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# The Determination of 5-Iodouracil Using the *o*-Hydroxyhydroquinonephthalein–Palladium(II) Complex[†]

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**Synopsis.** A simple and sensitive spectrophotometric procedure was studied for the determination of 5-iodouracil (IU) using the *o*-hydroxyhydroquinonephthalein(Qn.Ph.)–palladium(II) complex and avoiding any effect of iodide ions. This method could be used in the concentration range of 0—15 μg/10 ml of IU, where the Sandell sensitivity was 0.0020 μg/cm$^2$ for at 630 nm; the coexistence of the iodide ion did not interfere with the determination of IU, even when it was present in a 500-fold excess.

The determination of organic iodine compounds involves, in general, a preliminary ashing step to release iodide ions.[1,2] Moreover, organic iodine compounds tend to decompose upon exposure to ambient light and so liberate iodide ions. Therefore, it is important that organic iodine compounds be determined without any interference by iodide ions.

We have already reported simple and sensitive spectrophotometries[3–5] for the determination of cephalexin, ampicillin, *etc.* based on the decrease in the absorbance of the *o*-hydroxyhydroquinonephthalein(Qn.Ph.)–palladium(II) complex solution.

This paper will describe a procedure for the determination of 5-iodouracil(IU) using the Qn.Ph.–palladium(II) complex without any effect of the coexistence of iodide ions.

## Experimental

*Apparatus.* The absorption spectra and absorbance were measured with a Shimadzu Model 240 recording spectrophotometer using matched 1.0-cm quartz cells. A Hitachi-Horiba Model F-7 AD glass electrode pH meter was used for the pH measurements.

*Reagents.* A stock solution($1.0\times10^{-3}$ M, 1 M=1 mol dm$^{-3}$) of IU(Tokyo Kasei Kogyo Co., Ltd., Tokyo) was prepared by dissolving IU in a 20% methanol solution, while the working solution was made by the suitable dilution of this stock solution as required. A $5.0\times10^{-4}$ M aqueous solution of palladium(II) and a $5.0\times10^{-4}$ M methanol solution of Qn.Ph. were prepared according to the method of a previous report.[3] A $1.0\times10^{-2}$ M solution of hexadecyltrimethylammonium chloride(HTAC) was prepared by dissolving the respective compound in water. A 0.1 M sodium carbonate-0.1 M sodium hydrogencarbonate buffer solution[6] was used for the pH adjustment. All the other reagents were of an analytical grade.

*Standard Procedure.* 0.5 ml of the $5.0\times10^{-4}$ M palladium(II) solution, 2.0 ml of the $1.0\times10^{-2}$ M HTAC solution, 2.0 ml of the buffer solution(pH 9.8), 0.75 ml of the $5.0\times10^{-4}$ M Qn.Ph. solution, and a solution containing up to 15 μg of IU were placed in a 10-ml calibrated flask. The mixture was diluted to 10 ml with water, kept at 60 °C for 15 min, and then cooled 5 min in water. The difference in absorbance [$\Delta A$] between the Qn.Ph.–palladium(II)–IU and the Qn.Ph.–palladium(II) solutions was measured at 630 nm against water.

## Results and Discussion

*Absorption Spectra.* The absorption spectra of Qn.Ph.–palladium(II) in the presence of IU at pH 9.8 are shown in Fig. 1. On the addition of IU to the Qn.Ph.–palladium(II) solution, the decrease in the absorbance of the Qn.Ph.–palladium(II) complex at 630 nm was proportional to the concentration of IU.

*Effect of pH and Surfactants.* The effects of the pH and the surfactants were examined. The maximum and constant $\Delta A$ value was obtained in the pH range from 9.3 to 10.5 with a 0.1 M sodium carbonate–0.1 M sodium hydrogencarbonate buffer solution. Subsequent measurements were carried out by the addition of 2.0 ml of the pH buffer solution(pH 9.8). Compared with nonionic surfactants, such as poly(vinyl alcohol) and poly(*N*-vinylpyrrolidone), or an anionic surfactant such as sodium dodecyl sulfate, the $\Delta A$ value in the presence of a cationic surfactant was more than 30 times as large. HTAC was superior to the other cationic surfactants examined in terms of sensitivity; the maximum and constant $\Delta A$ value could be obtained over the range of 1.5—2.5 ml of the $1.0\times10^{-2}$ M HTAC solution in the final 10 ml.

*Effects of Palladium(II) and Qn.Ph. Concentration.* The effect of the amount of Qn.Ph. on $\Delta A$ was examined by varying the molar ratio of Qn.Ph. to palladium(II), the amounts of palladium(II) and IU

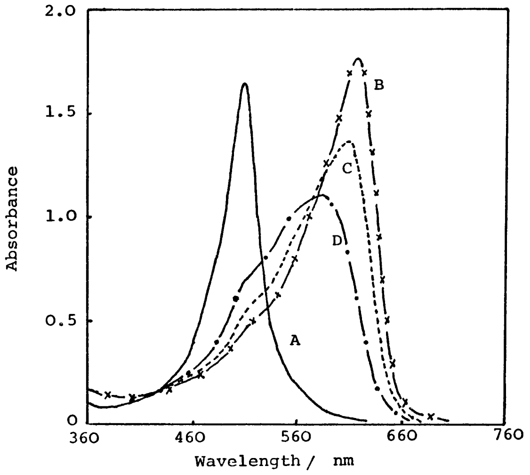

Fig. 1. Absorption spectra of Qn.Ph. and Qn.Ph.–palladium(II) solutions in the presence of IU at pH 9.8.
Pd(II): $1.5\times10^{-5}$ M; Qn.Ph.: $2.25\times10^{-5}$ M; HTAC: $2.0\times10^{-3}$ M; Reference: water; curve A: Qn.Ph. solution; curves B, C, and D: Qn.Ph.-Pd(II) solutions(IU concentration: curve B, 0; curve C, $6.0\times10^{-6}$ M; curve D, $1.8\times10^{-5}$ M).

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[†] Application of Xanthene Derivatives for Analytical Chemistry. Part XXIX. Part XXVIII: Y. Fujita, I. Mori, and S. Kitano, *Chem. Pharm. Bull.*, **31**, 1289 (1983).

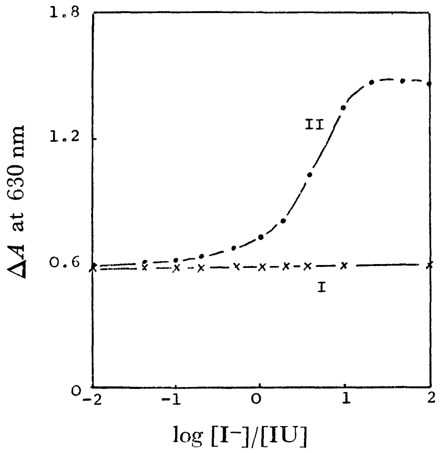

Fig. 2. Effect on $\Delta A$ with sequence of addition of iodide ion.
IU: $5.0\times10^{-6}$ M; Pd(II): $2.5\times10^{-5}$ M; Qn.Ph.: $3.75\times10^{-5}$ M; HTAC: $2.0\times10^{-3}$ M; pH: 9.8; Reference: water; sequence of addition of reagents, curve I, Pd(II)→HTAC→Buffer→Qn.Ph.→IU, iodide ion; curve II, IU, iodide ion→Pd(II)→HTAC→Buffer→Qn.Ph.

being kept constant. The molar ratio of palladium(II) 2:Qn.Ph. 3 was most effective. Further, the molar ratio of palladium(II) to Qn.Ph. was found to be 2:3 by using the molar ratio method. Accordingly, all further work was carried out with $2.5\times10^{-5}$ M palladium(II) and $3.75\times10^{-5}$ M Qn.Ph. solutions in the final 10 ml.

*Sequence of Addition.* The identical $\Delta A$ value was obtained in the determination of IU even when the order of the addition of the reagents was varied. However, the influence of iodide ions could be completely eliminated when a solution containing iodide ions was added to the solution obtained after the color reaction between palladium(II) and Qn.Ph. The effect on $\Delta A$ of the sequence of addition is shown in Fig. 2.

*Stability and Calibration Curve.* The maximum $\Delta A$ value at 630 nm was given by heating at 60 °C for 15 min. The $\Delta A$ value was constant for at least 90 min. The calibration curve for the determination of IU was prepared according to the standard procedure. It obeyed Beer's law over the range of 0—15 μg of IU in the final 10 ml. The Sandell sensitivity was estimated to be 0.0020 μg/cm$^2$ IU at 630 nm, and the effective "desorptivity" was $1.15\times10^5$ l mol$^{-1}$ cm$^{-1}$. When 5 solutions containing 11.3 μg/10 ml were determined by the standard procedure, the coefficient of variation was 0.95%.

*Effects of Foreign Substances.* The interfering effects of various substances were examined under the standard conditions. The results are summarized in Table 1. Various metal ions, such as iron(III), copper(II), zinc(II), and cobalt(II) reacted with Qn.Ph. to form a colored complex, thus interfering with the determination of IU. Large amounts of chloride, nitrate, sulfate, organic acids ions, *etc.* did not interfere, while small amounts of cyanide, thiosulfate and thiocyanate ions did so. Uracil, thiourea, purine, orotic acid, amino acids, *etc.* in small amounts affected the determination of IU. Large amounts of salicylic acid, glucose, phenol, urea, *etc.* did not, however, interfere. Uracil present up to 4 times the amount of IU could be permitted by determining the IU according to the standard addition method. As has been described above, iodide ions did not interfere, even in a 500-fold excess. Therefore, the present method could be used to determine IU in the presence of large amounts of iodide ions without a preliminary ashing step to release the iodide ions.

TABLE 1. EFFECTS OF FOREIGN SUBSTANCES

| Foreign substances | Added as | Added (μg/10 ml) | $\Delta A$ at 630 nm |
|---|---|---|---|
| — | — | — | 0.576 |
| Cu(II) | Nitrate | 1.6 | 0.432 |
| Zn(II) | Chloride | 3.2 | 0.408 |
| Fe(III) | Sulfate | 2.8 | 0.498 |
| $S^{2-}$ | Sodium | 1.6 | 0.660 |
| $SCN^-$ | Potassium | 5.8 | 0.618 |
| 2-Thiouracil | — | 0.6 | 0.618 |
| Thiourea | — | 0.4 | 0.640 |
| Uracil | — | 8.4 | 0.652 |
| Barbital | — | 18.4 | 0.630 |
| Glycine | — | 7.5 | 0.655 |

IU taken: 11.3 μg/10 ml; Pd(II): $2.5\times10^{-5}$ M; Qn.Ph.: $3.75\times10^{-5}$ M; HTAC: $2.0\times10^{-3}$ M; pH: 9.8; Reference: water.

## References

1) "Muki Oyo Hishoku Bunseki(Inorganic Applied Colorimetric Analysis)," Kyoritsu Shuppan, Tokyo (1973), Vol. 3, p. 50.

2) "Commentary of the Japanese Pharmacopoeia," Tenth ed, Hirokawa Publishing Co., Tokyo (1981).

3) I. Mori, Y. Fujita, and K. Sakaguchi, *Chem. Pharm. Bull.*, **30**, 2599 (1982).

4) I. Mori, Y. Fujita, S. Kitano, and K. Sakaguchi, *Bunseki Kagaku*, **31**, E 305 (1982).

5) I. Mori, Y. Fujita, and S. Kitano, *Chem. Pharm. Bull.*, **31**, 1289 (1983).

6) D. D. Perin and B. Dempsey, "Buffer for pH and Metal Ion Control," trans. by K. Tsuji Kodansha, Tokyo (1981).