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ANALYSIS OF PLATINUM COMPLEXES BY LIQUID CHROMATOGRAPHY WITH QUENCHED PHOSPHORESCENCE DETECTION

C. GOOIJER*, A. C. VELTKAMP, R. A. BAUMANN, N. H. VELTHORST and R. W. FREI
Department of General and Analytical Chemistry, Free University, De Boelelaan 1083, 1081 HV Amsterdam (The Netherlands)
and
W. J. F. VAN DER VIJGH
Research Laboratory of Internal Medicine, Free University Hospital, De Boelelaan 1117, 1007 MB Amsterdam (The Netherlands)
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SUMMARY

Quenched biacetyl phosphorescence is shown to be a promising method for the detection of platinum(II) complexes in liquid chromatography. The analyses were performed on a solvent-generated anion-exchange column prepared by coating RP-18 material with hexadecyltrimethylammonium bromide. The detection limits calculated from the chromatograms for cisplatin (CDDP) and carboplatin (CBDCA) are $3.0 \cdot 10^{-7}$ *M* and $3.3 \cdot 10^{-7}$ *M*, respectively. Experiments on urine samples reveal that CDDP and CBDCA can be observed separately, although interference effects from phosphorescence-quenching compounds in the matrix are not negligible. This problem can probably be solved by using precolumn sample-handling techniques.

INTRODUCTION

Platinum coordination complexes are well known agents with antitumour activity[1]. Cisplatin [CDDP, *cis*-dichlorodiammineplatinum(II)] is widely used in the treatment of solid tumours[2]. Because of its toxic side effects, however, intensive research is being undertaken to obtain platinum compounds with a higher therapeutic index[3]. Carboplatin [CBDCA, *cis*-diammine-1,1-cyclobutanedicarboxylateplatinum(II)] and iproplatin [CHIP, *cis*-dichlorodiisopropylamine-*trans*-dihydroxyplatinum(IV)] seem most promising cisplatin analogues[4,5].

It is a challenge for analytical chemists to develop selective and sensitive methods to quantitate the administered platinum compounds and their metabolites in blood plasma and urine samples. Especially the combination of liquid chromatography with electrochemical detection has been applied[6–10]. A post-column reaction detector has also been developed[11]. The detection limits obtained for CDDP are sufficiently low. For other platinum compounds, however, the sensitivity of detection still seems to be a problem.

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We have developed a detection method based on quenched room temperature phosphorescence in liquids QRTPL[12–14]. In the present paper we show that various platinum-complexes can be quantitated sensitively with this detector. High-performance liquid chromatography (HPLC) of CDDP and CBDCA based on a solvent-generated anion-exchange system[15] is presented. Measurements of these compounds in urine indicate that the HPLC–QRTPL system is promising.

EXPERIMENTAL

Batch experiments

The batch experiments were carried out on a Perkin-Elmer MPF-44 fluorescence spectrometer. Details of the procedure, including the deoxygenation step, have been given before[16]. The bimolecular rate constants for the quenching components were determined as described[12]. The lifetimes of biacetyl in its triplet state were derived from the phosphorescence to fluorescence ratio as outlined in ref. 17.

HPLC

The experimental set-up has been described in detail before[18]. The modified eluent vessel of ref. 13 was used and if possible (see below) the electronic signal inverter of ref. 14.

The linear dynamic ranges for the platinum-compounds were determined with the Kontron SFM 22 fluorescence detector in combination with the signal inverter. For the other liquid chromatograms the Perkin-Elmer MPF-44 was applied together with a quartz flow-through cuvette (pathlength 1 cm, cell volume 20 μl).

The excitation wavelength was 415 nm and the emission wavelength 520 nm; the excitation and emission slit widths in the Kontron spectrometer were 10 nm, in the Perkin-Elmer 20 nm. Unfortunately, the home-made signal inverter[14] (inverting linearly spectrometer outputs from 100 to 8 mV) could not be used directly after the Perkin-Elmer MPF-44 because its amplifier output is less than 10 mV. For this reason, the non-inverted phosphorescence signal was recorded, although theoretically its decrease is not a linear function of the analyte concentration[12]. However, deviations from linearity for the platinum compounds under investigation are only visible at concentrations exceeding $1 \cdot 10^{-5}$ *M*.

The Perkin-Elmer MPF-44 was applied because of its favourable signal to noise ratio for the quenched phosphorescence signal. It is emphasized that the noise at the top of the phosphorescence signal is important since the decrease in the signal is measured, so that the source stability presumably plays an important rôle. Contrary to earlier reports[12], the most favourable limits of detection (LODs) were obtained using higher biacetyl concentrations than previously applied, *i.e.*, about 10^{-2} *M*.

The chromatography was performed on an ODS Hypersil column (10 cm × 4.6 mm I.D., particle size 5 μm spherical), prepared with hexadecyltrimethylammonium bromide (HTAB) according to Riley *et al.*[15]. As eluents, water–methanol mixtures were applied with a variable methanol content (0–10%, v/v), a variable concentration of citrate buffer (10^{-3}–10^{-2} *M*) and a variable pH (5.0–6.2), 10^{-2} *M* biacetyl and $2 \cdot 10^{-5}$ *M* HTAB.

Chemicals

Reagent-grade samples were used throughout. Deionized water was distilled before use. Crystalline samples of cisplatin, carboplatin and iproplatin were obtained from the Academic Hospital of the Free University (Amsterdam). Stock solutions in 0.15 *M* sodium chloride were applied within 3 days.

RESULTS AND DISCUSSION

Batch experiments

The QRTPL detection method is based on measurement of the room temperature phosphorescence of biacetyl present as a solute in the (deoxygenated) eluent. Analytes able to interact with biacetyl in the excited (electronic triplet) state cause a decrease in the phosphorescence signal intensity from I_0 to I. This decrease can be calculated[12] from

$$\frac{I_0}{I} = 1 + k_A \tau_0^B [A] \tag{1}$$

where τ_0^B is the triplet state lifetime (sec) of biacetyl in the absence of analyte, k_A the bimolecular rate constant of the quenching reaction (M^{-1} sec^{-1}) and [A] the analyte concentration (M).

It is obvious from eqn. 1 that the sensitivity of the QRTPL detector for a particular analyte is determined by k_A. Previously[12] we have estimated that, assuming $\tau_0^B = 5 \cdot 10^{-4}$ sec the LOD (expressed in M) that can be reached is equal to 10 k_A^{-1}. Of course the sensitivity depends also on τ_0^B and thus on the amount of oxygen and quenching impurities in the eluent.

Table I gives k_A values for a number of platinum-compounds. It is seen that the rate constants for CDDP and CBDCA, although they are smaller than for $PtCl_4^{2-}$, give rise to interesting LOD values, *i.e.*, between 10^{-8} and 10^{-7} M. Presumably the quenching is based on electron transfer from the platinum ion to the excited biacetyl molecule. Therefore we expect that platinum(II) compounds can be observed via QRTPL quite generally, but not platinum(IV) compounds. This is in line with the fact that, for CHIP, a platinum(IV) derivative, we did not observe any quenching.

The above data suggest the application of QRTPL as a platinum(II) detector

TABLE I

k_A VALUES AND EXPERIMENTAL LODs OF SOME PLATINUM COMPOUNDS MEASURED BATCHWISE

Biacetyl concentration: 1 10^{-2} M.

Compound	k_A (M^{-1} sec^{-1})	*LOD (M)*	*Solvent*
$PtCl_4^{2-}$	$6 \cdot 10^9$	*	Water
CDDP	$7 \cdot 10^8$	$2 \cdot 10^{-8}$	0.15 *M* Aqueous NaCl
CBDCA	$4 \cdot 10^8$	$8 \cdot 10^{-8}$	0.15 *M* Aqueous NaCl
CHIP	$<10^6$	*	0.15 *M* Aqueous NaCl

* Not measured.

in liquid chromatography, provided that τ_0^B in the eluents applied is similar to that in water or in 0.15 *M* aqueous sodium chloride (see eqn. 1). Especially the presence of HTAB containing the heavy bromine atom might influence τ_0^B. Fortunately, only a slight reduction was observed; for a typical eluent composition, τ_0^B was found to be $3 \cdot 10^{-4}$ sec (in water, $4 \cdot 10^{-4}$ sec).

Liquid chromatography

A liquid chromatogram obtained for 20 µl CDDP ($6.1 \cdot 10^{-6}$ *M*) and CBDCA ($8.3 \cdot 10^{-6}$ *M*) in about 0.15 *M* sodium chloride, with QRTPL detection without signal inversion, is shown in Fig. 1. The peaks of CBDCA and CDDP are clearly seen; their retention times are 2.5 and 4.1 min, respectively. Peak 1 probably originates from Br^- generated by ion exchange between Cl^- in the sample and HTAB on the column. The k_A value for Br^- has been reported[19] to be $6 \cdot 10^7$ M^{-1}; for Cl^- it is only $1.2 \cdot 10^3$ M^{-1} sec^{-1}. As a result of the ion-exchange process, the column contains, besides HTAB, a small amount of HTACl. This explains, presumably, the increase of the phosphorescence intensity in the range *ca.* 5.5–8.1 min. Chloride ions are gradually eluted from the column so that in the eluent HTAB is partially replaced by HTACl which leads to an increase in τ_0^B and thus to an increase in I_0[17]. The origin of peak 2 is not completely clear. It might be caused by the lack of biacetyl in the sample plug.

The signal $I_0 - I$ varies linearly with the CBDCA and CDDP concentrations up to about 1×10^{-5} *M*. LODs calculated from the chromatograms are $3.0 \cdot 10^{-7}$ *M* for CDDP (1.8 ng in 20 µl) and $3.3 \cdot 10^{-7}$ *M* for CBDCA (2.4 ng in 20 µl). These data compare favourably with LODs obtained via other detection techniques, see Table II.

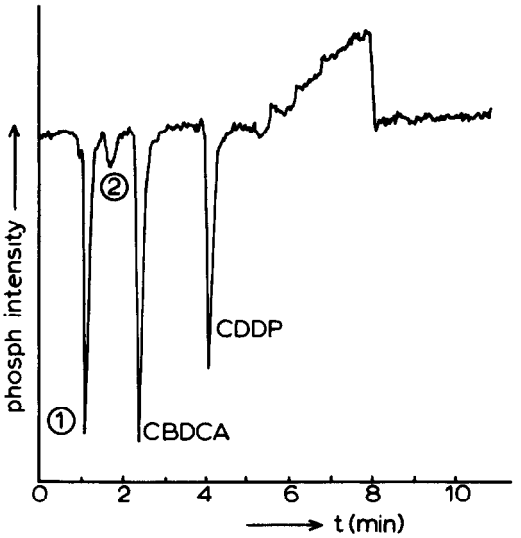

Fig. 1. Quenched phosphorescence chromatogram of CDDP ($6.1 \cdot 10^{-6}$ *M*) and CBDCA ($8.3 \cdot 10^{-6}$ *M*) in 0.15 *M* aqueous NaCl. The chromatographic conditions are given in the text; the methanol content of the mobile phase was 1%, v/v, the citrate buffer concentration $1 \cdot 10^{-3}$ *M* and the pH 5.0. Injection volume: 20 µl. Flow-rate: 1.1 ml/min. For peaks 1 and 2, see text

TABLE II

COMPARISON OF LODs (ng/ml) FOR CDDP AND CBDCA DERIVED FROM LIQUID CHROMATOGRAMS WITH DIFFERENT DETECTION METHODS

Ref.	*Detection method*	*CDDP*	*DBDCA*
11	UV	20,000	20,000
11	UV after derivatization	40	1200
8	Electrochemical	15	15 After precolumn derivatization
10	Chloride-assisted electrochemical	50	Not measured
This work	QRTPL	90	122

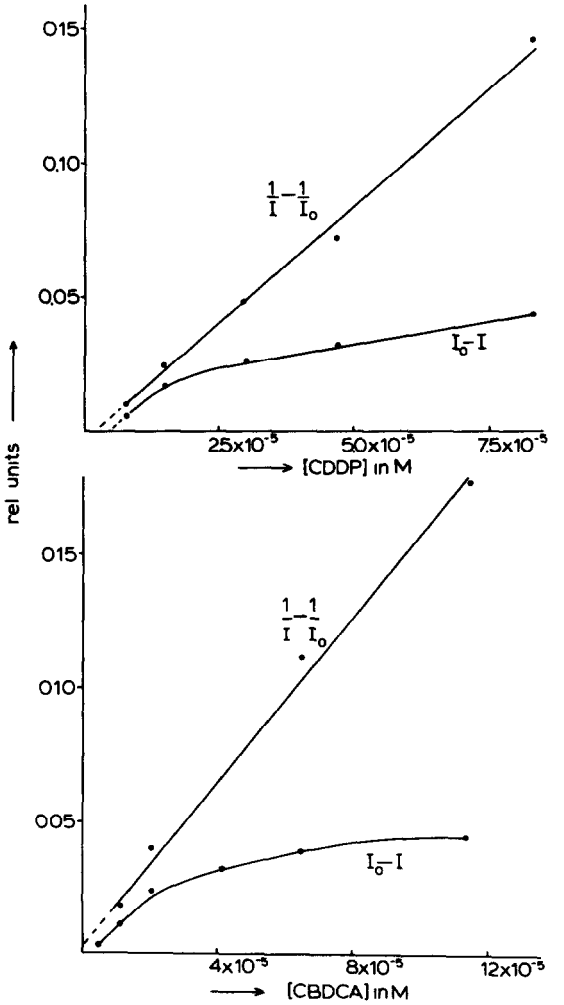

Fig. 2. Calibration curves of the non-inverted and the inverted phosphorescence signals for CDDP and CBDCA. Conditions as in Fig. 1.

The linearity of the QRTPL detector can be considerably extended if the inverted signal, I^{-1}, is measured instead of I. Calibration curves for CDDP and CBDCA obtained with the Kontron SFM 22 fluorimeter combined with the home-made signal inverter are shown in Fig. 2. The plots are linear up to about 10^{-4} M. At higher concentrations problems were encountered with regard to solubility.

CDDP and CBDCA in urine

It is interesting to find out whether the HPLC–QRTPL combination is also applicable to urine samples. Matrix effects must be expected because urine is known to contain quenching compounds such as ureas[20].

In Figs. 3 and 4 are shown chromatograms of urine samples. No pretreatment was employed: the urine sample spiked with CDDP and CBDCA was only diluted in eluent (in Fig. 3, seven times; in Fig. 4, eleven times). The main difference between the experimental conditions is the methanol content of the eluent: in Fig. 3 no methanol is used; in Fig. 4 the methanol fraction is 10%, v/v. The pH difference is only of minor importance.

In Fig. 3 only the CDDP peak is observed. Apparently, the CBDCA signal is hidden under the strong matrix signal with a retention time of around 2.8 min. However, in Fig. 4 both the CDDP and the CBDCA peak can be observed separately, although the interference effects are not negligible.

These results imply that precolumn sample handling both for clean-up of urine and preconcentration of the platinum compounds is necessary. We expect that such a procedure will be successful because the retention behaviour of the urine compo-

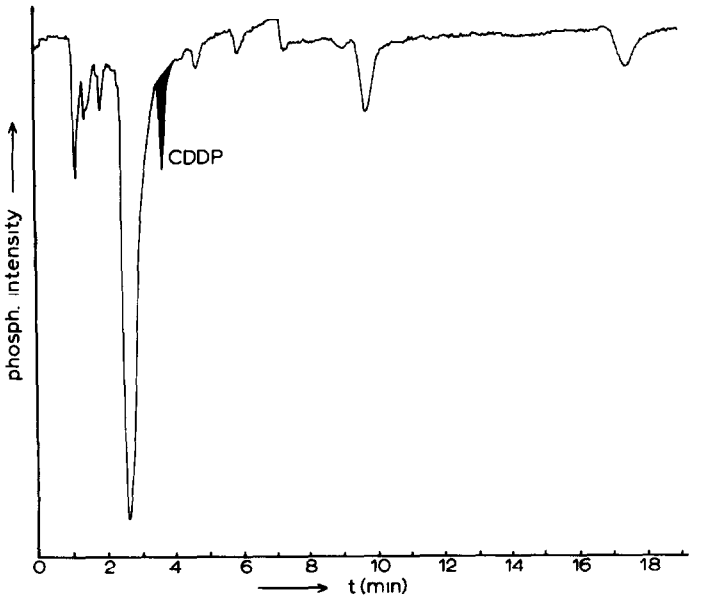

Fig. 3. Quenched phosphorescence chromatogram of urine (diluted 1:7 in eluent) spiked with CDDP ($1.0 \cdot 10^{-5}$ M) and CBDCA ($1.4 \cdot 10^{-5}$ M). Chromatographic conditions are given in the text; the eluent contained no methanol, the citrate buffer concentration was $1 \cdot 10^{-2}$ M and the pH 5.0. Injection volume 20 μl. Flow-rate: 1.1 ml/min.

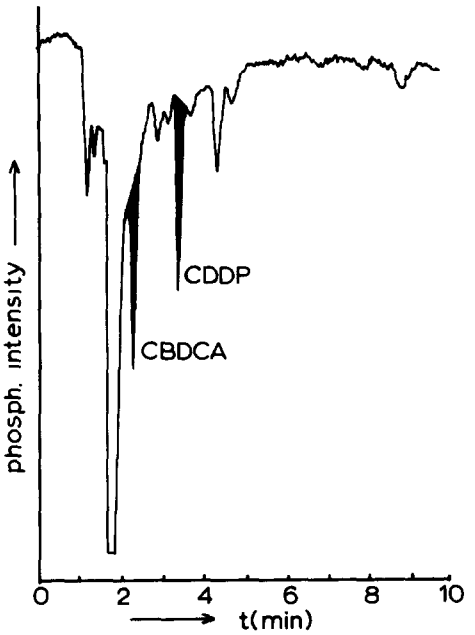

Fig. 4. Quenched phosphorescence chromatogram of urine (diluted 1:11 in eluent), spiked with CDDP ($1.7 \cdot 10^{-5}$ *M*) and CBDCA ($2.5 \cdot 10^{-5}$ *M*). Chromatographic conditions are given in the text; the methanol content of the mobile phase was 10%, v/v, the citrate buffer concentration $1 \cdot 10^{-2}$ *M* and the pH 6.2. Other conditions as in Fig. 3.

nents differs significantly from that of platinum derivatives. In fact the influence of methanol as visualized in Figs. 3 and 4 indicates that in addition to the ion–dipole interactions also hydropohobic interactions play a rôle.

CONCLUSIONS

Quenched-RTPL detection in HPLC is promising for the quantitation of platinum(II) compounds. Its sensitivity for CDDP and CBDCA is almost equal (LODs $3.0 \cdot 10^{-7}$ and $3.3 \cdot 10^{-7}$ *M*); its linearity is more than two decades.

Measurements in urine require on-line sample-handling techniques for clean-up and preconcentration. Experiments along these lines are currently underway.

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