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Note

# A note on the solution chemistry of carboplatin in aqueous solutions

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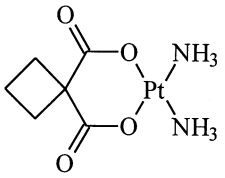
**Abstract**

The aging of carboplatin solutions in water, 3.5 and 103 mM NaCl was studied using thin layer chromatography and thin layer electrophoresis. A number of Pt-containing species can be separated, which were not observed in previous work with HPLC. 

*Keywords:* Solution chemistry; Carboplatin; Aqueous solutions

Carboplatin (*cis*-diammine 1,1-cyclobutanedicarboxylato platinum (II), CBDCA), is a second generation cisplatin analogue (Scheme 1). It is more soluble, and exhibits lower toxicity (Reedijk, 1996) than cisplatin.




Scheme 1. Carboplatin (CBDCA).

Several authors have reported the study of carboplatin hydrolysis in aqueous solutions, in the presence and absence of chloride. Cheung et al. (1987) found that when carboplatin is dissolved in 0.9% NaCl about 5% was lost after 24 h, and Perrone et al. (1989) conducted a similar study to test whether the formation of cisplatin could account for this loss of carboplatin. They found 0.7% was converted after 24 h. This contrasts with the results of Krull et al. (1983), who found, using reductive liquid chromatography-electrochemical detection (LCEC), that a carboplatin solution in 0.9% NaCl to be stable for 7 days. Wenclawiak and Wollmann (1996) obtained one unidentified carboplatin hydrolysis product after 3 weeks ag-

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ing in 100 mM NaCl at 37°C. Prat et al. (1994) reported that carboplatin solutions in water and 5% glucose are very stable when protected from the light. A degradation pathway of carboplatin dissolved in water and 5% glucose solutions under illumination, as studied by high performance liquid chromatography (HPLC), was published by Pujol et al. (1997), where they identified as the only platinum species produced the diamminediaquoplatinum (II) cation. We have recently separated the products formed in aged cisplatin solutions under light and dark conditions in water, 3.5 and 103 mM NaCl by thin layer chromatography (TLC) and thin layer electrophoresis (TLE) obtaining more separated species than reported previously (Lederer and Leipzig-Pagani, 1998), and thus wanted to examine also the aging of carboplatin solutions with these techniques. The purpose of this communication is to report that with TLC and TLE we have obtained more species in aged CBDCA aqueous solutions than reported in the literature so far. We report here our preliminary qualitative findings.

Carboplatin was purchased as one of its pharmaceutical preparations, Paraplatin® (Bristol-Myers Squibb), and consists of a 10 mg/ml carboplatin solution in water. It was stored in the dark at 4°C. Sodium perchlorate and 4-nitrosodimethylaniline were obtained from Fluka (Buchs, Switzerland), and were used without further purification.

Carboplatin solutions (5 mg/ml) were made in distilled water, 3.5 and 103 mM NaCl by mixing equal volumes of the pharmaceutical preparation with an NaCl solution of appropriate concentration. The solutions were aged at room temperature (20–22°C) in ordinary laboratory light conditions (not in direct sunlight) and in the dark.

Electrophoresis was performed on a Multiphor II Electrophoresis system with a cooling table (Pharmacia Biotech, Uppsala, Sweden) powered by an EPS 3500 electrophoresis power supply using Merck 5577 microcrystalline cellulose thin layers (E. Merck, Darmstadt, Germany) as support, bridged by Whatman 3MM (Whatman International, Maidstone, UK) paper strips. The thin layers and paper strips were impregnated with the electrolyte, dried with absorbant paper, and the sample (3–5 $\mu$l) was applied either as distinct spots or as a thin line using known volume micropipettes. Both the electrolyte and the eluent used throughout were 0.1 M $NaClO_4$.

Thin layers used for chromatography were Merck 5577 microcrystalline cellulose thin layers. Standard ascending chromatography techniques were used.

Both the chromatograms and the electropherograms were revealed by exposing them to $I_2$ vapours followed by spraying with a 1% solution of 4-nitrosodimethylaniline (4-NDMA) in acetone. Most Pt species appear as red spots or bands against a yellow background. Although 4-nitrosodimethylaniline is not a universal reagent for all Pt complexes (it does not react with, e.g. $Pt(NH_3)_4^{2+}$), it gave the best contrast/detection limit under the conditions used, and is specific for several metals (especially the Pt group metals), so any bands or spots observed here correspond to Pt species.

The densitometer setup is made up of a CCD camera with an acquisition device (Cybertech CS-1, Cybertech, Germany) which numerises images in 480 × 374 pixels with 256 grey shades, linked to an IBM PC running the Cybertech Wincam 2.1 data treatment program under Windows 3.1. Diffuse room lighting was used throughout.

Chromatography using 0.1 M $NaClO_4$ as eluent gives less resolution than in the case of cisplatin, as the $R_f$ values are usually over 0.5 (Fig. 1). TLC with this eluent is nevertheless informative, because it allows comparisons with previously obtained results (Lederer and Leipzig-Pagani, 1998).

As migration distance standard in TLE sulfanilic acid azochromtrop was used (Fluka 86100), and no significant mobility change occured between pHs 6 and 8 (Scheme 2).

As can be seen in Fig. 2a, it appears by TLE that in the dark there is little change, apart from a faint anionic band seen for the 103 mM NaCl sample. By TLC a different pattern emerges, as there is a more retained compound of perhaps comparable visual intensity (Fig. 1a); it exhibits the same $R_f$ value as cisplatin (with several eluents), which, being neutral, cannot be distinguished from carboplatin by TLE. The faint anionic band for this sample is thought to be the

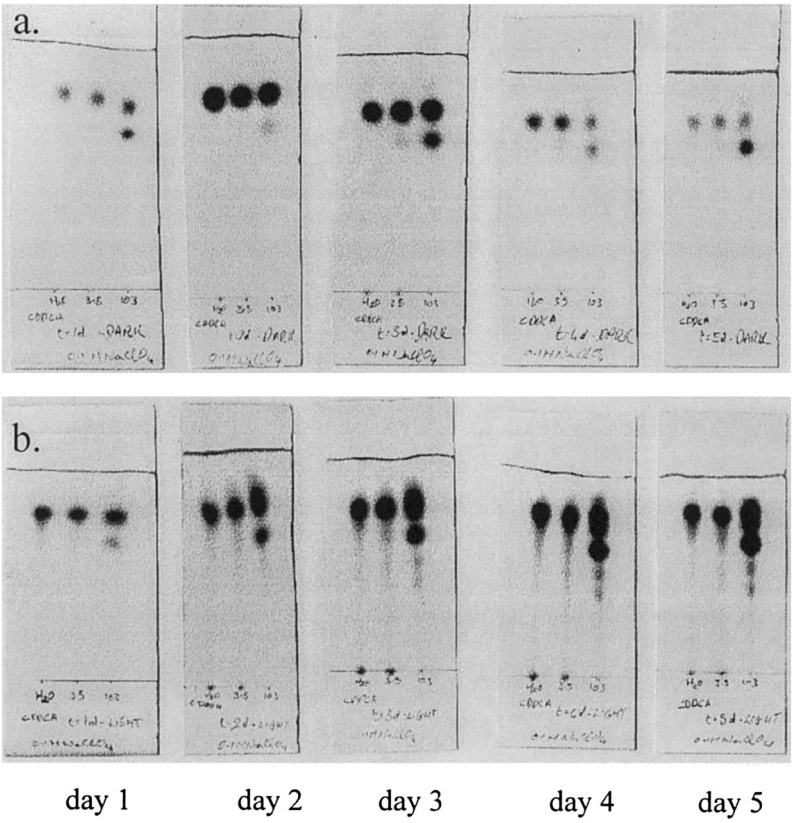
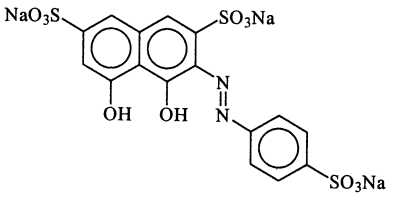



Fig. 1. Thin layer chromatograms of carboplatin aged in water, 3.5 and 103 mM NaCl: (a) in the dark; (b) under illumination. Merck 5577 microcrystalline cellulose thin layers, 0.1 M $NaClO_4$ eluent, $I_2$/4-NDMA detection.

monochloro substituted carboplatin (also seen in Fig. 3a—lane 8). Quantitative evaluation of the quantity of cisplatin produced under these conditions after 2 days was $\approx 3.7\%$ (chromatogram and densitometric evaluation not shown).

Carboplatin solutions aged in the light show a more complex pattern, especially by TLE. To attempt to identify the species seen, a comparative electropherogram was performed (Fig. 3a).




Scheme 2. Sulfanilic acid azochromtrop.

While previous studies of aged carboplatin solution reported usually two to three peaks in chromatograms our electrophoretic separations yield three cationic and up to five anionic species. Neutral species remain on the application point and therefore cannot be separated from carboplatin.

It is not possible to assign a putative structure on the sole basis of electrophoretic mobility. It is however possible to compare the mobilities with those of known species.

The fastest anionic species which by the way can be observed only with carboplatin aged in 103 mM NaCl solution has the same mobility as pure $[PtNH_3Cl_3]^-$ or the fastest anion observed in aged *cis*-$Pt(NH)_2Cl_2^0$ and thus should be a monovalent anion. It certainly moves much less than $PtCl_4^{2-}$ (Fig. 3a—lane 1). A second anionic species moves much slower than this fastest band, is

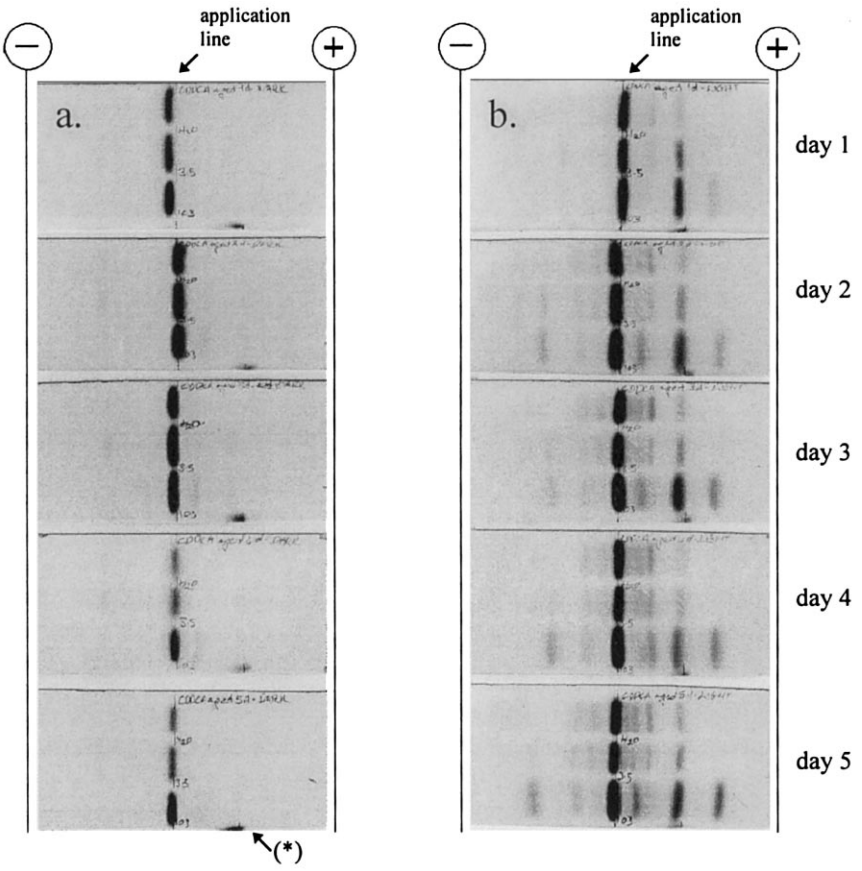



Fig. 2. Thin layer elctropherograms of carboplatin aged in water, 3.5 and 103 mM NaCl: (a) in the dark; (b) under illumination. Merck 5577 microcrystalline cellulose thin layers, 0.1 M $NaClO_4$ electrolyte, 500 V, analysis time $\approx 11$ min, sulfanilic acid azochromtrop mobility standard (*), $I_2$/4-NDMA detection.

present in all carboplatin samples aged in the light (even the sample aged in water), and has increasing intensity with increasing $Cl^-$ concentration. It is not present in aged *cis*-$Pt(NH)_2Cl_2^0$ nor are three other anionic bands which move very slowly.

The fastest cationic band (Fig. 3a—lane 7) has the same mobility as the slower cationic band of the solution of *cis*-$Pt(NH)_2Cl_2^0$ reacted with 2.1 equivalents of $AgNO_3$ and as one of the fast cationic bands in aged *cis*-$Pt(NH)_2Cl_2^0$. So it seems to be a monovalent cationic species, possibly *cis*-$Pt(NH_3)_2(H_2O)Cl^+$. As for the other species, multimeric and hydroxo species with and without the organic ligand are the best guess. Although unlikely, Pt(IV) complexes could also have been formed. In general, these do not react readily with the reagent used and are thus less likely to be responsible for the observed zones.

The multitude of charged species could have relevance in both pharmacy and environmental problems, and as they have not been reported in previous investigations, it is the purpose of this note to draw attention to their existence.

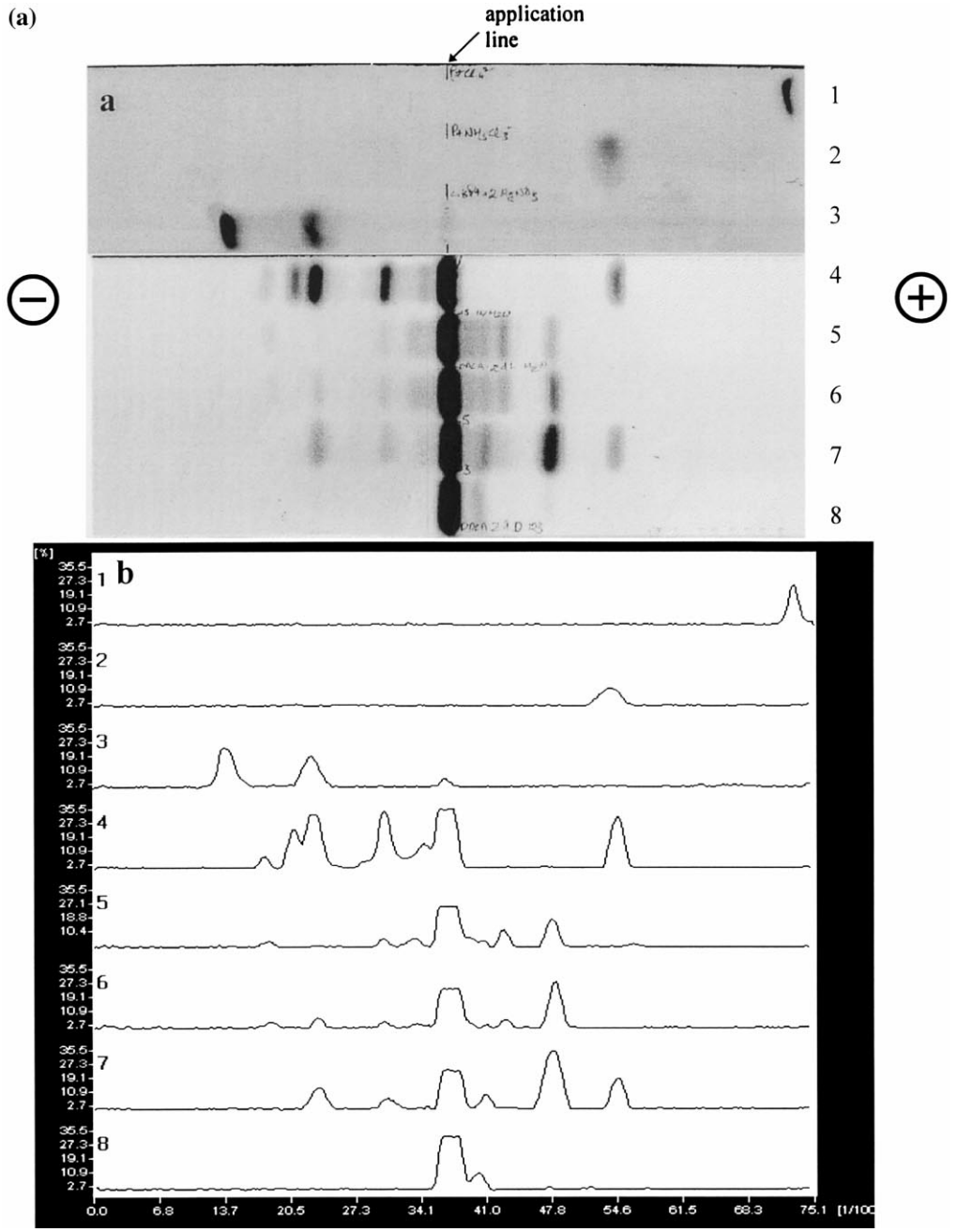



Fig. 3. (a) Electropherogram of: 1. $K_2PtCl_4$ freshly dissolved in 2.4 M HCl; 2. $KPtNH_3Cl_3$ freshly dissolved in 2.4 M HCl; 3. cisplatin + 2.1 eq. $AgNO_3$ aged 2 days; 4. cisplatin in water aged more than 2 weeks (light); 5. carboplatin in water aged 2 days (light); 6. carboplatin in 3.5 mM NaCl aged 2 days (light); 7. carboplatin in 103 mM NaCl aged 2 days (light); 8. carboplatin in 103 mM NaCl aged 2 days (dark). Merck 5577 microcrystalline cellulose thin layers, 0.1 M $NaClO_4$ electrolyte, 500 V, analysis time $\approx$ 11 min, sulfanilic acid azochromtrop mobility standard, $I_2$/4-NDMA detection. (b). Densitometric evaluation of lanes 1–8 in (a). It should be noted that (a) consists of two separate and distinct electropherograms obtained under the same conditions.

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