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Spectrophotometric Determination of Creatinine using *o*-Hydroxyhydroquinonephthalein-Palladium(II) Complex[1]

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A sensitive spectrophotometric method for the determination of creatinine was established by using *o*-hydroxyhydroquinonephthalein(Qn.Ph.)-palladium(II)[Pd(II)] complex in the presence of polyvinyl alcohol (PVA) and sodium dodecyl sulfate (SDS) in weakly acidic media. This method is based on the decrease in absorbance of Qn.Ph.-Pd(II) complex at 615 nm, and could be used in the concentration range of 0.3—4 μg/10 ml of creatinine, where the Sandell sensitivity was estimated to be 0.00053 μg/cm². Jaffe chromogens scarcely affected the determination of creatinine. Recovery of creatinine added to human urine was examined.

Keywords——creatinine; spectrophotometry; *o*-hydroxyhydroquinonephthalein-palladium(II) complex; polyvinyl alcohol; sodium dodecyl sulfate

Recently, the determination of creatinine has become increasingly important in order to correct measured values[2] of cystathionine, hydroxyproline, δ-aminolevulinic acid, *etc.* The most widely used method for the determination of creatinine in routine analysis is the Folin–Wu method[3] utilizing the Jaffe reaction.[4] Though this method is simple and rapid, it suffers from interference by Jaffe chromogens,[5] such as protein, glucose, ascorbic acid, acetone bodies, *etc.* Therefore, it is necessary to remove these interfering substances by ether extraction or preliminary treatment with iodine.[5] In addition, this method is unsatisfactory as regards sensitivity.

In our previous papers, simple, rapid and sensitive spectrophotometric methods for the determination of cephalexin, ampicillin and thiourea utilizing the decrease in absorbance of the *o*-hydroxyhydroquinonephthalein(Qn.Ph.)-palladium(II)[Pd(II)] complex have been reported.[6] A similar phenomenon is observed in the reaction between creatinine and the Qn.Ph.-Pd(II) complex.

In this paper, suitable conditions for the spectrophotometric determination of creatinine using Qn.Ph.-Pd(II) complex were investigated.

Experimental

Reagents——A stock solution (1.0×10^{-3} M) of creatinine was prepared by dissolving creatinine (Merck) in water, and the working solution was prepared by suitable dilution of this stock solution as required. A 5.0×10^{-4} M aqueous solution of Pd(II) and a 1.0×10^{-3} M methanol solution of Qn.Ph. were prepared as described in a previous report.[6] A 1.0% polyvinyl alcohol (PVA, *n*: 2000) solution and a 1.0% sodium dodecyl sulfate (SDS) solution were prepared by dissolving the compounds in water. A buffer solution of pH 5.5 was made by mixing appropriate amounts of 0.2 M acetic acid and 0.2 M sodium acetate. Other reagents were of analytical reagent grade. All the solutions were prepared with deionized water.

Apparatus——Absorption spectra were taken and absorbances were measured with Shimadzu Model UV-200 and 240 recording spectrophotometers with 1.0-cm silica cells. A Hitachi-Horiba Model F-7 AD glass electrode pH meter was used for pH measurements.

Standard Procedure——A creatinine solution containing 0.3—4 μg was placed in a 10-ml calibrated flask; 1.0 ml of 1.0% PVA solution, 1.0 ml of 1.0% SDS solution, 0.5 ml of 5.0×10^{-4} M Pd(II) solution, 3.0 ml of the buffer solution (pH 5.5) and 0.75 ml of 1.0×10^{-3} M Qn.Ph. solution were added. Then the solution was diluted to the mark with water and kept at 60°C for 45 min. After the solution had been cooled to room temperature, the difference of absorbance ($=\Delta A$) was calculated between the Qn.Ph.-Pd(II)-creatinine and the Qn.Ph.-Pd(II) solutions; absorbances were measured at 615 nm against water.

Results and Discussion

The absorption spectra of the Qn.Ph.-Pd(II) solution in the presence and the absence of creatinine are shown in Figure 1. On addition of creatinine to the Qn.Ph.-Pd(II) solution, the absorption peak at 615 nm was lowered significantly, and the magnitude of the decrease in absorbance was proportional to the concentration of creatinine.

The effect of pH on the reaction system was examined. Maximum and constant ΔA was obtained in the pH range of 5.3 to 5.7, the amount of creatinine being kept constant. Walpole buffer (0.2M acetic acid–0.2M sodium acetate) solution was found to be satisfactory for this purpose. Later measurements were carried out by addition of 3.0 ml of Walpole buffer (pH 5.5) solution.

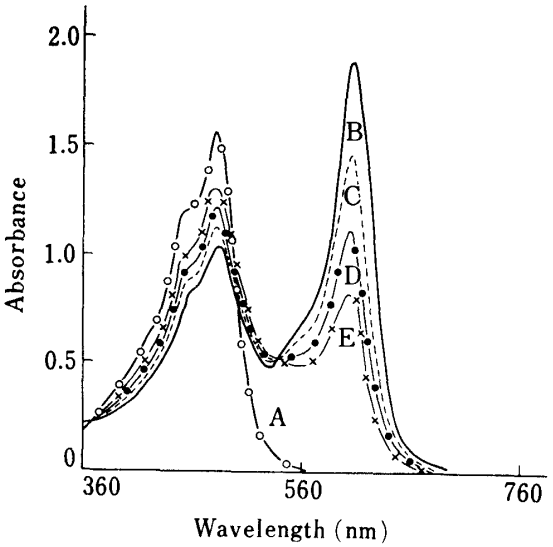

Fig. 1. Absorption Spectra of Qn.Ph. and Qn. Ph.-Pd (II) Solutions in the Presence and Absence of Creatinine at pH 5.5

Pd(II), 2.0×10^{-5} M; Qn.Ph., 5.0×10^{-5} M; 1.0 ml of 1.0% PVA and 1.0 ml of 1.0% SDS solution/10 ml; reference, water; curve A, Qn.Ph. solution; curve B, C, D and E, Qn.Ph.-Pd(II) solutions (creatinine concentrations: curve B, 0; curve C, 2.0×10^{-6} M; curve D, 4.0×10^{-6} M; curve E, 6.0×10^{-6} M).

As regards surfactants, the use of PVA and SDS gave the best sensitivity; maximum ΔA could be obtained by addition of 1.0 ml of 1.0% PVA solution and 1.0 ml of 1.0% SDS solution in the final 10 ml.

The effect of the amounts of Qn.Ph. and Pd(II) on ΔA was also examined. The results indicated that the molar ratio of Qn. Ph. to Pd(II) should be greater than 1.5. Accordingly, all further work was carried out with 2.5×10^{-5} M Pd(II) and 7.5×10^{-5} M Qn.Ph. solutions in the final 10 ml.

Experiments on the effect of time and temperature proved that on heating at 60°C, maximum and constant ΔA was obtained at 45—60 min. ΔA remained unchanged for at least 3 h after the solution had cooled to room temperature.

The calibration curve for the determination of creatinine was prepared by the standard procedure. It was found to be applicable to the determination of creatinine in the concentration range of 0.3—4 μg in the final 10 ml. The Sandell sensitivity was estimated to be 0.00053 μg/cm^2 for an absorbance unit of 0.001, and the effective "desorptivity" was 2.3×10^5 l mol^{-1} cm^{-1}.

Under the standard conditions, various substances were examined for interference. Fe(III), Cu(II) and amino acids such as glycine and glutamine interfered in small amounts, and human albumin, uric acid and hippuric acid interfered in large amounts. The interference from amino acids could be overcome fairly well by addition of *o*-phthalaldehyde. In this case, the standard addition method can be used. On the other hand, up to 10- to 20- fold excess of Ca(II), Mg(II), creatine, taurine, ammonia and ascorbic acid did not affect the absorbance in the determination of creatinine. Na(I), K(I), chloride, sulfate, phosphate, urea, glucose, acetone bodies, *etc.* did not interfere at 100—1000 fold excess. The results are summarized in Table I.

The precision was estimated for 2.3 μg of creatinine, and the coefficient of variation for 5 determinations was 2.1%.

Recovery of creatinine added to human urine without preliminary treatment was also examined. Recovery (the average of 5 determinations for 2.3 μg of creatinine) was 102.5%.

Though this proposed method has slight disadvantages in terms of simplicity and rapidity,

TABLE I. Effects of Other Substances

Substances	Added (μg/10 ml)	Creatinine found (μg/10 ml)
—	—	2.3
Fe (III) sulfate	1.1	2.0
Ca (II) nitrate	40.1	2.3
Glycine	0.8	2.6
Histidine	1.6	2.6
Glutamine	1.5	2.5
Taurine	25.0	2.3
Creatine	26.2	2.3
Hippuric acid	35.8	2.4
Uric acid	33.6	2.4
Human albumin	10.0	2.4
Glucose	90.1	2.3
Caffeine	76.7	2.3

Creatinine, 2.3 μg/10 ml; Pd(II), 2.5×10^{-5} M; Qn. Ph., 7.5×10^{-5} M; 1.0 ml each of 1.0% PVA and SDS solutions/10 ml; pH, 5.5; reference, water.

the sensitivity is over 10 times higher than that of the Folin–Wu method,[4] and Jaffe chromogens scarcely affected the determination of creatinine. Therefore, application of this method to biological fluids should be feasible.

References and Notes

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