatographic performance.

*Propensity for conversion of cisplatin to oligomeric species **4** and **5***

Eight samples of cisplatin (1.00 g/l) were prepared in which drug was dissolved in a solution

containing sodium chloride 9 g/l (0.154 M) and mannitol (10 g/l). Samples were packaged in glass vials; four were stored for 10 months at 40 °C and four at 5 °C. All samples were then refrigerated (5 °C) for an additional 12 months and subsequently analyzed for loss of cisplatin and appearance of oligomeric degradates (**4** and **5**).

Results and Discussion

Chromatography

The chromatographic conditions for monitoring **1** have been described by Riley et al. (1981), but methods for measuring **2, 3, 4** and **5** by HPLC have not been published previously. The mobile phases used for the elution of **2–5** are given in Table 1. All 4 species were maintained in the ionized conditions during elution by use of an acidic phosphate buffer (40 mM pH 2.65) as the mobile phase. Ion pairing agent was added to facilitate retention for the hydrolysis products on a C_{18}-reverse-phase column. Attempts to use a single chromatographic system to resolve and detect all 4 compounds were unsuccessful; 3 different mobile phases were required to monitor the species of interest. The performance of the HPLC separation was highly sensitive to slight variations in the composition of the mobile phase. Small changes in pH or in the ratio of buffer-to-ion pairing agent led to substantial changes in retention time and caused band broadening. A relatively high concentration of ion pairing agent and salt in the mobile phase was consistently used since it minimized sensitivity of the chromatographic system to the electrolyte content of the sample matrix.

Chromatograms obtained from fresh solutions of **2, 3, 4** and **5** in 0.150 M NaCl are shown in Fig. 2. Their elution volumes are given in Table 1. It should be noted that **3** and **4** could not be chromatographically resolved. Their coelution is not entirely unexpected since both species are divalent and they may therefore be expected to interact similarly with the ion pairing agent and stationary phase. All other compounds of interest were resolvable under the chromatographic conditions given in Table 1.

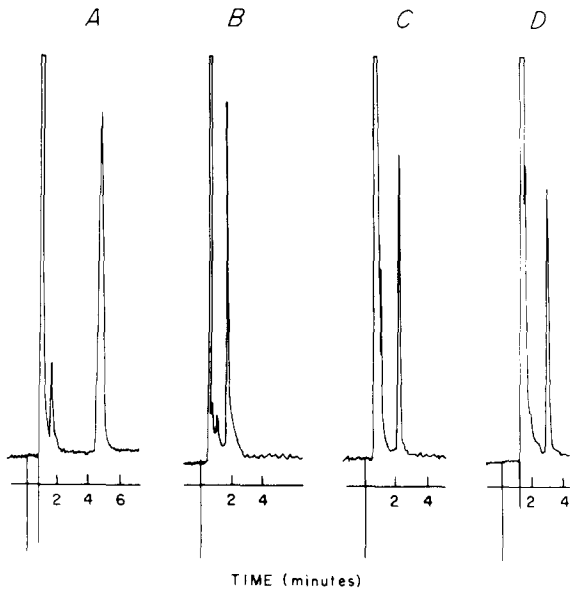

Fig. 2. Chromatograms for platinum(II) complexes. Mobile phases according to Table 1, flow as given for kinetic studies except for **5** where a flow rate of 1.2 ml/min was used. Chromatogram labelled A shows elution of **4** at 50 mg/l; B = **5** at 130 mg/l; C = **3** at ca. 100 mg/l; D = **2** at ca. 100 mg/l.

*Reactivity of cisplatin and oligomeric species **4** and **5***

The degradation of oligomeric species **4** and **5** was studied as a function of pH in solutions of varying sodium chloride concentrations. In these solutions, **4** and **5** degraded to cisplatin (in some cases, kinetic analysis involved monitoring formation of cisplatin in addition to measuring loss of **4** or **5**). The k_{obs} for disappearance of **4** ($k_{obs,D}$) and **5** ($k_{obs,T}$) are described as a function of pH in Fig. 3. The rate of degradation of the oligomeric species proceeds more rapidly at low pH; **4** and **5** are more than an order of magnitude more stable at neutral pH. Figs. 4 and 5 show the dependence of $k_{obs,D}$ and $k_{obs,T}$ on chloride concentration at different pHs (k_{obs} values were determined in duplicate with an RSD of $\pm 5\%$). Increasing concentrations of chloride in solution accelerated the degradation of **4** and **5**, resulting in their conversion to cisplatin.

In addition to monitoring oligomer loss, the formation of cisplatin from **4** and **5** was studied in 0.150 M sodium chloride solutions and the results

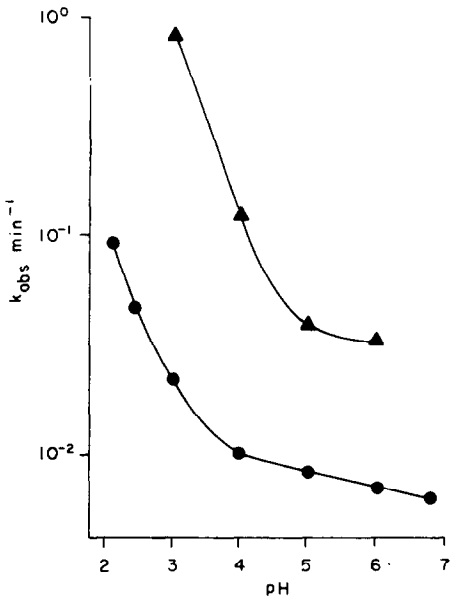

Fig. 3. Rate constants for degradation **4** and **5** as function of pH in 0.15 M NaCl solution. $T = 30.0\,^{\circ}$C, ●, $k_{obs,D}$; ▲, $k_{obs,T}$.

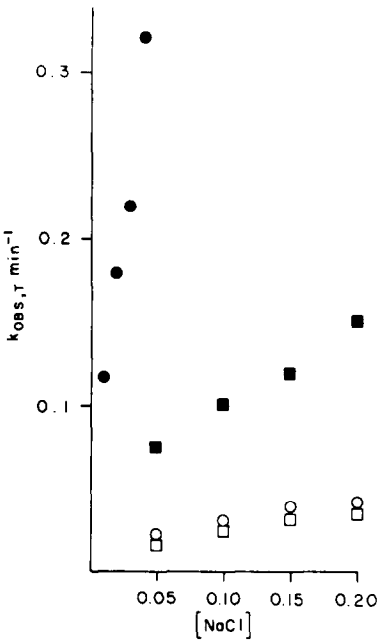

Fig. 5. $k_{obs,T}$ measured as a function of different chloride ion concentrations, [Cl^-] in M. ●, pH 3.00; ■, pH 4.00; ○, pH 5.00; □, pH 6.00.

are summarized in Figs. 6 and 7. In all cases, loss of **4** and **5** could be accounted for in terms of cisplatin formed. Since **4** and **5** are quantitatively converted to cisplatin under these conditions, these reactions can be treated as being irreversible. Additionally, cisplatin has been shown to form oligomeric species **4** and **5** when reagents are added that facilitate precipitation of chloride ions

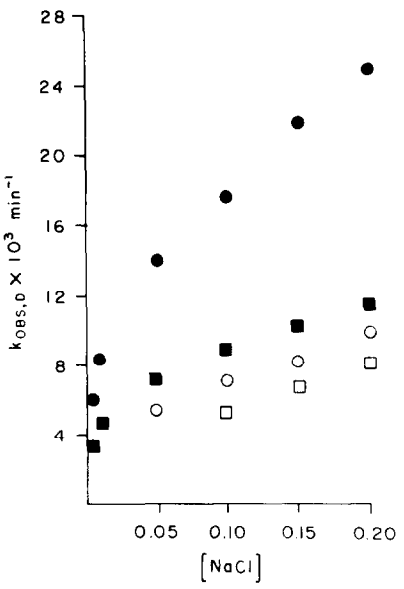

Fig. 4. $k_{obs,D}$ measured as a function of different chloride ion concentrations, [Cl^-] in M. ●, pH 3.00; ■, pH 4.00; ○, pH 5.00; □, pH 6.00.

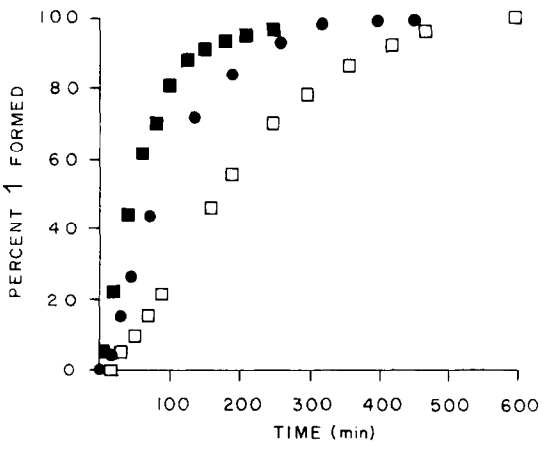

Fig. 6. Rate of formation of **1** from **4** determined in 0.150 M NaCl solution measured as a percentage of theoretical yield. $T = 30.0\,^{\circ}$C ■, pH 3.00; ●, pH 4.00; □, pH 5.00.

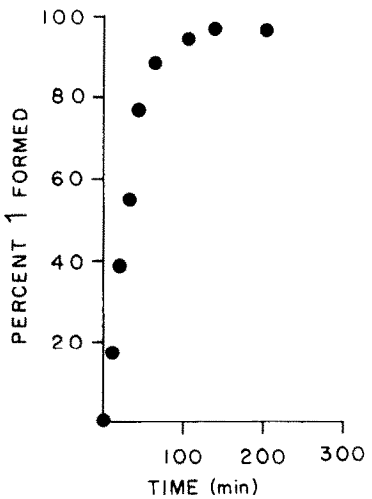

Fig. 7. Formation of **1** from **5** determined in 0.150 M NaCl solution measured in terms of percentage of theoretical yield. T = 30.0 °C.

from the solution (Faggiani et al., 1977a, 1978) thus establishing the interconvertability of **1**, **4** and **5** (Fig. 1).

Degradation of **4** (measured as $k_{obs,D}$) was determined using an analytical method in which the diaquated species, **3**, and the dimeric species, **4**, coelute. However, since the rate of degradation of **3** is much faster than the degradation of **4** in sodium chloride solutions (500 times faster at pH 4.0 and $[Cl^-] = 0.15$ M) **3** never accumulates sufficiently to interfere with the determination of $k_{obs,D}$. The first-order kinetic behavior observed when the disappearance of **4** was followed in sodium chloride solutions further supports the hypothesis that significant concentrations of **3** are not generated in these systems. Degradation of **4** was followed at three different pHs: 3.0, 4.0 and 5.0 in solutions containing 0.150 M NaCl. In these solutions, **4** was converted to cisplatin with an average yield of 98% (Fig. 6). When similar studies were carried out using solutions of **5** at pH 4.0, cisplatin was formed in 96% yield (Fig. 7). The difference in the yield of cisplatin obtained from **4** and **5** is not statistically significant. The less than quantitative (97%) recovery of cisplatin can be accounted for in terms of aquated species in equilibrium with **1** in the reaction medium. The equilibrium between cisplatin and its aquated analogs is described by Equations 1 and 2:

$$\underset{\mathbf{3}}{(NH_3)_2Pt(OH_2)_2^{2+}} \overset{k_{1_{Cl^-}}}{\underset{k_{-1}}{\rightleftharpoons}} \underset{\mathbf{2}}{(NH)_2PtClOH_2^+} + H_2O \quad (1)$$

$$\underset{\mathbf{2}}{(NH_3)_2PtClOH_2^{2+}} \overset{k_{2_{Cl^-}}}{\underset{k_{-2}}{\rightleftharpoons}} \underset{\mathbf{1}}{(NH_3)_2PtCl_2} + H_2O \quad (2)$$

Literature values for the equilibrium constant for reaction 2, K_2 (where $K_2 = k_{-2}/k_2$), have been reported to be ca. 3.7×10^{-3} M (Reishus and Martin, 1961; Green et al., 1979; Perumareddi and Adamson, 1968; Lee and Martin, 1976). The equilibrium constant for reaction 1, K_1 (where $K_1 = k_{-1}/k_1$) has been reported to be 4×10^{-4} M and 2×10^{-4} M at 25 °C and 35 °C, respectively, by Reishus and Martin (1961) and 1.11×10^{-4} M and 1.88×10^{-3} M by Lee and Martin (1976) at the same temperatures. Assuming that K_1 is smaller than 2×10^{-3} M at 30 °C, a solution of cisplatin in 0.150 M NaCl contains only negliglible concentrations of **3**; the monoaquated species accounts for approximately 3% of the total concentration of platinum compounds and cisplatin represents the remaining 97%.

Degradation of the aquated species, **2** and **3**, show a first-order dependency on chloride concentration, and in saline solution reactions of **2** and **3** can be treated as being irreversible (i.e., $k_1[Cl] \gg k_{-1} + k_2\,[Cl^-] \gg k_{-2}$). k_1 and k_2 were calculated from plots of k_{obs} vs Cl^- concentration to be 25.1 $M^{-1} \cdot min^{-1}$ and 0.62 $M^{-1} \cdot min^{-1}$, respectively. These values were in general agreement with those reported previously (Perumareddi and Adamson, 1968; Greene et al., 1979; Reishus and Martin, 1961).

Stability of cisplatin in chloride-containing formulations

The high reactivity of cisplatin towards nucleophiles (Riley et al., 1982; Garren and Repta, 1985) gives cause for concern as to its stability in solution formulations. Cisplatin is pro-

tected from degradation by the high chloride concentration in saline solutions but if other nucleophiles exist in the solution, rapid degradation can occur (Garren and Repta, 1985; Long et al., 1980).

Solutions of cisplatin (1 mg/ml) were stored at either 5 °C or 40 °C for 10 months and then maintained in a refrigerator for an additional 1 year. Samples stored at 40 °C showed no physical change, but those stored at 5 °C did show a precipitate which could be redissolved by sonicating the vials at 40 °C for ca. 20–30 min. All samples were analyzed by HPLC and extent of loss of cisplatin and formation of **4** and **5** were monitored. None of the solutions had detectable concentrations of **4** or **5**. This observation is consistent with the kinetic studies in which it was shown that **4** and **5** were converted to cisplatin and the mono-aquated species (**2**) in 0.150 M NaCl solutions at pH 4.0. At the end of the storage period, the concentration of cisplatin remaining in the vials stored at 5 °C was 0.96 g/l, ± 0.04 (ca. 4% loss), while those stored at 40 °C had lost an average of 15% of initial concentration of drug (to 0.85 ± 0.19 g/l). The 4% degradation that was observed in solutions kept at 5 °C can be accounted for by the equilibrium of cisplatin with **2**. The additional degradation noted in vials stored at 40 °C for 10 months cannot be explained by the equilibrium between cisplatin and aquated species. Since oligomeric species, **4** and **5**, are not formed in such solutions, it would appear that products other than those previously considered are formed.

Conclusions

HPLC assays were developed to monitor cisplatin, its aquated products (**2** and **3**) and oligomeric species, **4** and **5**, which were used to assess the interconvertability of these species in aqueous sodium chloride solutions used as prototypical formulations. These methods afforded detection limits of 1 mg/l for both **4** and **5**. Oligomeric species, **4** and **5**, were not found in aged solutions of cisplatin but degradation of cisplatin stored at 40 °C was observed to proceed through an unidentified route. Under refrigeration conditions, saline solutions of cisplatin were stable for over 1 year. The oligomeric products, **4** and **5**, of concern because of their potential toxicity liability, were found to be unstable in NaCl solution, degrading rapidly to cisplatin. In all sodium chloride solutions of cisplatin, **4** and **5** were completely absent from the solution; in NaCl solutions of **4** and **5**, these species were quantitatively converted to cisplatin within 7 h at 30 °C.

The above results show that in sodium chloride solutions, **1** is not transformed into oligomeric species **4** and **5** at the minimum detectable levels of 1 mg/l. This would represent a maximum conversion of **1** to **4** or **5** of $< 0.1\%$ and suggest that these species do not present a toxicity risk in clinically relevant formulations.

Acknowledgements

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