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Note

Quantitative analysis of *cis*-dichlorodiammineplatinum(II) by high-performance thin-layer chromatography

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In our attempt to optimize the synthesis of *cis*-dichlorodiammineplatinum(II) (cisplatin, DDP), a widely used anticancer drug, we needed a simple, rapid, specific and sensitive determination of this compound. High-performance liquid chromatography coupled with pre-column derivatization¹, post-column derivatization², polarographic detection³ and flameless atomic absorption spectrophotometry (FAAS)⁴ have been used to determine cisplatin in various matrices. These methods are specific and sensitive, but have several disadvantages. While some of them are time-consuming, others are more sophisticated and tedious.

Qualitative work with flat-bed chromatography methods has been successfully used. Basolo *et al.*⁵ described the use of solvent mixtures of water and acetone or ethanol on cellulose filter paper to separate the *cis*- and *trans*-[Pt(NH₃)₂X₂](X = Cl, Br or I) complexes. In a recent paper, Janjić *et al.*⁶ studied the effect of the geometrical configuration of square-planar platinum complexes on their *R_F* values obtained by paper chromatography. These flat-bed chromatography methods encompass only qualitative work on cellulose matrix.

This paper describes a high-performance thin-layer chromatographic (HPTLC) procedure for the rapid separation of several platinum compounds; it also provides a simple and sensitive technique for the quantitative determination of cisplatin.

EXPERIMENTAL

Reagents and solvents

All reagents and solvents were analytical grade. N,N-Dimethyl-4-nitrosoaniline was obtained from Janssen Chimica. K₂PtCl₄, *trans*- and cisplatin were purchased from Johnson Matthey and used without further purification. The aquo de-

derivatives of cisplatin, consisting of a mixture of cis -[Pt(NH₃)₂Cl(H₂O)]⁺ and cis -[Pt(NH₃)₂(H₂O)₂]²⁺, were prepared by dissolving cisplatin in doubly distilled water. This solution was allowed to stand for at least 48 h prior to use.

Apparatus

The HPTLC separations were carried out on pre-coated HPTLC silica gel 60 plates (10 × 10 cm, without fluorescence indicator, E. Merck, Darmstadt, F.R.G.). The aqueous solutions were spotted in 200- μ l volumes by means of a Camag Nano-Applicator*. Samples were applied using a data-pair technique. The plates were developed in twin-trough chambers (Camag, Switzerland).

Dipping is carried out in a commercially available chamber (Desaga, Heidelberg).

In situ quantitation was carried out by scanning with a Zeiss PMQ 3 chromatogram spectrophotometer, equipped with a recorder and an electronic integrator (Minigrator, Spectra-Physics). The scanning speed and the recorder speed were 30 mm/min.

Thin-layer chromatography

Two mobile phases were used in this study: solvent A, acetone-water (95:5, v/v); solvent B, acetone-toluene-water (76:20:4, v/v). The migration distance was *ca.* 4 cm and the migration time was only 5 min.

After development, the plates were dried in a stream of warm air to remove all traces of solvent before the colour reaction.

Post-chromatographic derivatization

The platinum compounds were visualized by dipping the dried plate in a 0.2% (m/v) solution of *p*-nitrosodimethylaniline in diethyl ether for *ca.* 10 sec. Excess reagent was wiped from the back of the plate, which was then placed in an oven at 105°C for 15 min. The plates were allowed to cool to room temperature before scanning at 515 nm.

RESULTS AND DISCUSSION

Several solvent systems were tried in preliminary experiments with conventional cellulose and silica gel TLC plates. The best separation of *trans*- and *cis*platin and aquo derivatives was obtained with solvent B on silica gel plates. However, interference of K₂PtCl₄ was observed, which could be eliminated by passing the sample through a Bond Elut™ SAX disposable column. If K₂PtCl₄ is present in the sample and if dilution of the sample must be avoided, then solvent system A can be used. Fig. 1 shows the separations achieved for a synthetic mixture.

The spots were visualized by dipping the plate in a solution of *p*-nitrosodimethylaniline. This reagent was also used by Kirkland and Yoe for the spectrophotometric determination of chloroplatinate in solution⁷. However, we could not use ethanol as solvent for the nitrosodimethylaniline, because spot diffusion occurred.

* Note by the Editor: The metal parts of this applicator could react with the platinum compounds. Its use here is hence not advisable.

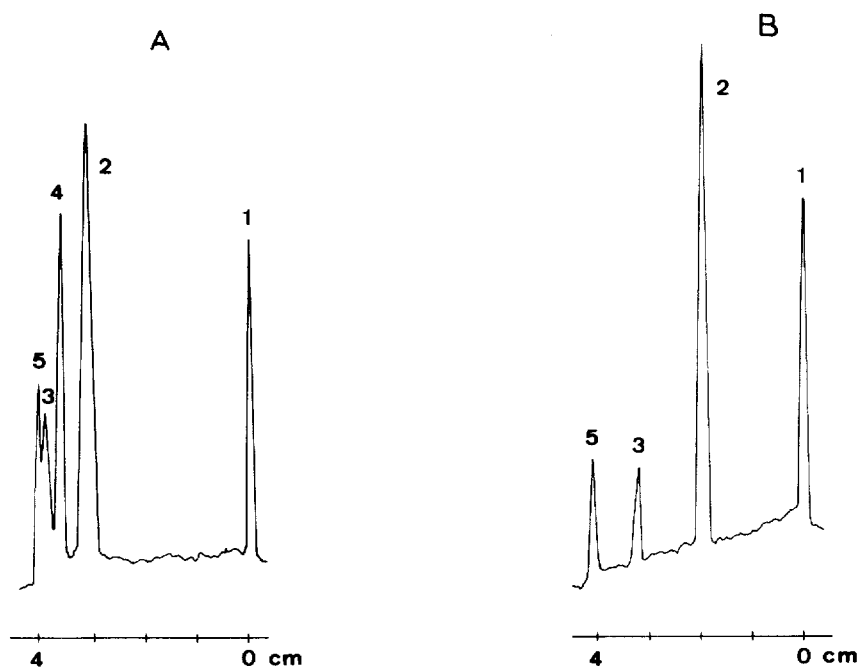


Fig. 1. HPTLC chromatograms of platinum compounds. (A) Solvent system A [acetone-water (95:5)]; (B) solvent system B [acetone-toluene-water (76:20:4)]. Peaks: 1 = aquo derivatives; 2 = cisplatin; 3 = transplatin; 4 = K_2PtCl_4 ; 5 = solvent front.

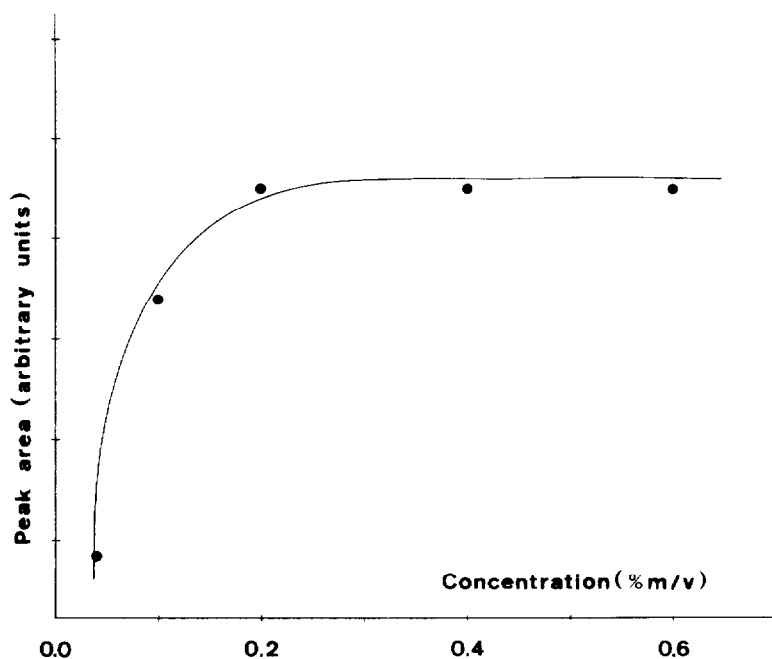


Fig. 2. Effect of concentration of *p*-nitrosodimethylaniline on the intensity of platinum-*p*-nitrosodimethylaniline colour (constant temperature, 100°C; heating time, 10 min; cisplatin concentration, 100 ng/spot).

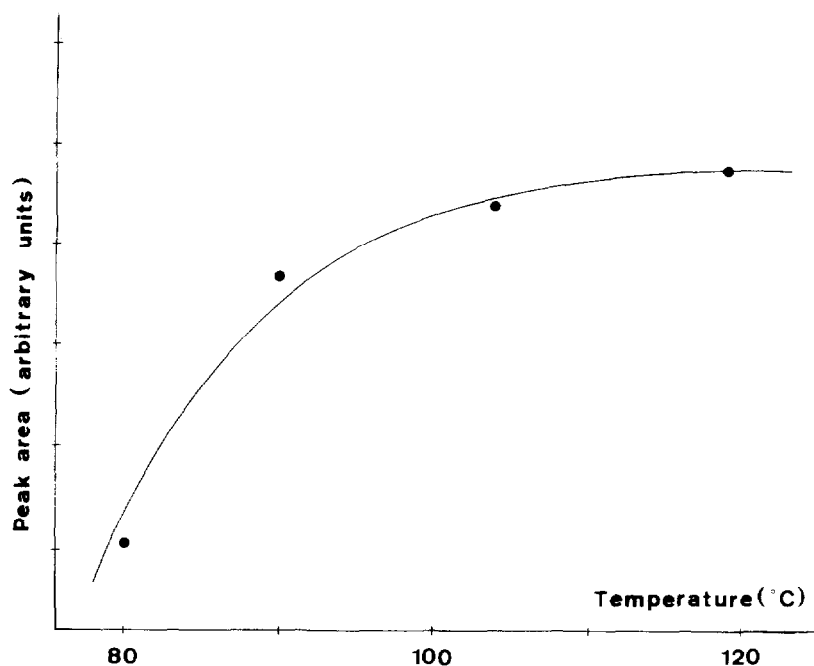


Fig. 3. Effect of temperature on the intensity of platinum-*p*-nitrosodimethylaniline colour (constant concentration, 0.2%; heating time, 10 min; and cisplatin concentration, 100 ng/spot).

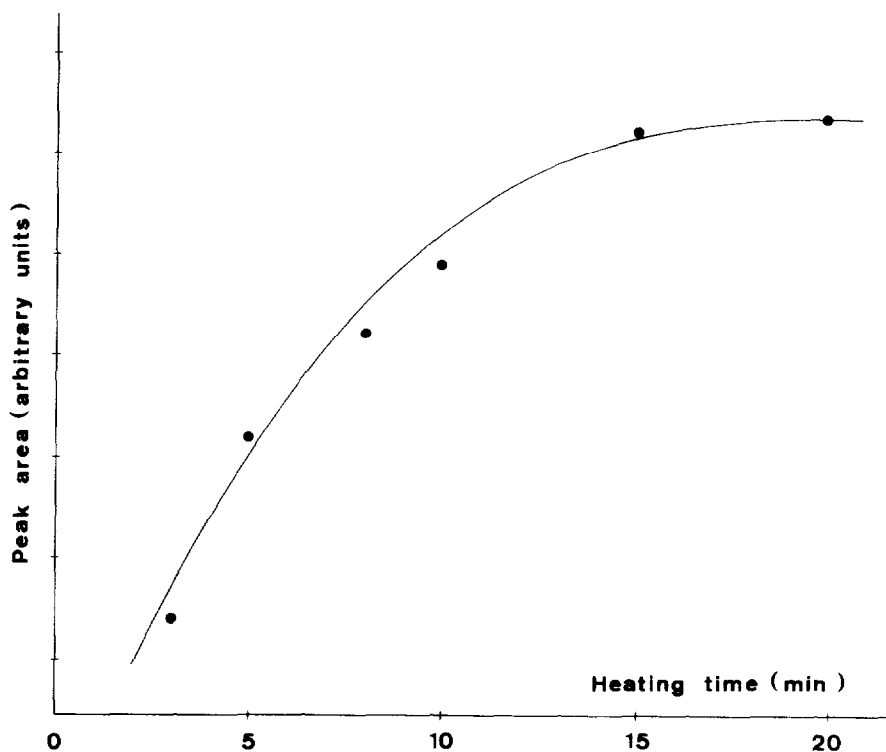


Fig. 4. Effect of heating time on the intensity of platinum-*p*-nitrosodimethylaniline colour (constant concentration, 0.2%; temperature, 105°C; cisplatin concentration, 100 ng/spot).

With dioxane, no colour reaction was observed, and tetrahydrofuran gives a significant less intense colour. The best results were obtained with diethyl ether, but it was found necessary to leave the plate in the dipping solution for *ca.* 10 sec. If a shorter dipping time is used, the solution streaks and spikes are seen when the plate is scanned.

The reagent concentration, the heating temperature and the time were optimized by a "one-factor-at-a-time" approach, as can be seen in Figs. 2-4. The optimum conditions concerning the intensity of the colour were limited by darkening of the background, which increases noise. This resulted in a lower precision. So the following compromise was chosen: dipping in a 0.2% (m/v) solution of *p*-nitrosodimethylaniline in diethyl ether (10 sec), after which the plates were placed in an oven at 105°C for 15 min. Cisplatin and its derivatives give red spots on a yellow background.

Quantitation was carried out with the Zeiss PMQ 3 spectrophotometer in the simultaneous transmission (60%)–remission (40%) mode. The absorption spectrum obtained by scanning the spot on the plate at different wavelengths showed a maximum at 515 nm.

The concentration of cisplatin in the unknown samples were calculated from a calibration graph. Fig. 5 shows scans of cisplatin in the different reference solutions (solvent system A) for the determination of the calibration curve (Fig. 6). To obtain

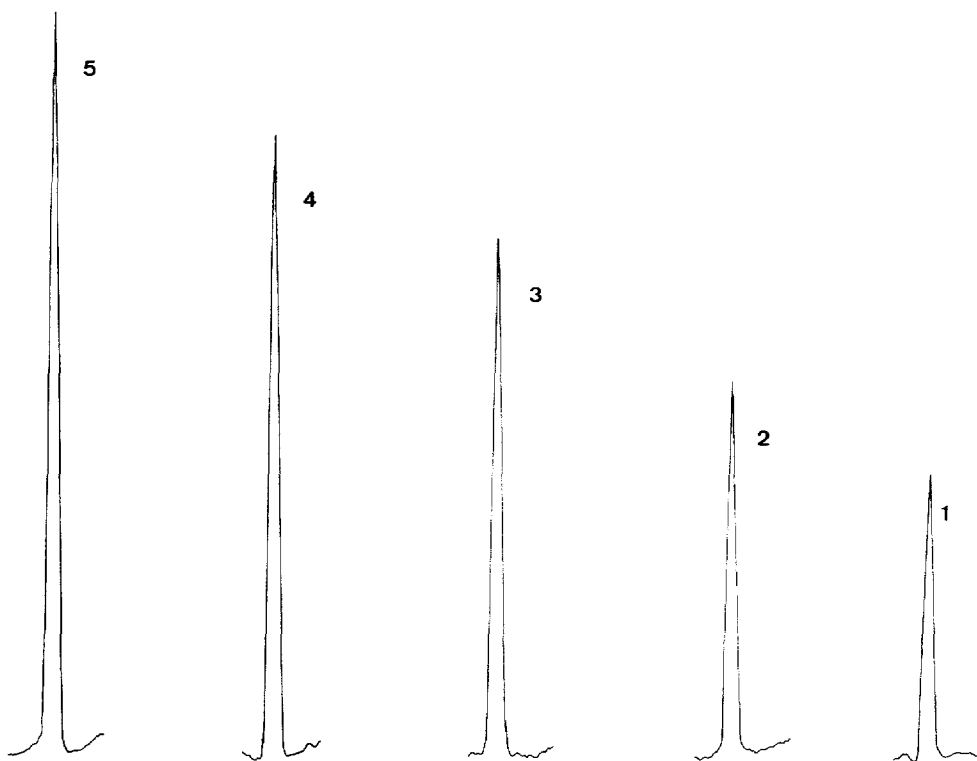


Fig. 5. Scans of cisplatin standard spots (solvent system A). Peaks: 1 = 31.96 ng/spot; 2 = 42.61 ng/spot; 3 = 56.81 ng/spot; 4 = 75.75 ng/spot; 5 = 101.00 ng/spot.

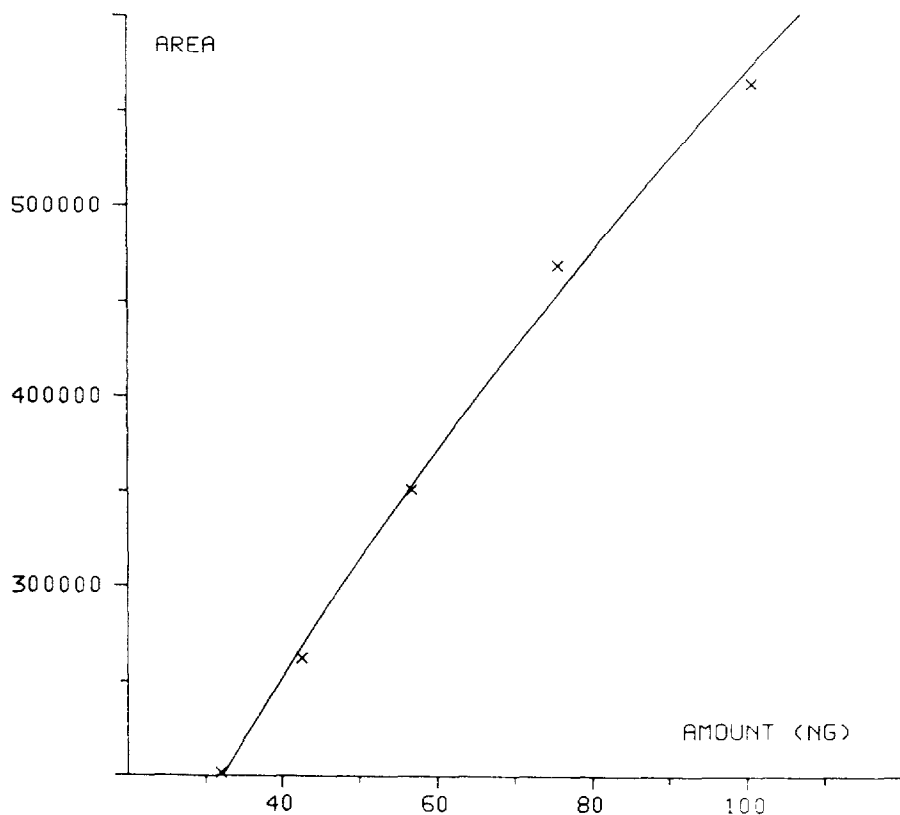


Fig. 6. Calibration curve for cisplatin.

the best curve-fitting, the mean deviation of the linear, quadratic and quadratic-logarithmic regression were compared⁸. The calibration curve is best described by the quadratic-logarithmic approach: $\ln y = a_0 + a_1 \ln x + a_2(\ln x)^2$.

The intensity of spot colour varies from day to day and is difficult to control accurately. However, if a calibration curve is included on each plate, reproducible results are obtained with a relative standard deviation of 3.5% (concentration 75 ng/spot; $n = 6$). The detection limit is *ca.* 14 ng per spot.

We used this HPTLC method for the optimization of the synthesis of cisplatin. However, preliminary experiments with urine indicate that the method should also be applicable to the determination of cisplatin in biological fluids, even though we have not yet fully investigated this aspect.

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