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Stability of carboplatin in 5% glucose solution in glass, polyethylene and polypropylene containers

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Abstract: The degradation of carboplatin (3.2 mg ml^{-1}) in 5% glucose infusion solution at 25°C and protected from light was investigated. The effects of the material of the container and temperature were also studied. Solutions were prepared in 5% glucose solution and stored in glass bottles, polyethylene (PE) and polypropylene (PP) containers at 40, 50 and 60°C and at 25°C ± 1°C. Samples were assayed by an HPLC method to determine the residual carboplatin concentration at each time of sampling. Carboplatin degradation followed pseudo-first-order kinetics and no dependence on the nature of the container was found. After 1 month at 25°(±1°)C the change in carboplatin concentration was <2% of the initial concentration in 5% glucose. These results are in agreement with those predicted by the application of the Arrhenius equation.

Keywords: *Carboplatin; high-performance liquid chromatography (HPLC); stability; degradation.*

Introduction

Carboplatin, [cis-diammine(cyclobutane-1,1-dicarboxylato)platinum], an analogue of cisplatin, was synthesized to obtain a drug with antitumour properties similar to those of cisplatin, but with less severe side-effects (renal toxicity, nausea and vomiting). The structures of carboplatin and cisplatin are shown in Fig. 1.

The antitumour activity of carboplatin in ovarian cancer [1] and small cell lung cancer [2] has been demonstrated. Carboplatin is used mainly in hospitals for administration by intravenous infusion [3]. The aim of this work was to determine the stability of carboplatin in 5% glucose infusion solutions so that the solution could be administered under the best conditions. A high-performance liquid chromato-graphic (HPLC) method was used for analytical measurements of the drug. The effects of the material of the container and the temperature on the degradation of carboplatin in 5% glucose solution were studied. Kinetics measurements were made under different experimental conditions and the rate constant at 25°C was determined by application of the Arrhenius equation.

Experimental

Materials

Carboplatin for injection (Paraplatin®), was obtained from Bristol-Myers Laboratoires (Barcelona, Spain) and was prepared in 5% glucose solution in glass bottles (Grifols Laboratoires, Barcelona, Spain), in polyethylene (PE) bags (Palex Laboratoires, Barcelona, Spain) and polypropylene (PP) bags (Ern Laboratoires, Valencia, Spain). 1,1-Cyclobutanedicarboxylic acid was obtained from ICN Chemicals (Corbera de Llobregat, Spain). Aqueous solutions containing cis-[Pt(NH$_3$)$_2$ClH$_2$O]$^+$ and cis-[Pt(NH$_3$)$_2$(H$_2$O)]$^{2+}$ were prepared by heating cisplatin solutions (1.0 mg ml^{-1}) for 3 h at 75°C [4]. HPLC grade methanol was supplied by Tecknocroma (Sant Cugat del Vallés, Spain). Double-distilled water was used after filtration through a Milli-
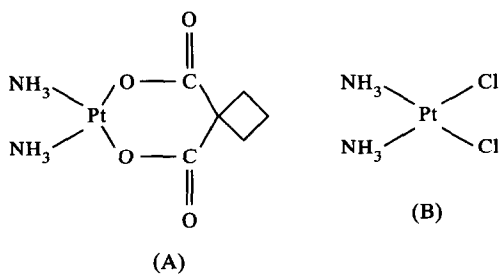

Figure 1
Molecular structure of (A) carboplatin and (B) cisplatin.

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Q system (Millipore, Barcelona, Spain) and a second vacuum filtration in a helium atmosphere.

Chromatographic assay method

All assays were performed by HPLC at ambient temperature (25°C ± 1°C). This procedure was carried out in a liquid chromatograph with an isocratic pump and diode-array UV light detector (model HP-1090, Hewlett Packard, Barcelona, Spain) coupled to an integrator (model HP-3396 D, Hewlett Packard, Barcelona, Spain) and a Hewlett Packard Think Jet printer.

A 250 × 4.6 mm i.d. column packed with 5-μm Spherisorb-Ph (Tecknocroma) was used as the stationary phase. The mobile phase had a flow rate of 0.8 ml min^{-1} under isocratic conditions of water–methanol (98:2, v/v). The UV dtector was set at 210 nm. Under these conditions, the retention time for carboplatin was 3.1 min.

A Crison pH-meter model 2001 (Alella, Spain) was used for pH measurements.

In order to assess the reproducibility and linearity of the method, calibration curves were constructed using 10 samples of carboplatin in 5% glucose solution in the concentration range of 0.4–4 mg ml^{-1}. Each sample was injected in triplicate. Linearity was determined by linear regression analysis: slope = 15390200 ± 294420 UA g^{-1} ml ($P < 0.05$); the correlation coefficient r was >0.999; the intercept of calibration graph was not significant and the reproducibility of assays by analysis of variance ($P > F$: 4.21 × 10^{-6}).

The stability-indicating capability of the method was demonstrated by using samples of carboplatin (3.2 mg ml^{-1}) in 5% glucose solution. One sample was heated at 60°C for 1 month; the decomposition of drug was >35%. Hydrochloric acid (0.1 M) was added to another sample; the final pH of the solution was 1.5. Sodium hydroxide solution (0.05 M) was added to a third sample; the final pH of the solution was 12.2. In all cases, the decomposition product peaks were well resolved from the peak of the intact drug. Figure 2 shows the chromatograms obtained. Three degradation products of carboplatin (P$_1$, P$_2$ and P$_3$) appeared either at elevated temperatures or under extreme pH conditions. All presented a
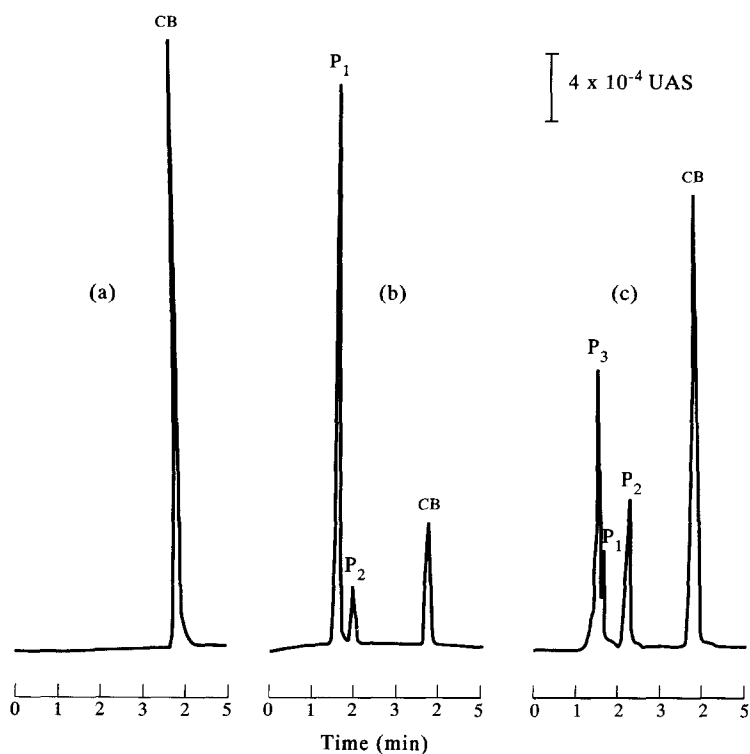

Figure 2

Chromatograms of (a) carboplatin (CB), 3.2 mg ml^{-1}, in 5% glucose solution (pH 6.4); (b) carboplatin, 3.2 mg ml^{-1}, in 5% glucose solution with added 0.1 M hydrochloric acid (pH 1.5); (c) carboplatin, 3.2 mg ml^{-1}, in 5% glucose solution with added 0.05 M sodium hydroxide (pH 12.2).

lower retention time than that of carboplatin. These degradation products were identified by comparison with solutions of known products. P_1 and P_3 corresponded to the cyclobutane-dicarboxylate anion and its protonated forms, respectively. P_2 corresponded to the complexes cis-$[Pt(NH_3)_2ClH_2O]^+$ and cis-$[Pt(NH_3)_2(H_2O)]^{2+}$ obtained by heating for 3 h at 75°C fresh cisplatin solutions (1.0 mg ml^{-1}) [4]. Peak purity of all peaks was proved by using photodiode array detection and absorbance indexing.

For quantitative measurement of the drug, the initial concentration was determined by comparing the peak area of carboplatin with those of external standard solutions. This initial concentration of carboplatin was designated as 100%; all subsequent concentrations were expressed as percentages of the initial concentration.

Kinetics measurements

Paraplatin solution (10 mg ml^{-1} of carboplatin in distilled water) was quantitatively transferred to glass bottles, PE and PP bags containing 5% glucose solution to achieve a final carboplatin concentration of 3.2 mg ml^{-1}.

Before carboplatin solution was added to the containers, volumes of intravenous fluid to compensate for overfill of the container were withdrawn. The carboplatin solutions were aseptically added to containers so that the final concentration in the admixtures would be approximately equal to the target concentration. All solutions were gently inverted three times to ensure proper mixing and stored at 40, 50 and 60°C, protected from light. Before storage of each solution the initial pH was determined.

To study the stability of carboplatin in 5% glucose solution at room temperature (25°C ± 1°C), triplicate solutions of carboplatin (3.2 mg ml^{-1}) in 5% glucose solution in glass, PE and PP containers were prepared as indicated

previously and stored at room temperature, protected from light. The admixtures were usually inspected by one of the investigators for visual evidence of changes.

Aliquots were taken at specific time intervals and the concentration of carboplatin was determined in triplicate by the HPLC assay. The pH of each sample was also determined at each time interval.

Results and Discussion

The degradation of carboplatin in 5% glucose solution was found to observe pseudo-first-order kinetics. The observed rate constants (k_{obs}) for the overall degradation of carboplatin were calculated by linear regression from the slope of linear plots of the logarithm of residual carboplatin concentration against time. All k_{obs} values obtained in this study are shown in Table 1. A statistical method [5] was used to determine the confidence limits of all evaluated constants. Values of $P < 0.05$ were considered to be significant.

The pH of solutions was monitored as indicated above. The initial pH of solutions was 6.0. This value decreased with time to pH 3.5 when more than 35% of carboplatin had degraded.

Examination of the k_{obs} values showed that the nature of the material of the container had no apparent effect on the degradation of carboplatin in 5% glucose infusion solution.

The temperature dependence on the degradation of carboplatin in 5% glucose solution was determined by application of the Arrhenius equation [6]. From the slope and intercept of a plot of the logarithm of the observed pseudo-first-order rate constants against the reciprocal of absolute temperature, the activation energy was 100.67, 111.56 and 112.23 kJ mol^{-1} and the frequency factor was 7.29×10^{13}, 4.96×10^{15} and 1.46×10^{14} day^{-1}

Table 1
Observed rate constants (±95% confidence limits) for carboplatin* degradation in 5% glucose solution in polyethylene (PE), polypropylene (PP) and glass containers at 40, 50 and 60°C

Containers	k_{obs} (day^{-1})		
	40°C	50°C	60°C
PE	$6.15 \times 10^{-4} \pm 3.19 \times 10^{-5}$	$3.91 \times 10^{-3} \pm 4.76 \times 10^{-4}$	$1.28 \times 10^{-2} \pm 5.67 \times 10^{-4}$
PP	$4.98 \times 10^{-4} \pm 1.33 \times 10^{-5}$	$2.74 \times 10^{-3} \pm 3.94 \times 10^{-4}$	$7.99 \times 10^{-3} \pm 4.07 \times 10^{-4}$
Glass	$8.55 \times 10^{-4} \pm 5.57 \times 10^{-5}$	$4.10 \times 10^{-3} \pm 2.75 \times 10^{-3}$	$9.97 \times 10^{-3} \pm 1.93 \times 10^{-3}$

* Initial concentration of carboplatin 3.2 mg ml^{-1}.

Table 2
Predicted rate constants (±95% confidence limits) for carboplatin degradation in 5% glucose solution polyethylene (PE), polypropylene (PP) and glass containers at 25°C

Containers	k_{25} (days^{-1})
PE	$2.94 \times 10^{-4} \pm 2.56 \times 10^{-5}$
PP	$1.59 \times 10^{-4} \pm 1.61 \times 10^{-5}$
Glass	$3.47 \times 10^{-4} \pm 8.68 \times 10^{-5}$

Table 3
Stability of carboplatin* in 5% glucose solution in glass bottles at room temperature (25°C ± 1°C)

Time (days)	% Initial concentration†
0	100.00 ± 2.29
1	99.88 ± 2.53
2	100.54 ± 3.28
3	101.01 ± 0.84
9	99.40 ± 1.84
20	99.45 ± 1.12
30	98.91 ± 1.25

*Initial concentration 3.2 mg ml^{-1}.
†Mean $\pm$ 1.96 $\times$ SE ($n = 6$).

for carboplatin in 5% glucose solutions in glass, PE and PP bags containers, respectively. From these values the stability of carboplatin in 5% glucose infusion solution at 25°C was predicted. Table 2 shows the predicted degradation constants at 25°C for carboplatin in each type of container. From these values, the time in which 10% of carboplatin has been degraded is estimated to be more than 300 days in all cases. The decrease in concentration of carboplatin at 25°C in 5% glucose solution was predicted to be <2% after storage for 1 month.

The predicted stability of carboplatin at 25°C was compared with the stability determined at 25°C. Three samples were stored for 1 month at room temperature protected from light. The initial concentration of carboplatin was 3.2 mg ml^{-1} in glass containers (Table 3). Only one type of container was studied in this case because the stability of carboplatin study had been found to be independent of the container material.

The predicted stability at 25°C yielded the same results for the first month as that determined at 25°C ± 1°C. Carboplatin was stable in 5% glucose solutions at 25°C in any kind of container protected from light for at least 1 month.

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