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Short communication

Determination of platinum by ion-pair reversed-phase high-performance liquid chromatography with 4,4′-bis(dimethylamino)thiobenzophenone

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Abstract

Platinum (II) was separated and determined by ion-pair reversed-phase high-performance liquid chromatography with spectrophotometric detection at 520 nm. 4,4′-Bis(dimethylamino)thiobenzophenone (TMK) was used as a precolumn complexing agent. The optimum conditions for the separation and determination of the Pt(II)–TMK complex were investigated. The complex was separated on a Nucleosil C_{18} column (250 mm × 4.0 mm I.D.) with tetrahydrofuran–water (48:52, v/v) containing 0.035 *M* acetic acid–sodium acetate buffer (pH 3.5), 0.20 *M* sodium perchlorate and $8.5 \cdot 10^{-5}$ *M* TMK. The proposed method was applied to the determination of platinum in cisplatin and carboplatinum samples.

1. Introduction

The separation and determination of mixtures of platinum metals as their complexes with organic reagents by high-performance liquid chromatography (HPLC) has received increasing attention and has been used in platinum metals analysis in recent years. Sometimes this technique has advantages over other instrumental methods of analysis, e.g., atomic absorption spectrometry or voltammetry, with regard both to detection limits and time requirements, which are of special importance in routine analyses of large numbers of sample.

The complexing agents that have been used for HPLC determination of platinum metals can be divided into two groups. The first group contains a nitrogen donor atom, such as 4-(2-pyridylazo)resorcinol [1], 1-(2-pyridylazo)-2-naphthol [2,3], 2-(5-bromo-2-pyridylazo)-2-diethylaminophenol [4], 2-(5-bromo-2-pyridylazo)-5-(N-propyl-N-sulphopropylamino)phenol [5], 1-(2-thiazolylazo)-2-naphthol [6], 2-(2-thiazolylazo)-5-diethylaminophenol [7], 2-(6-methylbenzolthiazolylazo)-5-diethylaminophenol [8] and 8-hydroxyquinoline [9,10]. The second group contains a sulphur donor atom, such as malconitriledithiol [11], disubstituted dithiocarbamate [12–14], 1-hydroxy-2-pyridinethione [15] and diphenylthiourea [16]. Amongst the chromophoric agents, only diethyldithiocarbamate (DDTC) has been used for the determination of platinum in cisplatin [*cis*-dichlorodiammineplatinum (II)], an anti-cancer agent, as a complex of platinum based on precolumn derivatization [17–21]. To date, there have been no

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reports on the separation and determination of platinum in carboplatinum [*cis*-diammine-1,1-cyclobutyldicarboxylate platinum(II)], another anti-cancer drug, as a complex of platinum with any chromophoric agent using HPLC.

The sensitive co-colour reaction of Pt(IV) and Pd(II) with 4,4′-bis(dimethylamino)thiobenzophenone (TMK) in the presence of ascorbic acid and Triton X-100 has been used previously for the spectrophotometric determination of platinum and palladium [22]. Using this reagent, the conditions for the precolumn derivatization and for the separation and determination of platinum in cisplatin and carboplatinum as complexes with TMK by ion-pair (IP) reversed-phase (RP) HPLC were investigated in this work.

2. Experimental

2.1. Apparatus

Liquid chromatography was performed using a Shimadzu Mode LC-4A HPLC instrument equipped with an SPD-1 spectrophotometric detector and a Chromatopac C-R2A data processor. A Nucleosil C_{18} column (250 mm × 4.0 mm I.D.) with a particle size of 5 μm was used in all experiments. A Shimadzu UV-240 recording spectrophotometer was used for spectral measurements. A Shanghai Model pH S-2 pH meter was also used.

2.2. Reagents and solutions

A stock standard solution of Pt(IV) was prepared by dissolving platinum wire (99.95%) in aqua regia on a hot-plate, evaporating nearly to dryness. The residue was treated with concentrated hydrochloric acid and evaporated to a small volume. After repeating this process three times to destroy oxides of nitrogen, the reaction mass was dissolved in and diluted with 1 *M* hydrochloric acid to give a concentration of 1.00 mg ml^{-1} of platinum. Working standard solutions of the required strength were prepared by appropriate dilution with water. A stock standard solution of Pd(II) was prepared by dissolving $PdCl_2$ in 1 *M* hydrochloric acid to give a concentration of 1.00 mg ml^{-1} of palladium. Working standard solutions of the required strength were prepared by appropriate dilution with water.

4, 4′ - Bis(dimethylamino)thiobenzophenone (Third Chemicals Factory of Shanghai) was dissolved in ethanol to give a concentration of 0.04% (w/v), stored in a brown-coloured bottle and kept in a refrigerator (a solution was prepared freshly every week). An ascorbic acid solution (8%, w/v) was prepared by dissolving the compound in water, adjusting the pH to 7 with dilute sodium hydroxide solution, and then kept in a refrigerator (a solution was prepared freshly every 3 days).

The mobile phase was tetrahydrofuran–water (48:52, v/v) containing 0.035 *M* acetic acid–sodium acetate buffer (pH 3.5), 0.20 *M* sodium perchlorate and $8.5 \cdot 10^{-5}$ *M* TMK, prepared freshly each day. All other chemicals were of analytical-reagent grade.

2.3. Procedure

To a slightly acidic solution containing 0.2–25 μg of Pt(IV) in a 10-ml calibrated flask, add 10 μg of Pd(II) solution and 1 ml of 8% ascorbic acid solution and mix thoroughly. After 1 min, add 2 ml of 2 *M* acetic acid–sodium acetate buffer solution (pH 3.5) and mix again thoroughly. After a further 3 min, add 1.5 ml of 0.04% TMK solution and 4 ml of 10% Triton X-100 solution and heat the mixture in a boiling water-bath for 20 min. After cooling immediately in tap water, dilute to volume with water. Filter the solution through a 0.3-μm membrane (mixed cellulose) and inject a 20-μl aliquot of the filtered solution on to the column. Elute the Pt(II)–TMK complex with the tetrahydrofuran–water mobile phase at a flow-rate of 0.6 ml min^{-1} and detect the complex in the eluate at 520 nm. Determine the amount of platinum by measuring the peak height.

Sample analysis

A cisplatin sample, or a carboplatinum sample, was dissolved in concentrated nitric acid–perchloric acid (1:10) on a hot-plate and evaporated nearly to dryness. This process was repeated twice to destroy organic chain bonds in the sample. The resulted residue was dissolved in 2 ml of 1 *M* hydrochloric acid by warming on the hot-plate. The solution was transferred into a 50-ml calibrated flask and diluted to volume with 1 *M* hydrochloric acid. An aliquot of this solution was diluted to volume with water in another 50-ml calibrated flask. A 1-ml aliquot of the last sample solution was taken and the Pt(II)–TMK complex was formed by reaction with TMK and determination by IP-RP-HPLC as described above.

3. Results and discussion

3.1. Selection of conditions for pre-column derivatization and detection wavelength of the Pd(II)–TMK complex

In a solution containing Pd(II), ascorbic acid and Triton X-100 in the pH range 2.5–4.2, buffered with acetic acid–sodium acetate, Pt(II) forms a violet complex with TMK at 100°C, exhibits an absorption maximum at 520 nm and has a molar absorptivity of $2.90 \cdot 10^5$ l mol^{-1} cm^{-1}. Ascorbic acid has the function of reducing Pt(IV) to Pt(II), which reacts with TMK to form the Pt(II)–TMK complex; Pd(II) plays a catalytic role in accelerating the colour development and making the reaction more completely. Although Pd(II) alone reacts with TMK to form an orange-red complex, the complex is labile and is decomposed rapidly at 100°C, and the amount of Pd(II) present in the sample solution does not affect the peak height of the Pt(II)–TMK complex.

In order to accelerate both the decomposition of the Pd(II)–TMK complex and the colour development of the Pt(II)–TMK complex, the sample solution had to be heated in a boiling water-bath for 15–20 min. As the reagent and the complex are insoluble in water, the concentration of Triton X-100 as a solubilizing agent in the sample solution should not be less than 4%. Reagents should be added to the solution containing Pt(IV) and Pd(II) as in the sequence described, as any change in the sequence affects the colour development of the Pt(II)–TMK complex.

The Pt(II)–TMK complex formed under the above-mentioned conditions had a stable peak height for at least 3 h.

3.2. Effect of concentration of tetrahydrofuran in the mobile phase

Some organic solvents, such as methanol, ethanol, isopropyl alcohol, acetone, acetonitrile and tetrahydrofuran, combined with water, were investigated as binary and ternary mobile phases. The tetrahydrofuran–water binary system was found to be suitable for the separation of the Pt(II)–TMK complex. A simple tetrahydrofuran–water mobile phase, however, gave a poor peak shape and low sensitivity; moreover, with a delay of injection of the sample solution, the peak height of the Pt(II)–TMK complex decreased distinctly because part of the complex was decomposed on the column. When acetic acid–sodium acetate buffer, sodium perchlorate and TMK were added to the mobile phase in order to suppress the decomposition of the complex, an excellent peak shape and high sensitivity were obtained. The effect of the concentration of tetrahydrofuran in the mobile phase on the retention time and peak height of the complex is shown in Fig. 1. The optimum results were obtained with tetrahydrofuran–water (48:52, v/v).

3.3. Effect of pH of buffer added to the mobile phase

The effect of the pH of the buffer to be added to the mobile phase containing 0.20 *M* sodium perchlorate and $8.5 \cdot 10^{-5}$ *M* TMK was examined in the pH range 2.5–5.0 by using acetic acid–sodium acetate. A good peak shape and a higher peak of the Pt(II)–TMK complex were obtained in the pH range 3.0–4.0, but below or above this

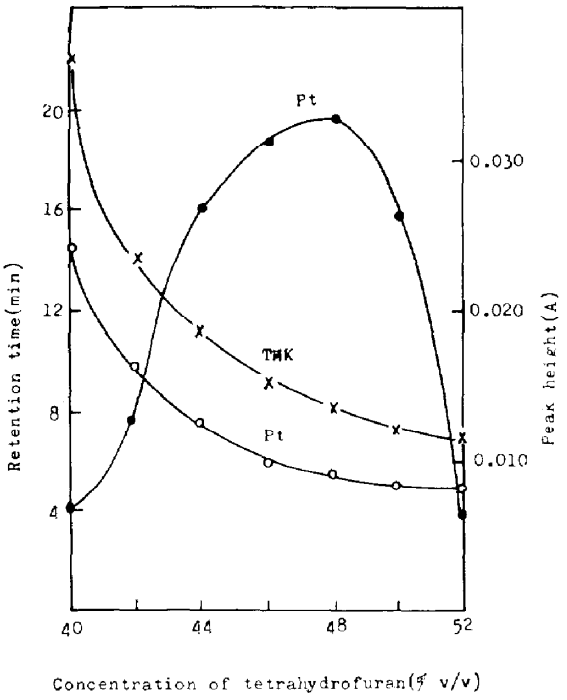

Fig. 1. Effect of concentration of tetrahydrofuran in the mobile phase on the retention time and the peak height of the complex of Pt(II)–TMK. Mobile phase, 0.035 *M* acetic acid–sodium acetate (pH 3.5), 0.20 *M* sodium perchlorate and $8.5 \cdot 10^{-5}$ *M* TMK, flow-rate, 0.6 ml min^{-1}; column, Nucleosil C_{18} (250 mm × 4.0 mm I.D.). × = Retention time of TMK; ○ = retention time of Pt; ● = peak height of Pt.

range the peak shape and peak height became worse because protonation and decomposition of the ligand occurred at high and low pH, respectively. Therefore, acetic acid–sodium acetate buffer solution of pH 3.5 was added to the mobile phase to give a concentration of 0.035 *M*.

3.4. Effect of TMK concentration in the mobile phase

If there was no TMK in the mobile phase, the peak height of the Pt(II)–TMK complex was very low. Because it is poorly stable, the complex injected may be gradually dissociated owing to dilution by a large volume of mobile phase without adding TMK. To suppress the decomposition of the complex, TMK was added to the mobile phase. An almost constant peak height was obtained at TMK concentrations greater $5 \cdot 10^{-5}$ *M*. The optimum concentration was obtained with $8.5 \cdot 10^{-5}$ *M* TMK in the mobile phase.

3.5. Effect of concentration of sodium perchlorate in the mobile phase

The effect of the concentration of sodium perchlorate in the mobile phase was investigated. As expected, the greater the concentration of sodium perchlorate, the longer was the retention time and the higher was the peak of the Pt(II)–TMK complex, and the better the resolution of peaks for the complex and unreacted reagent became. It is thought that the retention time of the complex increases on increasing the distribution of the complex to the stationary phase, based on the formation of an ion pair between the complex cation [22], $[Pt(TMK)_4]^{6+}$, and perchlorate anion. A concentration of 0.2 *M* sodium perchlorate in the mobile phase was selected.

3.6. Chromatogram and calibration graph

A typical chromatogram for the separation of the Pt(II)–TMK complex and unreacted reagent is shown in Fig. 2. The peak-height calibration graph was linear over the Pt(IV) concentration range 0.02–2.5 $\mu g\ ml^{-1}$, which is represented by the equation y (absorbance) $= 0.0274x(\mu g\ ml^{-1}) + 0.0084$. The absolute detection limit, calculated as the amount injected that gave a signal that was three times the background noise (i.e., a signal-to-noise ratio of 3:1), was 0.11 ng.

3.7. Effect of foreign ions

The effect of possible interferences was studied by adding each foreign ion in turn to the sample before precolumn derivatization of the Pt(II)–TMK complex. The maximum levels (in μg) of foreign ions which gave a change of less than ±5% in the peak height of the Pt(II)–TMK complex that could be tolerated in the determination of 10 μg of platinum were as follows:

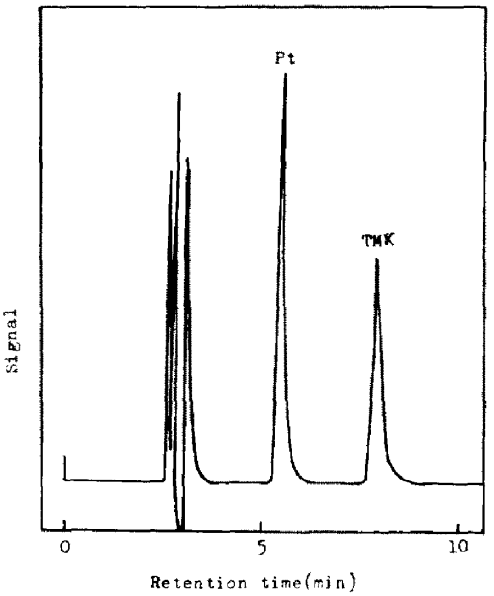

Fig. 2. Chromatogram of Pt(II)–TMK complex on a Nucleosil C_{18} column (250 × 4.0 mm I.D.) with a 20-μl injection of a solution containing 10 ppm of Pt(IV) and 20 ppm of Pd(II) using tetrahydrofuran–water (48:52, v/v) containing 0.035 *M* acetic acid–sodium acetate buffer (pH 3.5), 0.2 *M* sodium perchlorate and $8.5 \cdot 10^{-5}$ *M* TMK as the mobile phase at a flow-rate of 0.6 ml min^{-1}. Detection wavelength, 520 nm.

Cd(II), Co(II), Fe(II, III) and Ni(II), 40; Ru(IV), Rh(III), Pd(II), Os(VIII) and Ir(IV), 20; and Ag(I), Au(III), Cu(II) and Hg(II), 10. Of the metal ions tested, only Pb(II) caused a serious interference in the determination of platinum.

Table 1
Determination of platinum in cisplatin and carboplatinum

Added (μg)		Found[a] (μg)	
Cisplatin	Carboplatinum	Cisplatin	Carboplatinum
0.50	0.50	0.48	0.49
1.00	1.00	1.02	0.98
5.00	5.00	5.03	4.92
10.0	10.0	10.2	9.84
20.0	20.0	20.3	19.5

[a] Average of two parallel determinations.

3.8. Application to the determination of platinum in cisplatin and carboplatinum

The results for the determination of platinum in cisplatin and carboplatinum are given in Table 1 and show good agreement with the standard values, with satisfactory accuracy.

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