ELSEVIER

International Journal of Pharmaceutics 146 (1997) 263–269

international journal of pharmaceutics

Notes

Degradation pathway of carboplatin in aqueous solution

Montserrat Pujol*, Victoria Girona, Josefina Prat, Montserrat Muñoz, Jordi De Bolós

Unitat de Físico-Química, Facultad de Farmacia, Universitat de Barcelona, Avgda Joan XXIII s/n, 08028 Barcelona, Spain

Received 19 August 1996; accepted 25 October 1996

Abstract

A degradation pathway for carboplatin in aqueous solution is described. Degraded solutions of carboplatin in water and in 5% glucose solution were analysed by high performance liquid chromatography; carboplatin and its degradation products were well separated. Three degradation products of carboplatin have been determined either in pure water and 5% glucose solution and they have been identified as 1,1-cyclobutanedicarboxilate anion, its protonated forms and *cis*-diamminediaquoplatinum (II) complex. © 1997 Elsevier Science B.V.

Keywords: Carboplatin; Cisplatin; Cytostatic platinum complexes; HPLC

Carboplatin (*cis*-diammine 1,1-cyclobutanedicarboxylato platinum II), an analogue of cisplatin (*cis*-diamminedichloroplatinum II) is a drug with antitumor properties similar to those of cisplatin (Van Echo et al., 1984). Carboplatin, unlike cisplatin, causes no renal or neurological toxicity and is used mainly in hospitals for parenteral administration by intravenous infusion (Cheung et al., 1987).

The cisplatin degradation pathway is well described in the literature (Lee and Martin, 1986; Pujol et al., 1991); an aquation process occurs to yield mono and diaquo complexes either under illumination or dark conditions. Several approaches to the degradation pathway of platinum derivatives have been made and the general degradation pathway for platinum complexes can be found in the literature (Banerjea et al., 1957; Lee and Martin, 1986). The degradation products of carboplatin protected from light have been identified and reported in a previous work (Prat et al., 1994) but not under illumination conditions.

The purpose of this report is to study the degradation kinetics of carboplatin under illumi-

* Corresponding author.

PII S0378-5173(96)04788-6

nation conditions, to identify the degradation products by light and to postulate a degradation pathway for carboplatin in aqueous solution at differents temperatures in the presence and absence of light to check with the general one. The study was done in pure water and also in 5% glucose infusion solution because this is the most utilised infusion solution for carboplatin administration in hospitals.

Carboplatin bulk powder and cisplatin bulk powder were a gift from Bristol Myers Laboratories (Barcelona, Spain) and Wasserman Laboratories (Barcelona, Spain), respectively. HPLC grade methanol was supplied by Tecknokroma (San Cugat del Vallès, Spain). 1,1-Cyclobutanedicarboxilic acid was obtained from ICN Chemicals (Corbera de llobregat, Spain). Carboplatin was prepared in pure water and in 5% glucose solution in glass bottles (Grifols Laboratoires, Barcelona, Spain). Aqueous solutions containing *cis*-$[Pt(NH_3)_2ClH_2O]^+$ and *cis*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ were prepared by incubating cisplatin (Riley et al., 1981). Double-distilled water was used after filtration in a Milli-Q system (Millipore, France) and a second vacuum filtration in a helium atmosphere.

All assays were performed by the HPLC method described by Prat et al., 1994, which has been demonstrated to be suitable for degradation stability studies by heat of carboplatin solutions. In order to know if the HPLC method used is aceptable for carboplatin determinations in degradation studies by light, carboplatin solutions in 5% glucose infusion solution and in pure water—to produce a final concentration of 3.2 $mg \cdot ml^{-1}$—in glass bottles were prepared. This concentration was chosen from hospital protocols. Three series of eight 10-ml sample solutions (Initial concentration of carboplatin = 3.2 mg ml^{-1}) were exposed to light intensity of 6.0×10^{-6} Einstein min^{-1} at 25°C either in pure water and 5% glucose solution. Each sample was exposed for different times and then stored at 4°C until analysis. A 250 xenon lamp of Applied Photophysics was used as light source and the light intensity was measured by use of a uranil oxalater actinometer solution. All samples were assayed in triplicate for the percentage of intact carboplatin remaining and for the visualytation of the degradation products.

The retention time for carboplatin was found to be 3.9 ± 5% min (Fig. 1a) The relative standard deviation for replicate injections was less than 1.0% and the assay results were linear for carboplatin in the concentration range tested 0.2–4.0 mg ml^{-1}.

Carboplatin was stable in 5% glucose infusion solution and in pure water for 1 month, at least under refrigeration conditions. This allowed storage of the samples at 4°C until analysis.

Either under illuminated or dark conditions, the concentration of carboplatin decreases with time, following a first order kinetics. In Table 1 the rate parameters for carboplatin degradation in 5% glucose intravenous solution and in pure water under different experimental conditions, are summarised. The presence of light accelerates the descompositon of drug in pure water and in 5% glucose solution too.

The retention time for cisplatin obtained from a solution of cisplatin in pure water (1.5 mg ml^{-1}) was 3.2 ± 5% min (Fig. 1b) and the retention time for cyclobutanedicarboxilic acid (1 mg ml^{-1}) in pure water (pH = 2.7) was 1.9 min ± 5% (Fig. 1f).

In Fig. 1d and e, chromatograms obtained from more tan 40% degraded solutions of carboplatin and cisplatin are shown, respectively. Three degradation products of carboplatin (P1, P2 and P3) appeared either under temperature or light

Fig. 1. Chromatograms corresponding to (a) carboplatin (3.2 mg ml^{-1}) in aqueous solution (pH = 6.4) (b) cisplatin (1.5 mg ml^{-1}) in aqueous solution (pH = 6.7) (c) carboplatin (1.6 mg ml^{-1}) and cisplatin (0.75 mg ml^{-1}) aqueous solution (d) solution sample of carboplatin (3.2 mg ml^{-1} initial concentration) in aqueous solution when about 50% degradation was reached (pH = 7.1) (peaks: P1 = protonated forms of 1,1-cyclobutanedicarboxilate anion, P2 = *cis*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ complexe, P3 = 1,1-cyclobutanedicarboxilate anion and CB = intact carboplatin) (e) solution sample of cisplatin (initial concentration of 1.5 mg ml^{-1}) in aqueous solution when about 50% degradation was reached (pH = 6.9) (peaks: P2 = mixture of *cis*-$[Pt(NH_3)_2ClH_2O]^+$ and *cis*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ complexes and C = intact cisplatin) and (f) 1,1-cyclobutanedicarboxilic acid (1 mg ml^{-1}) in aqueous solution (pH = 2.8).

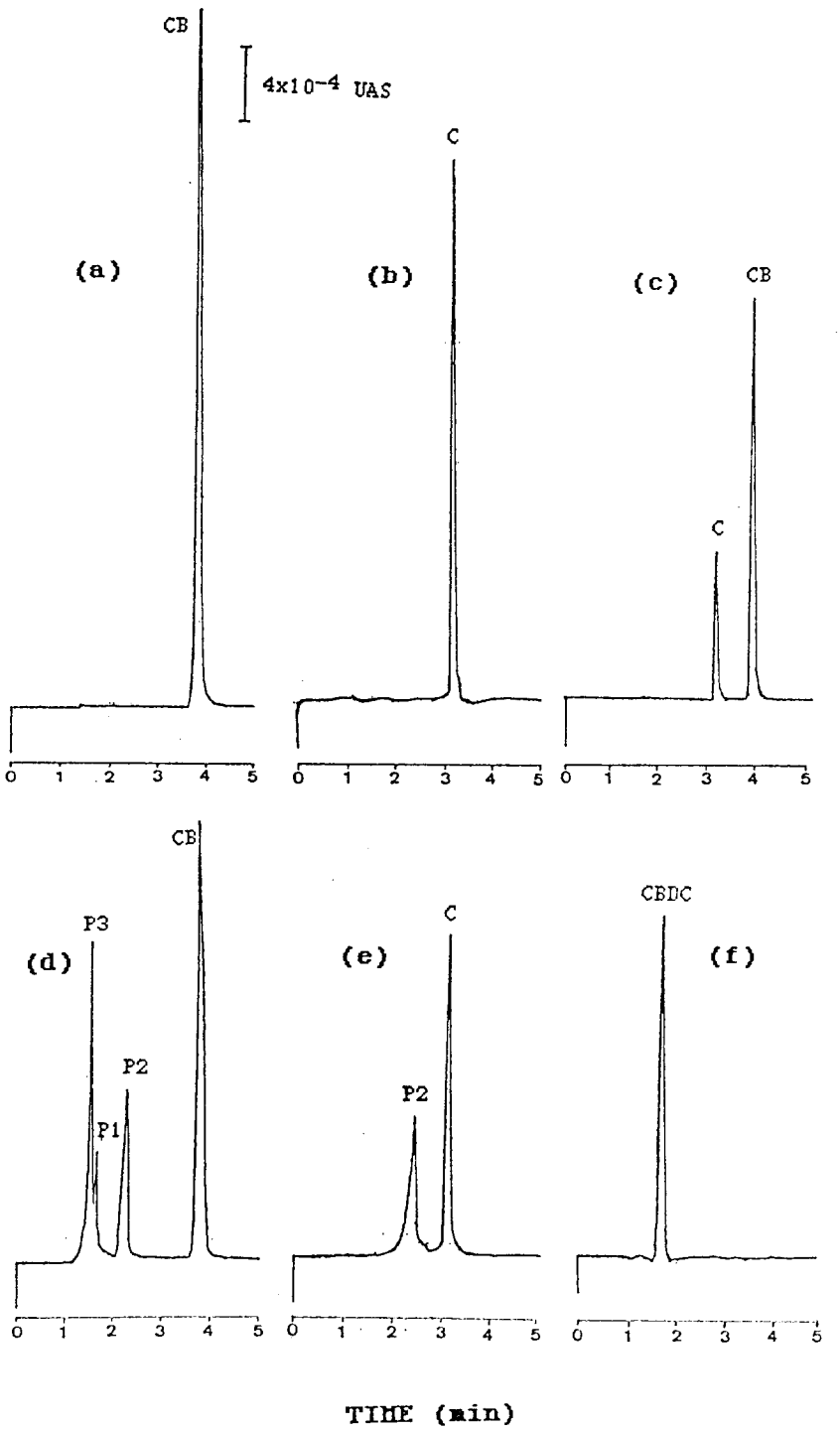
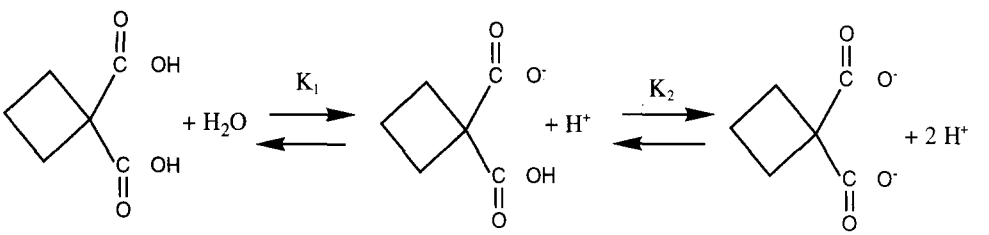


Fig. 1.

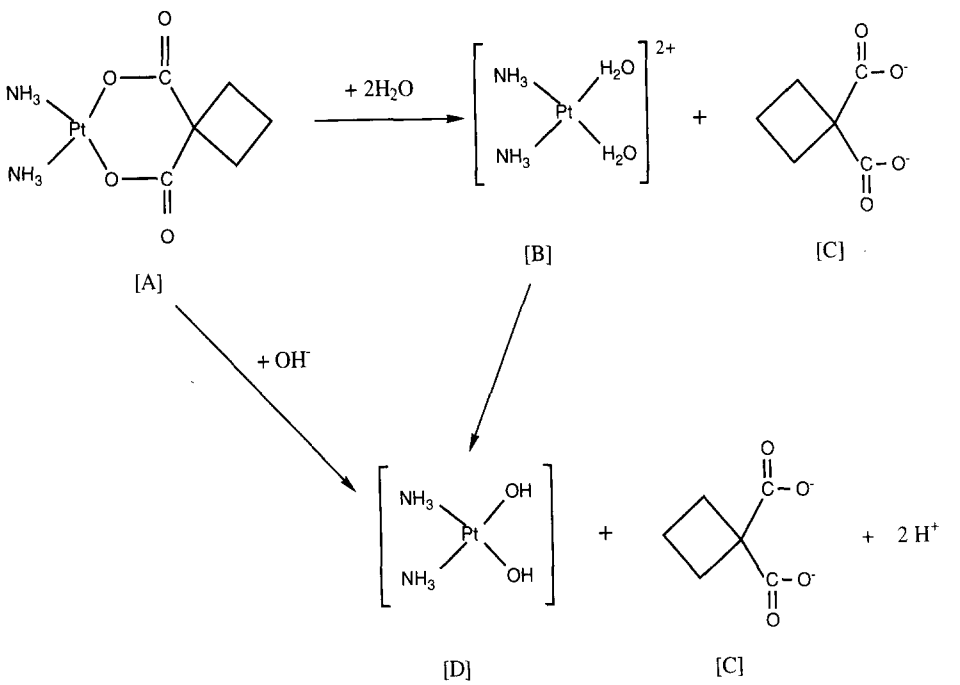
Table 1
Rate parameters for carboplatin degradation in 5% glucose solution and in pure water

	$I \times 10^6$ (Einstein min^{-1}	T (°C)	$k_{obs} \times 10^3$ (min^{-1})
Carboplatin in pure water initial concentration: 3.2 mg ml^{-1}	6.00	25	6.0
	Dark	60	0.056
Carboplatin in 5% glucose solution initial concentration: 3.2 mg ml^{-1}	6.00	25	5.7
	Dark	60	0.069
	49.0*	25	49.0

*Intensity corresponding to daylight in laboratory conditions.



Scheme 1.



Scheme 2.

degradation conditions. All of them present lower retention time than carboplatin, ($T_{r(P1)} = 1.9$ min, $T_{r(P2)} = 2.5$ min and $T_{r(P3)} = 1.6$ min). From degraded solution of cisplatin only one degradation

product at 2.5 min corresponding to a mixture of *cis*-$[Pt(NH_3)_2ClH_2O]^+$ and *cis*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ complexes, was obtained. Peak purity for carboplatin, cisplatin and its degradation products were proved by using photodiode array detection and absorbance indexing.

The pH of solution was monitored through the degradation process and changes with time were observed. The initial pH for carboplatin (3.2 mg ml^{-1}) either in 5% glucose infusion solution and water was 6.4. In pure water the initial pH of carboplatin solution changes to 7.2 when the 50% degradation of drug was reached. This change of pH can be accounted for considering the acid-base equilibria established in solution, between 1,1-cyclobutanedicarboxylate anion and their protonated forms ($pK_1 \approx 2$ and $pK_2 \approx 7$) as shown in Scheme 1.

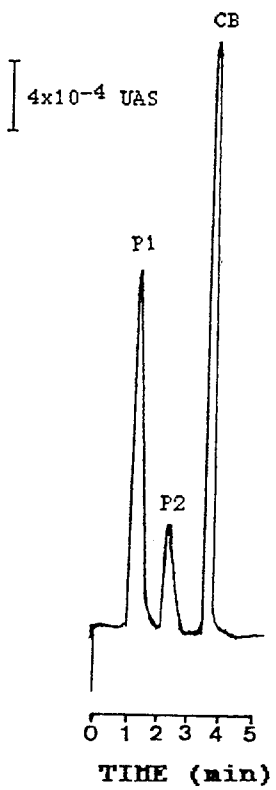


Fig. 2. Chromatogram of carboplatin degraded solution (pH = 3.5) in 5% glucose intravenous solution.

P1 and P3 were identified with a pattern solutions of 1,1-cyclobutanedicarboxilic acid (1 mg ml^{-1}). In pure water (pH = 2.7) shows only one peak at 1.9 min and in 5.10^{-2} M sodium hydroxide solution (pH = 12.3) shows two peaks at 1.6 and 1.9 min corresponding to 1,1-cyclobutanedicarboxylate anion and its protonated forms, respectively. This explains why in carboplatin alkaline solutions there appeared two peaks at 1.6 and 1.9 min. P2 was assignated to complexes *cis*-$[Pt(NH_3)_2ClH_2O]^+$ and *cis*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ obtained by heating at 75°C for 3 h freshly cisplatin solutions of 1.5 mg ml^{-1} (Riley et al., 1981).

Based on knowledge of the aquation process of platinum complexes (Banerjea et al., 1957; Lee and Martin, 1986; Pujol et al., 1991) and the experimental results, the degradation reaction for carboplatin in aqueous solution can be postulate as Scheme 2, where carboplatin [A] reacts with water to yield *cis*-diamminediaquoplatinum (II) complex [B] and 1,1-cyclobutane dicarboxilate anion [C].If the oxidation process continues the *cis*-diamminediaquoplatinum(II) complex [B] becomes *cis*-diamminedihydroxideplatinum (II) [D], as occurs with cisplatin degradation process in aqueous solution. On the other hand if the carboplatin degradation takes place in alkaline media, the OH^- ions are the nucleophylic agents and carboplatin yields *cis*-diamminedihydroxideplatinum (II) [D] and 1,1-cyclobutane dicarboxilate anion [C] while the initial pH of solution decreases with time.

When the degradation process of the drug takes place in 5% glucose aqueous solution and the 50% of carboplatin degradation was reached, the pH of solution decreases with time to 3.5. Fig. 2 shows a chromatogram for carboplatin degraded aqueous solution in the presence of 5% glucose. Only two degradation products appear, corresponding to P1 and P2. It seems the glucose in solution favours the apperance of H^+ ions in solution (second step in Scheme 2).

Fig. 3 shows chromatograms obtained with the proposed method in different experimental condi-

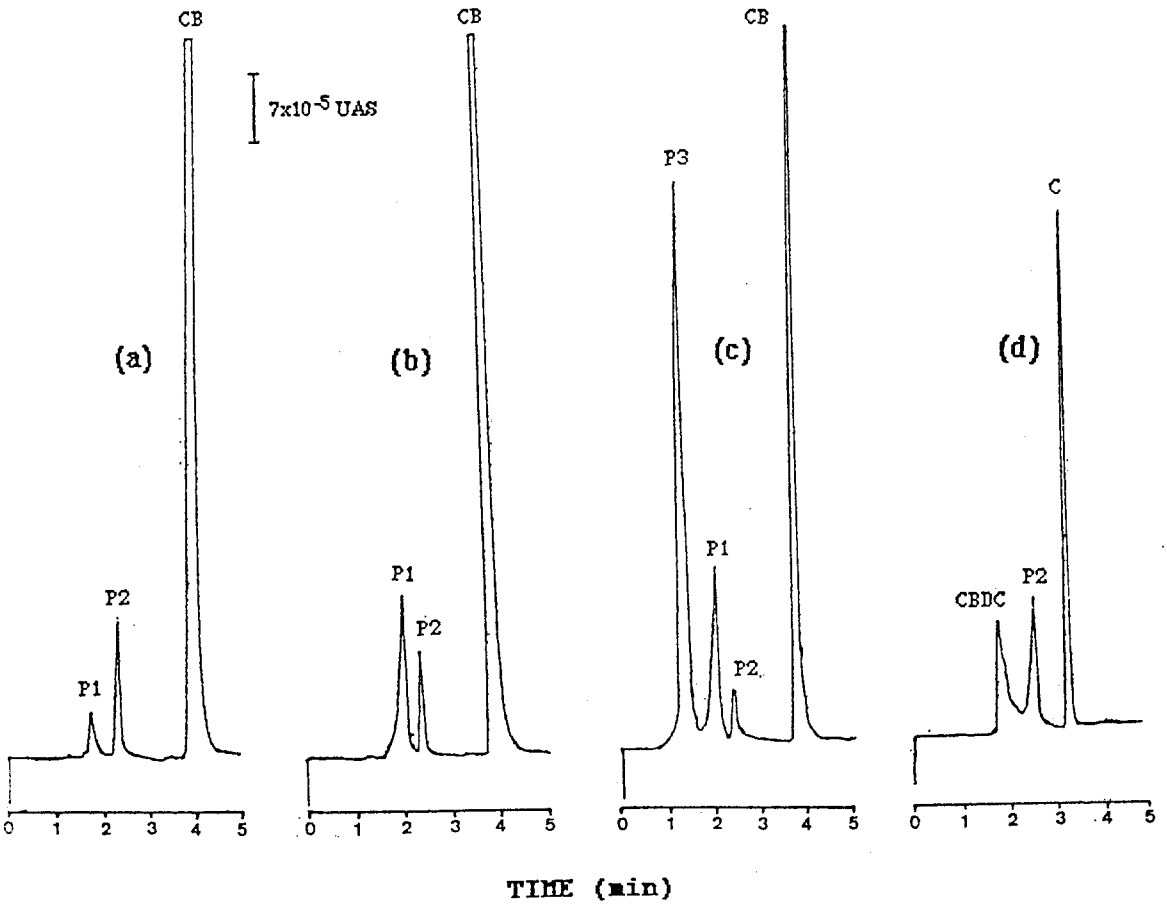


Fig. 3. Chromatograms of (a) carboplatin degraded solution (pH = 7.0) (b) a mixture (pH = 3.9) of 5 ml of above carboplatin degraded solution and 5 ml of 1,1-cyclobutanedicarboxylic acid (1 mg ml^{-1} in pure water) (c) a mixture (pH = 12.0) of 5 ml of carboplatin degraded solution and 5 ml of 1,1-cyclobutanedicarboxylic acid solution (1 mg ml^{-1} in 0.05 M sodium hidroxide) and (d) a mixture (pH = 3.8) of 5 ml of cisplatin aqueous solution (1.5 mg ml^{-1}) and 5 ml of 1,1-cyclobutanedicarboxylic acid solution (1 mg ml^{-1} in pure water).

tions. (They confirm the above discussion.) Fig. 3a shows a chromatogram of degraded carboplatin solution (pH = 7.1). When we added 5 ml of cyclobutanedicarboxylic acid (1 mg ml^{-1}) to a 5 ml of above degraded solution of carboplatin, to make a new solution of pH = 3.9, a peak corresponding to P1 was observed (Fig. 3b). If we added 0.05 M sodium hydroxide solution to allow a pH of 12, P1 changed and a second peak, P3, appeared (Fig. 3c) while P2 decreased. On the other hand if we added 5 ml of cyclobutanedicarboxylic acid (1 mg ml^{-1}) to 5 ml of cisplatin (1.5 mg ml^{-1}) degraded solution to make a solution of pH = 3.9, the peak corresponding to protonated forms of cyclobutanedicarboxylate anion appeared (Fig. 3d).

The above results allow us to explain why chromatograms obtained from degraded solutions of carboplatin in 5% glucose solution that had a pH = 3.5 (Fig. 2) appeared as only two peaks, P1 and P2, while chromatograms from carboplatin degraded solutions in pure water with pH = 7.1 appeared as three peaks, P1, P2 and P3. P1 and P3 corresponding to the cyclobutanedicarboxylate anion and its protonated forms, respectively, as indicated previously.

The results obtained in this work are in good agreement with published ones, the degradation kinetics of carboplatin is accelerated by light (Torres et al., 1996). On the other hand, carboplatin in aqueous solution follows the same degradation pathway in dark conditions as under illumination conditions. The general degradation mechanism is similar to that reported for cisplatin and related compounds

Acknowledgements

This work was supported by Grant 94/1138 from 'Ministerio de Sanidad y Consumo de España'.

References

Banerjea, D., Basolo, F. and Pearson, R., Mechanism of substitution reactions of complex ions. XII. Reactions of some platinum (II) complex with various reactants. *J. Am. Chem. Soc.*, 79 (1957) 4055–4062.

Cheung, Y.W., Cradock, J.C., Rao Vishnuvajjala, B. and Flora, K.P., Stability of cisplatin, iproplatin, carboplatin and tetraplatin in commounly used intravenous solutions. *Am. J. Hosp. Pharm.*, 44 (1987) 124–130.

Lee, K.W. and Martin, Jr., D.S., *Cis*-dichlorodiammineplatinum (II). Aquation equilibria and isotopic exchange of chloride ligands with free chloride and tetrachloroplatinate (II). *Inorg. Chim. Acta.*, 17 (1986) 105–110.

Prat, J., Pujol, M., Girona, V., Muñoz, M. and Solé, L.A., Stability of carboplatin in 5% glucose solution in glass, polyethylene and polypropylene containers. *J. Pharm. Biomed. Anal.*, 12 (1994) 81–84.

Pujol, M., Girona, V., Trillas, M. and Domènech, X., Kinetics of cisplatin photoaquation in aqueous solution. *J. Chem. Res.*, 9 (1991) 258–259.

Riley, C.M., Sternson, L.A. and Repta, A.J., High-performance liquid chromatography of inorganic platinum (II) complexes using solvent-generated anion exchangers. II. The effect of electrolytes on solute retention. *J. Chromatogr.*, 219 (1981) 235–244.

Torres, F., Girona, V., Pujol, M., Prat, J. and De Bolós, J., Stability of carboplatin in 5% glucose solution exposed to light. *Int. J. Pharm.*, 129 (1996) 275–277.

Van Echo, D.A., Egorin, M.J., Whitacre, M.Y., Olman, E.A. and Aismer, J., Phase I Clinical and pharmacologic trial of carboplatin daily for 5 days. *Cancer Treat. Rep.*, 68 (1984) 1103–1114.