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Application of *o*-Hydroxyhydroquinonephthalein-Iron(III) Complex to Determination of Pyridone Carboxylic Acids¹⁾

YOSHIKAZU FUJITA, ITSUO MORI,* KINUKO FUJITA
and TAKESHI TANAKA

*Osaka University of Pharmaceutical Sciences, 2-10-65,
Kawai, Matsubara, Osaka 580, Japan*

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A simple spectrophotometric method for the determination of pyridone carboxylic acids (PCA) using *o*-hydroxyhydroquinonephthalein(Qnph)-iron(III) complex was developed. The present method is based on the ternary complex formation among Qnph, iron(III) and PCA in the presence of sodium dodecylbenzenesulfonate in weakly basic media. In the determination of nalidixic acid(NA), Beer's law held up to *ca.* 20 μg of NA in the final volume of 10 ml, with an apparent molar absorptivity of $1.3 \times 10^5 \text{ l mol}^{-1} \text{ cm}^{-1}$ for NA $\{1.8 \times 10^5 \text{ l mol}^{-1} \text{ cm}^{-1}$ for piromidic acid (PA), $6.6 \times 10^4 \text{ l mol}^{-1} \text{ cm}^{-1}$ for pipemidic acid}. The proposed method was applied to the assay of NA or PA in pharmaceutical preparations and calf serum. The use of a membrane filter preconcentration technique was also investigated in an attempt to improve the proposed method.

Keywords—spectrophotometry; pyridone carboxylic acid; nalidixic acid; piromidic acid; naphthyridine; *o*-hydroxyhydroquinonephthalein; iron(III); ternary complex; anionic surfactant; membrane filter

Pyridone carboxylic acids (PCA)²⁾ have recently attracted much interest as new antibacterial agents, and various compounds have been prepared. Some work has been on the determination of PCA by gas-liquid chromatography³⁾ and high-performance liquid chromatography,⁴⁾ but little work has been done on simple chemical methods, such as spectrophotometry⁵⁾ and fluorometry.⁶⁾ In addition, most of the reported methods³⁻⁶⁾ are procedures for the determination of only nalidixic acid (NA).

On the other hand, we have shown that the absorbances of xanthene dye-iron(III) complexes are remarkably enhanced by addition of substances having the donor atoms O, O, and we reported⁷⁾ some simple and highly sensitive methods for the determination of phosphate ion, organophosphorus compounds, citric acid and 1,2-diphenols by using the *o*-hydroxyhydroquinonephthalein(Qnph)-iron(III) or phenylfluorone(Phfl)-iron(III) complexes. In further investigations, similar phenomena were observed when PCA, especially naphthyridines such as NA and piromidic acid (PA), were used instead of phosphate ion, citric acid and so on.

In this work, a simple spectrophotometric method for the determination of PCA using the Qnph-iron(III) complex was developed. The proposed method was applied to the assay of NA or PA in pharmaceutical preparations and calf serum. The use of a membrane filter preconcentration technique⁸⁾ was also investigated in an attempt to improve the proposed method.

Experimental

Reagents, Materials and Apparatus—Stock solutions ($1.0 \times 10^{-2} \text{ M}$, $\text{M} = \text{mol l}^{-1}$) of NA and PA were prepared by dissolving appropriate quantities of NA (Maruishi Pharmaceutical Co., Ltd.) and PA (Dainippon Pharmaceutical

Co., Ltd.) in small amounts of 1 N sodium hydroxide solution and were diluted to 10 ml with water. The working solutions were made by diluting these stock solutions as required. These solutions were stored in a cool, dark place. A Qnph solution and an iron(III) solution were prepared as 1.0×10^{-3} M methanol solution and 5.0×10^{-4} M aqueous solution, respectively, as described in the previous reports.^{7a,b)} A 1.0% aqueous solution of sodium dodecylbenzenesulfonate (SDBS, Tokyo Kasei Kogyo Co., Ltd.) was also prepared. A 0.2 M ammonia–0.2 M ammonium chloride buffer solution was used for pH adjustment. All other reagents and materials were of analytical reagent grade, and deionized water was used. All apparatus used was as described in the previous reports.^{7a,b)}

Standard Procedure—The following components were added to a 10-ml volumetric flask; a sample solution containing up to *ca.* 20 μ g NA or PA, 1.0 ml of 1.0% SDBS solution, 0.5 ml of 5.0×10^{-4} M iron(III) solution, 3.0 ml of ammonium buffer solution (pH 8.2) and 0.5 ml of 1.0×10^{-3} M Qnph solution. The mixture was made up to the total volume of 10 ml with water, kept at 60°C for 25 min, and cooled for 5 min in water. The absorbance of the Qnph–iron(III)–NA (or –PA) solution (Solution A) was measured at 615 nm against a similarly prepared Qnph–iron(III) solution (Solution B).

Results and Discussion

Figure 1 shows the absorption spectra of Solutions A and B, and their absorption difference (curve C) under the standard conditions. The absorption maximum of the curve C was at 615 nm, which was selected for the determination of PCA. A constant absorbance was obtained between pH 7.8–8.6 with 0.2 M ammonia–0.2 M ammonium chloride buffer solution.^{7b)}

The effect of various surfactants was examined. Surfactants used were nonionic [Tween 20, polyvinyl alcohol and gum arabic], cationic [hexadecyltrimethylammonium chloride and hexadecylpyridinium chloride] and anionic surfactants [SDBS, sodium dodecyl sulfate (SDS), sodium bis-(2-ethylhexyl)sulfosuccinate, sodium choleate and sodium oleate]. Compared with other surfactants, SDBS and SDS gave about 5–10 times better sensitivity. SDBS was somewhat superior to SDS as regards the stability of the ternary complex and rapidity of color development. A constant absorbance was observed upon addition of more than 0.5 ml of 1.0% SDBS solution in the final volume of 10 ml.

The effect of xanthene dyes in this reaction system was studied. Among the xanthene dyes⁹⁾ tested—Qnph, 4,5-dibromohydroxyhydroquinonephthalein, 3',4',5',6'-tetrachlorohydroxyhydroquinonephthalein, Phfl, salicylfluorone, pyrogallol red and gallein—, Qnph was the most effective dye in terms of sensitivity and reproducibility. In this reaction system, only iron(III) (or iron(II)) was effective among various metal ions tested—iron(III,II), copper(II), zinc(II), manganese(II), palladium(II), zirconium(IV) and aluminum(III). Maximum absorbance was obtained when the molar ratio of iron(III) to Qnph was 1:2.

The color formation in this reaction system did not occur instantaneously at room

Fig. 1. Absorption Spectra

Curve A, Solution A [Qnph–Fe(III)–NA]; curve B, Solution B [Qnph–Fe(III)]; curve C, Solution A minus Solution B; NA, 7.5×10^{-6} M; Fe(III), 2.5×10^{-5} M; Qnph, 5.0×10^{-5} M; SDBS, 1.0 ml of 1.0% SDBS solution; pH 8.2; reference, water.

TABLE I. Apparent Molar Absorptivities for Some PCA

Compound	PCA classification	$\epsilon^a)$
NA	Naphthyridine	1.3×10^5
PA	Naphthyridine	1.8×10^5
Pipemidic acid ^{b)}	Naphthyridine	6.6×10^4
Norfloxacin ^{c)}	Quinoline	4.7×10^3
Ofloxacin ^{d)}	Quinoline	7.5×10^3

a) Apparent molar absorptivity ($1 \text{ mol}^{-1} \text{ cm}^{-1}$) at 615 nm. *b)* Dainippon Pharmaceutical Co., Ltd. *c)* Kyorin Pharmaceutical Co., Ltd. *d)* Daiichi Pharmaceutical Co., Ltd.

TABLE II. Determination of NA and PA in Commercial Pharmaceutical Preparations

Sample	Amount (mg)		Recovery test ^{a)}	
	Nominal	Found ^{a)}	Added ($\mu\text{g}/10 \text{ ml}$)	%
A	250	250.0	5.8	98.6
B	50	51.9	5.8	102.8
C	250	248.7	7.2	101.1
D	200	200.5	7.2	102.1

Sample A, NA tablet (in 1 Tab. $\approx$ 380 mg); sample B, NA dry syrup (in 1 g); sample C, PA tablet (in 1 Tab. $\approx$ 300 mg); sample D, PA dry syrup (in 1 g). *a)* Mean of 5 determinations.

temperature. Experiments on the effect of time and temperature showed that maximum and constant absorbance was obtained on heating at 60 °C for 20–30 min. The absorbance remained unchanged for at least 2 h after Solutions A and B had been cooled to room temperature.

Calibration curves were constructed for PCA at 615 nm under the standard conditions. In the determination of NA, a good linear relationship was obtained over the range up to *ca.* 20 μg of NA in the final volume of 10 ml. The apparent molar absorptivity for NA was $1.3 \times 10^5 \text{ l mol}^{-1} \text{ cm}^{-1}$, which is about 5 times higher than that obtained by the method ^{5b)} using copper(II) and sodium diethyldithiocarbamate. The relative standard deviation ($n=5$) was 1.4% at 11.6 μg of NA. The other PCA were also determined under the same conditions. The sensitivities for naphthyridines were far larger than those for quinolines, as shown in Table I.

The iron(III)-to-Qnph ratio and the NA-to-iron(III) ratio as determined by the molar ratio method and the continuous variation method were 1 : 2 and 1 : 1, respectively. Thus, the ternary complex formed in this reaction system may be expressed as $(\text{NA}) (\text{Fe}^{\text{III}}) (\text{Qnph})_2$.

Under the standard procedure, the effects of various interfering substances on the determination of 11.6 μg of NA were examined. The coexistence of metal ions such as zinc(II), copper(II) and cobalt(II) interfered with the determination when present in an equimolar amount with respect to NA. These interfering metal ions could be masked by addition of iminodiacetic acid solution. Among the anions tested, phosphate ion and hydroxypolycarboxylate ions such as citrate and tartrate gave positive errors in small amounts, but other anions did not interfere at 100- to 200-fold molar excess with respect to NA. Caffeine, taurine, cephalixin, lactose and glucose did not interfere in 100-fold molar excess. The coexistence of up to 200 μg of human albumin had no effect.

The proposed method was applied to the assays of NA and PA in commercial pharmaceutical preparations, and the experimental results were in good agreement with those

obtained by the ultraviolet (UV) method.^{5c)} Recoveries of NA and PA added to the samples were satisfactory (98—103%). The results are given in Table II. On the other hand, recovery of 5.8 μg of NA added to calf serum was examined, but was low (ca. 50—70%).

Next, the use of a membrane filter preconcentration technique⁸⁾ was investigated in an attempt to improve the proposed method with respect to sensitivity, *etc.*

This procedure did not much improve the sensitivity as compared with that in aqueous media, but had the advantage that the absorbance of the reagent blank was remarkably low. The recommended procedure is as follows: Solutions A and B obtained according to the standard procedure were each filtered through a membrane filter (nitrocellulose of 0.20 μm pore size), and the filtered substances were washed with about 20 ml of water. The membrane filters were dissolved in 5 ml of dimethyl sulfoxide, and then the absorbance at 650 nm was measured against the reagent blank.

In conclusion, though further investigations are necessary for the application of this method to biological samples, including development of the membrane filter preconcentration technique, the method should be useful and convenient for the simple determination and detection of PCA, especially naphthyridines such as NA and PA, in pharmaceutical preparations.

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References and Notes

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