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Direct Determination of *cis*-Dichlorodiammineplatinum(II) in Urine by Derivative Spectroscopy

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A derivative spectrophotometric method for the determination of the antitumor agent *cis*-dichlorodiammineplatinum(II) in urine has been developed. The method is based on the complexation of *cis*-dichlorodiammineplatinum(II) with *N*,*N*′-bis(3-mercapto-2-quinoxalinyl)-1,3-propanediamine. Optimum conditions for quantification of *cis*-dichlorodiammineplatinum(II) were established. The complex formed was stable in urine, and the recovery of platinum from urine was 92—95%. Interference arising from urine can be avoided by measurement of the second-order derivative spectrum. The relative standard deviations of the present method were 3.9% and 1.9% for the determination of 0.2 and 2.0 ppm of platinum in urine, respectively.

Keywords——*cis*-dichlorodiammineplatinum(II); urine; platinum complex; derivative spectroscopy; antitumor drug

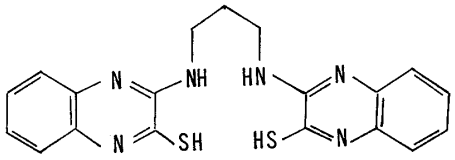
Introduction

The antitumor agent, *cis*-dichlorodiammineplatinum(II), *cis*-DDP, had recently been introduced into wide clinical use. In clinical practice, severe nephrotoxicity has been reported as a toxic side effect. Further, *cis*-DDP tends to accumulate in the body.[1] It is therefore important to monitor Pt(II) excreted into urine. The administration of *cis*-DDP with diuresis and fluid hydration has been carried out in order to avoid acute renal failure. The urinary excretion of *cis*-DDP during the first 24 h is rapid, but after that, *cis*-DDP is excreted very slowly over the long period. The majority of the platinum complex excreted into urine appears to be unchanged *cis*-DDP (90%).[2]

Several methods have been reported for the determination of *cis*-DDP in urine. The method based on high-performance liquid chromatography (HPLC) with an on-line spectrophotometric detector has the advantage of direct detection of *cis*-DDP, but its sensitivity is poor in the determination of urinary platinum.[3] Although non-flame atomic absorption spectrophotometry provides the necessary sensitivity for the determination of urinary platinum, it suffers from matrix effects arising from urine.[4-7] Recently a method involving HPLC with an electrochemical detector has been reported,[8-10] but the requirement for special apparatus restricts the use of this method.

This paper describes a very simple method for determination of urinary platinum. In the case of biological samples such as urine, solvent extraction can be difficult because of emulsification at the urine–solvent interface. Therefore, we have tried to utilize a direct color reaction between a reagent and *cis*-DDP. Such a reagent should be a chelating agent containing soft donor atoms, and in the present work, *N*,*N*′-bis(3-mercapto-2-quinoxalinyl)-1,3-propanediamine (MQP) was used. The reagent and its platinum complex were so insoluble in water that all experiments were carried out in 50% (v/v) dimethylformamide (DMF)–urine (or 50% DMF–water) solution.

It is well known that substitution reactions of platinum(II) complexes are slow.[11] This was also the case in the present work. However, the difference in reaction rate between



N,N'-bis-(3-mercapto-2-quinoxalinyl)-1,3-propanediamine

platinum(II) and other metal ions would be expected to provide a basis for a selective analytical method for platinum(II). Although the present method is time-consuming, the analytical procedure is very simple, and the selectivity for the determination of platinum is high.

Experimental

Apparatus——Spectral measurements were made with a Hitachi 557 dual-wavelength double-beam spectrophotometer using quartz cells of 1 cm path length. The apparatus allows the direct recording of first- to fourth-order derivative absorbance spectra.

Reagents——The chelating agent, MQP, was synthesized by allowing 2,3-quinoxalinedithiol to react with an equimolar amount of 1,3-propanediamine in ethanol.[12] The MQP stock solution (5×10^{-3}) was prepared by dissolving MQP in DMF. *cis*-DDP was prepared according to the literature.[13] The *cis*-DDP stock solution (300 ppm of *cis*-DDP) was prepared by dissolving *cis*-DDP in deionized water, just before use. A solution containing hydrolysis products of *cis*-DDP was prepared by allowing the *cis*-DDP stock solution to stand for more than 1 week without shielding from light. The concentration of platinum in *cis*-DDP stock solution was verified by atomic absorption spectrophotometery.[14] 2-(*N*-Morpholino)ethanesulfonic acid (MES) buffer solution was prepared by dissolving MES in deionized water and adjusting the pH appropriately with 10% sodium hydroxide and 10% hydrochloric acid solutions. All the other reagents were of analytical reagent grade and were used without further purification. Human urine was collected from volunteers not undergoing *cis*-DDP therapy.

General Analytical Procedure——Urine containing 1.50 ppm of *cis*-DDP (0.98 ppm at Pt) was adjusted to pH 6.5 by adding 10% NaOH or 10% HCl solutions. A 5 ml aliquot of the urine, 4.0 ml of DMF and 1.0 ml of the reagent solution (MQP stock solution) were added to a 10 ml cyclindrical glass vial fitted with a glass cap, and the solution was then heated on a water bath (*ca.* 85 °C) for 24 h. After cooling, the solution was centrifuged to remove the precipitates. The second-order derivative spectrum of the supernatant solution was measured against the reference solution (described below), and the distance between the maximum (525 nm) and the minimum (510 nm) of the second-order derivative spectrum was measured. The reference solution was prepared as follows; a mixture of 5.0 ml of the urine and 4.0 ml of DMF was heated at 85 °C for 24 h. The mixture was allowed to cool, 1.0 ml of the reagent solution was added, and the whole was centrifuged. The resultant solution did not show any ultraviolet (UV) spectral change upon standing for at least 3 h at room temperature (20—25 °C), and can be used as a reference solution.

Results and Discussion

The absorption pattern of Pt–MQP complex in urine is shown in Fig. 1. The reagent, MQP, did not show any absorbance at wavelengths beyond 460 nm. The absorption spectrum of the Pt–MQP complex showed absorption maxima at 472 and 504 nm (curve b). Therefore, measurement of the absorbance of the Pt–MQP complex can be conducted without interference from the reagent. However, a problem in the determination of urinary platinum seems to be caused by the color of the urine. As can be seen in Fig. 1, the absorbance of the urine amounts to about half of that of Pt–MQP complex at 504 nm. This decreases the analytical precision. For this reason, we examined the problem in detail. It was found that (a) the color of urine was enhanced by heating, (b) on heating, the change in the color of urine depended on urinary pH (the color generally became darker with increasing pH), (c) the color of urine was also affected by the DMF content added to the urine (for instance, there was a slight difference between the spectra of 50% (v/v) DMF–urine solution and 40% (v/v) DMF–urine solution). In order to cancel out the effect of the color arising from urine, the following experiments were conducted. First, the reaction rate of *cis*-DDP with the reagent was

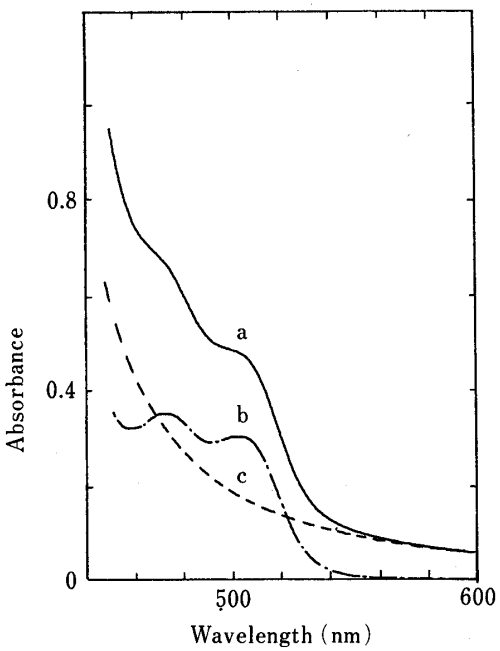


Fig. 1. Absorption Spectra of Pt–MQP Complex in Urine

[*cis*-DDP] = 3.0 ppm, pH = 6.5, and [MQP] = 5×10^{-4} M.
(a) Pt–MQP complex *vs.* aqueous blank; (b) Pt–MQP complex *vs.* urine blank; (c) Urine after heating at 85 °C for 24 h.

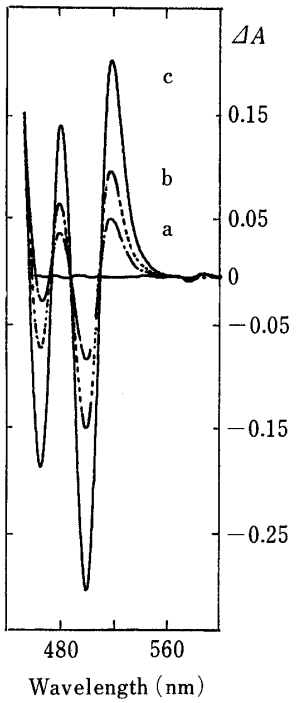


Fig. 2. Second-Order Derivative Spectra of Pt–MQP Complex in Urine ($\Delta\lambda = 5$ nm)

(a) 0.75 ppm of *cis*-DDP; (b) 1.5 ppm of *cis*-DDP; (c) 3.0 ppm of *cis*-DDP.

measured at room temperature, and it was found that the reaction of *cis*-DDP with MQP is negligible for up to 3 h because of the slow reaction rate. This means that blank-1 mentioned below can be used as a reference. Secondly, the following two urinary blank solutions were prepared by using platinum-free urine. Blank-1: 5.0 ml of platinum-free urine was mixed with 4.0 ml of DMF and then heated on a water bath for 24 h at 85 °C. The mixture was allowed to cool, 1.0 ml of reagent solution was added, and the whole was centrifuged for 5 min at 3000 rpm. The supernatant was used as blank-1. Blank-2: 5.0 ml of platinum-free urine, 1.0 ml of the reagent solution and 4.0 ml of DMF were mixed and then heated for 24 h at 85 °C. The supernatant after centrifugation was used as blank-2. The spectrum of blank-2 was measured against blank-1 as a reference. It showed absorbance values of 0.04 at 504 nm and 0.07 at 472 nm. This means that the effect of the color of the urine could not be cancelled out by using blank-1, probably because of the complicated changes of urinary pigments under heating. The absorbance of the Pt–MQP complex changes sharply in intensity with wavelength. On the other hand, the spectrum of urine merely shows a broad change with changing wavelength. Therefore, measurement of the derivative spectrum was expected to overcome the problem. The first-order derivative spectrum showed a slight decrease with decreasing wavelength. A completely horizontal baseline was observed in the second-order derivative spectrum (see Fig. 2). It was found that the effect arising from the color of urine could be completely avoided by measurement of the second-order derivative spectrum. Figure 2 shows second-order derivative spectra of the Pt–MQP complex in urine. It is clear that the distance (peak-to-peak value) between the maximum and minimum of the derivative spectra is unaffected by the color of urine and that the peak-to-peak value is proportional to the concentration of *cis*-DDP added to the urine. We concluded that the effect of the color of urine could be completely

cancelled out by measuring the second-order derivative spectrum when blank-1 is used as a reference.

In the present work, the reagent was allowed to react with three kinds of Pt compounds, K_2PtCl_4, *cis*-DDP and hydrolysis products of *cis*-DDP (dark blue solution). *cis*-DDP is easily hydrolyzed in water and forms various kinds of hydrolysis products. The hydrolysis products also yield several oligomers when they are allowed to stand for more than 1 week at room temperature without shielding from light, and the resultant solution shows a dark blue color. Therefore, the solution of *cis*-DDP was prepared just before used. The reaction solution of the reagent with K_2PtCl_4, and the hydrolysis products of *cis*-DDP gave exactly the same spectrum as *cis*-DDP (zero-order and second-order derivative spectra). This means that the present method can be applied to the determination of *cis*-DDP and also the hydrolysis products in urine.

Effect of pH

The pH of urine containing 15 μg of *cis*-DDP was varied from 4.0 to 7.5 by adding 10% NaOH and/or 10%HCl solutions. MQP and *cis*-DDP reacted quantitatively at these pH, *i.e.*, the peak to-peak value of the second-order derivative spectrum remained constant. In subsequent experiments, the pH of the urine sample was adjusted to pH 6.5, which is considered to be the pH of normal urine. The absorbance of the urine blank solution tends to increase with increasing pH, but the peak-to-peak value in the second-order derivative spectrum was unaffected.

Effect of Reagent Concentration

The reagent concentration was varied from 1.0×10^{-4} to 1.0×10^{-3} M at pH 6.5. The absorbance remained constant at concentrations above 2.0×10^{-4} M as shown in Fig. 3, *i.e.*, 5×10^{-4} M reagent sufficed for the purpose. All experiments were carried out with addition of 1 ml of 5×10^{-3} M reagent solution.

Effect of Heating Time

Generally, the reaction rate for complexation of platinum(II) complexes is so slow that the reaction should be conducted under heating. Figure 4 shows the time dependence of the reaction under heating at 85 °C. The reaction of *cis*-DDP with MQP was several times slower than that of K_2PtCl_4 with MQP because the Pt–N bond in *cis*-DDP is inert to substitution

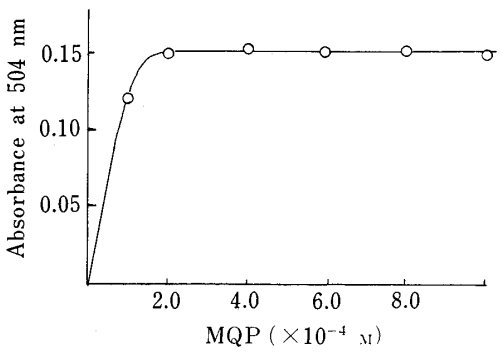


Fig. 3. Effect of MQP Concentration on Formation of Pt–MQP Complex in Urine

[*cis*-DDP] = 1.5 ppm, and pH = 6.5.

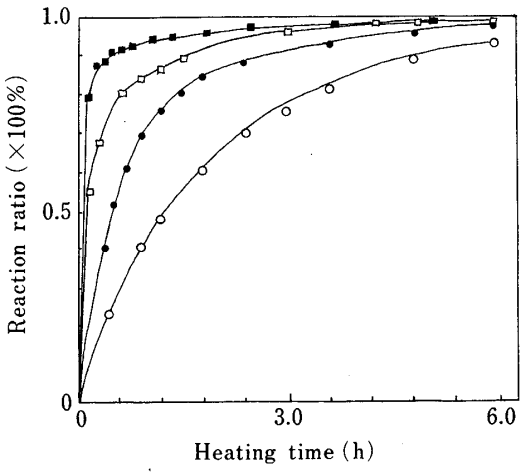


Fig. 4. , Effect on Heating Time on the Formation of Pt–MQP Complex

pH = 6.4, [MQP] = 5×10^{-4} M, [Pt] = 1.0 ppm.
○, *cis*-DDP in urine; ●, *cis*-DDP in MES buffer; □, K_2PtCl_4 in urine; ■, K_2PtCl_4 in MES buffer.

reaction. The reaction in MES buffer solution was several times faster than that in urine matrix. However, every reaction solution, after heating at 85 °C for 20 h, showed the same spectrum. In subsequent experiments, the reaction solution of platinum complexes with MQP was heated at 85 °C for 24 h.

The pattern in the absorption spectrum measured at various time periods was identical with the spectral pattern obtained after heating at 85 °C for 24 h. Therefore, the value shown in Fig. 3 appears not to arise from an intermediate, but to reflect the final product, Pt–MQP. Although the final product was heated for a further 24 h, the heating did not produce any measurable change in its absorption spectrum. It is therefore considered that the Pt–MQP complex is stable. On the other hand, the absorption spectrum of MQP itself changes slowly under heating, but apparently it did not have any effect on the spectrum of the Pt–MQP complex. After MQP itself had been heated at 85 °C for 24 h in MES buffer, *cis*-DDP was added to the solution and the mixture was then heated again at 85 °C for 24 h. The spectrum thus obtained was identical with the spectrum of the Pt–MQP complex shown in Figs. 1 and 2. Thus, the result of the analysis was not affected even though MQP itself was changing slowly under heating.

Recovery and Precision

A calibration curve was prepared by measuring the peak-to-peak value of the second-order derivative spectra of solutions prepared by the reaction of *cis*-DDP with the reagent in MES buffer solution ($Y=0.285\times[\mathrm{Pt}]+0.001$, $r=0.999$ ($n=5$)). Urine containing 1.5 ppm of *cis*-DDP (0.98 ppm of Pt) was treated according to the standard analytical procedure. The mean recovery was 92% ($n=8$). The precision of the present method was evaluated in terms of relative standard deviation. Eight independent analyses on *cis*-DDP-spiked urine samples containing 0.2 or 2.0 ppm of Pt gave relative standard deviations of 3.9% and 1.9%, respectively.

Effect of Various Ions

The metals normally present in urine did not interfere with the determination of *cis*-DDP in urine. Even if the trace metals in urine react with the reagent to give colored complexes, their effects may be cancelled out by using the urinary blank because of their relatively high reaction rate, compared with that of the platinum(II) complexes. This was confirmed by the following experiments in which urine was spiked with both *cis*-DDP and other transition metal ions. To 5 ml of urine containing 1.5 ppm of *cis*-DDP, 0.1 ml of 100 ppm Cu^{2+}, Cd^{2+}, Fe^{2+}, Zn^{2+}, or Hg^{2+} was added. Each mixture was treated according to the standard analytical procedure. A calibration curve was prepared by measuring the peak-to-peak values of the second-order derivative spectra of urine solutions containing 0.2—2.0 ppm of *cis*-DDP ($Y=0.263\times[\mathrm{Pt}]+0.002$, $r=0.999$ ($n=5$)). The recoveries of Pt in urine were 97% (Cu), 98% (Cd), 98% (Fe), 97% (Zn), and 103% (Hg). Accordingly it is concluded that these metals do not interfere with the determination of *cis*-DDP in urine.

Conclusion

A spectrophotometric method for determination of *cis*-DDP in urine was developed. Interference arising from urine could be removed by measurement of the second-order derivative spectrum. Although the present method is time-consuming, the sensitivity, selectivity, and reproducibility are high. Further, the analytical procedure is very simple. The recovery of *cis*-DDP from urine is approximately 90%. There does seem to be a very slight interference, especially in the case of concentrated urine such as first morning urine. However, the calibration curve obtained for urine spiked with *cis*-DDP showed excellent linearity, so the standard addition method may be recommended for clinical analysis.

Administration of *cis*-DDP is normally carried out together with administration of diuretics and a large amount of fluid hydration. For this reason, urine samples obtained after administration of *cis*-DDP appear to be almost colorless. In our trials, the use of urine collected after the volunteers had taken coffee showed a recovery of more than 95%.

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