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Determination of Chlorpromazine, Thiamine, Lincomycin, Ofloxacin and Theophylline by Ternary Complex Formation with Eosin and Palladium(II)¹⁾

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Color reactions among a drug, an organic dye and a metal ion were studied, and a simple and sensitive spectrophotometric method, without solvent extraction, has been established for the determination of some drugs by ternary complex formation with eosin and palladium(II) (probably involving an ion-association complex between $(\text{Pd}^{\text{II}}(\text{drug})_n)$ cation and eosin anion). Beer's law held up to *ca.* 2.0×10^{-5} M of each drug at 545 nm in the final volume of 10 ml, with apparent molar absorptivities of 5.7×10^4 l mol⁻¹ cm⁻¹ for chlorpromazine, 6.7×10^4 for thiamine, 5.5×10^4 for lincomycin, 4.0×10^4 for ofloxacin, and 3.5×10^4 for theophylline. The proposed method was applied to the determination of these drugs in pharmaceutical preparations. A fluorescence quenching method for the determination of these drugs by forming this ternary complex was also investigated for the purpose of enhancing the sensitivity of determination.

Keywords—spectrophotometry; fluorometry; ternary complex; eosin; palladium(II); chlorpromazine; thiamine; lincomycin; ofloxacin; theophylline

Of the methods available for enhancing the sensitivity of determination of organic compounds, the ion-pair complex formation system between an organic dye and an organic compound has often been used; suitable organic dyes²⁾ are bromothymol blue, bromophenol blue, bromocresol green, methyl orange, tropaeolin OO, eosin, zincon, chromazurol S, tetrabromophenolphthalein ethyl ester, *etc.* These methods are complicated since they require an extraction procedure due to the formation of water-insoluble complexes. The addition of a surfactant or a water-miscible organic solvent to avoid to any extraction procedure usually causes decomposition of the ion-pair complexes formed, and thus the organic compounds can not be determined any longer. In addition, these methods are not sensitive enough.

We have already reported³⁾ some simple and sensitive spectrophotometric methods, which do not require any process of solvent extraction, for the determination of various organic compounds with an organic dye and a metal ion.

In this research, color reactions of various drugs by utilizing the ternary complex formation among an organic dye, a metal ion and an organic compound were studied in aqueous media, and then suitable conditions for the spectrophotometric determination of some drugs (chlorpromazine (CP), thiamine (TA), lincomycin (LCM), ofloxacin (OFLX) and theophylline (TP)) by using eosin and palladium (II) (Pd(II)) were established. The proposed method was applied to the assays of these drugs in pharmaceutical preparations. A fluorescence quenching method to determine these drugs was also investigated.

Experimental

Reagents and Apparatus—Standard aqueous solutions (1.0×10^{-3} M, M = mol l⁻¹) of CP, TA, LCM, OFLX

and TP were prepared by dissolving appropriate quantities of CP·HCl (Sigma Chemical Ltd.), TA·HCl (Wako Pure Co., Ltd.), LCM·HCl (Japan Upjohn Ltd.), OFLX (Daichi Pharmaceutical Co., Ltd.) and TP (Tokyo Kasei Kogyo Co., Ltd.). Eosin and Pd(II) solutions were prepared as 2.0×10^{-3} M aqueous solutions, as described in the previous reports.³⁾ A 0.5% methylcellulose (MC) solution was prepared by dissolving MC (1500 cps, Kishida Chemical Co., Ltd.) in cold water. A buffer solution (pH 4.3) was prepared by mixing 0.2 M acetic acid and 0.2 M sodium acetate solutions. All other reagents and materials were of analytical grade, and were used without further purification. Deionized water was used. A Shimadzu model UV 240 spectrophotometer and a Shimadzu model RF-540 spectrofluorometer with 1.0-cm quartz cells were used. The pH measurements were made with a Hitachi-Horiba F-7 AD pH meter with a combination calomel glass electrode.

Standard Procedure for the Spectrophotometry—An aliquot of each drug solution (up to *ca.* 2.0×10^{-5} M) was transferred to a 10-ml calibrated flask. To this, 1.5 ml of a 0.5% MC solution, 3.0 ml of the buffer solution (pH 4.3), 0.75 ml of a 2.0×10^{-3} M eosin solution and 0.75 ml of a 2.0×10^{-3} M Pd(II) solution were added. The mixture was diluted to 10 ml with water, kept at 50 °C for 30 min, and then cooled for 5 min in water. The absorbance of the eosin–Pd(II)–drug solution (solution A) was measured at 545 nm against a similarly prepared eosin–Pd(II) solution (solution B).

Recommended Procedure for the Fluorometry—A sample solution containing up to *ca.* 7.5×10^{-6} M of each drug was placed in a 10-ml calibrated flask. To this solution, 1.5 ml of a 0.5% MC solution, 3.0 ml of the buffer solution, 0.5 ml of a 2.0×10^{-3} M eosin solution and 0.5 ml of a 2.0×10^{-3} M Pd(II) solution were added. The mixture was diluted to 10 ml with water and kept at 50 °C for 30 min. Solutions A and B were cooled to room temperature in water for 5 min, then the difference of relative fluorescence intensity between solutions A and B at 545 nm emission wavelength with excitation at 462 nm was measured.

Results and Discussion

On the addition of TA to the eosin–Pd(II) solution (solution B), a distinct difference of absorbance between the eosin–Pd(II)–TA solution (solution A) and solution B was observed at around 545 nm, and the absorbance difference of solution A against solution B was proportional to the concentration of TA. The absorbance of solution B at 545 nm was relatively low.

The effect of organic dyes was studied by measuring the difference of absorbance between organic dye–Pd(II)–TA or organic dye–Pd(II)–LCM and organic dye–Pd(II) solutions. Ion-association reagents such as eosin, phloxine, fluorescein, dibromofluorescein and bromophenol blue increased the absorbance of organic dye–Pd(II) solution, whereas chelating reagents such as *o*-hydroxyhydroquinonephthalein, pyrogallol red, zincon, chromazurol S and aluminon decreased its absorbance. Eosin was chosen on the basis of sensitivity.

The effect of metal ions was examined. Only Pd(II) was effective among various metal ions; Pd(II), iron(III), titanium(IV), zirconium(IV), aluminum(III), zinc(II), copper(II), manganese(II), silver(I), *etc.*

About 30 kinds of drugs were examined in the colored complex formation system with eosin and Pd(II). The results showed that compounds involving heterocyclic N-bases or S-bases were apt to form colored complexes. In this paper, suitable conditions for the determination of CP, TA, LCM, OFLX and TP are described.

Study of the Optimum Conditions for the Spectrophotometry

A maximum and constant absorbance of solution A against solution B was observed in the range of pH 3.8–4.5 with 3.0 ml of a 0.2 M acetic acid–0.2 M sodium acetate buffer solution.

Solution A without surfactant was unstable and gave a precipitate. In order to solubilize and stabilize the colored complex, the use of various surfactants was tried. Cationic surfactants such as cetylpyridinium chloride depressed the colored complex formation due to the formation of an ion-pair complex between eosin and the cationic surfactant. MC (1500 cps), which is a nonionic and water-soluble polymeric surfactant, was found to be the best dispersion agent with respect to sensitivity.

The effect of the amounts of eosin and Pd(II) was examined by varying the molar ratio of

TABLE I. Sensitivities, Reproducibilities and Complex Compositions of Some Drugs in the Proposed Method

Drug	$\epsilon^a)$ ($\times 10^4$)	R.S.D. ^{b)}	Mole ratio (Pd : eosin : drug)
CP	5.7	0.5	1 : 1 : 2
TA	6.7	1.0	1 : 1 : 2
LCM	5.5	0.8	3 : 3 : 1
TP	3.5	2.1	1 : 1 : 1
OPLX	4.0	2.1	1 : 1 : 1

a) Apparent molar absorptivity. b) Relative standard deviation ($n=5$).

eosin to Pd(II), the amounts of Pd(II) and CP being kept constant. A maximum absorbance was observed when the molar ratio of Pd(II) to eosin was approximately 1 : 1. Further, it was found that the eosin-to-Pd(II) ratio in the presence of a drug was 1 : 1. All further work was thus carried out with 1.5×10^{-5} M eosin and Pd(II) in the final volume, taking into consideration the determination limit of drugs.

The color development at room temperature was very slow, more than 24 h being required. The effects of temperature and time were examined by heating for 10–60 min at various temperatures (40, 50 and 60 °C); an almost constant absorbance was obtained at 50 °C for 25–60 min or at 60 °C for 20–50 min, followed by cooling to room temperature. As both of solutions A and B on heating at 60 °C formed jelly-like aggregates, which disappeared on cooling and agitating, the reproducibility was somewhat poor (the conditions are beyond the cloud point of MC⁴⁾). The absorbance of solution A against solution B kept at 50 °C for 30 min and cooled for 5 min in water remained constant for at least 24 h.

Calibration Curves and Nature of Ternary Complex

Under the standard procedure, calibration curves were prepared for CP, TA, LCM, OFLX and TP. Good linear relationship was obtained in the range up to *ca.* 2.0×10^{-5} M of each drug in the final volume of 10 ml. The apparent molar absorptivities (ϵ), the relative standard deviations (R.S.D., $n=5$) and the compositions of the ternary complexes obtained by the molar-ratio and continuous variation methods are listed in Table I.

Next, a study to establish the character of the ternary complex was performed. The acid dissociation properties of eosin in the presence of MC were determined spectrophotometrically⁵⁾ at an ionic strength of 0.1 at 20 ± 0.1 °C. Eosin exists in any of the following forms, depending on the pH in aqueous solution:

Where R denotes the undissociated parts of eosin. It was found that $\text{p}K_{a1}$, $\text{p}K_{a2}$ and $\text{p}K_{a3}$ in the presence of MC were -2.10 , 2.85 and 4.95 , respectively, and about 80% of eosin at pH 4.3 was estimated⁶⁾ to be HR^- . Moreover, this ternary complex without surfactant was fairly well extracted into *n*-butanol (dielectric constant, 17.7⁷⁾), but got an insoluble film was formed at the interface of the two phases in methyl isobutylketone (13.1). The color development was remarkably depressed by the coexistence of small amounts of methanol or cationic surfactant. On the other hand, Shcherboy *et al.*⁸⁾ reported a fluorometric method for the determination of Pd(II) with eosin and 1,10-phenanthroline (phen). On the addition of phen to solution B, the same colored complex ($\epsilon \approx 3.4 \times 10^4$) was produced. This reaction is based on the ion-association complex formation⁹⁾ between $[\text{Pd}(\text{phen})]^{2+}$ as a cationic component and eosin as an anionic counter-ion. From the results, it is deduced that the ternary complex formed in this

reaction system may be the ion-association complex between $(\text{Pd}^{\text{II}}(\text{drug})_n)$ cation and eosin anion.

Effect of Foreign Substances

The effect of foreign ions and substances on the determination of LCM or CP was examined. The following ions and substances caused no interference up to at least the concentrations indicated: 100-fold molar excess over LCM or CP of $\text{Ca}(\text{II})$, $\text{Mg}(\text{II})$, $\text{K}(\text{I})$, $\text{Na}(\text{I})$, fluoride, chloride, nitrate, sulfate, phosphate, glucose, lactose, succinate and urea; 10- to 20-fold molar excess of $\text{Cu}(\text{II})$, $\text{Co}(\text{II})$ and $\text{Zn}(\text{II})$. $\text{Fe}(\text{III})$, iodide, thiocyanate, tartrate, citrate, salicylate, ascorbate, glycine; caffeine, diphenhydramine and panthothenate interfere at 1- to 5-fold molar excess over LCM or CP. Human albumin caused positive errors. The interference of large amounts of $\text{Fe}(\text{III})$ could be removed by addition of fluoride ion solution. To overcome the effects of small amounts of positive interfering substances such as ascorbate, caffeine, diphenhydramine and pyridoxine, a standard addition method could be used. The results are summarized in Table II.

Application of the Proposed Method

The proposed method was applied to the determination of $\text{CP} \cdot \text{HCl}$, $\text{TA} \cdot \text{HCl}$, $\text{LCM} \cdot \text{HCl}$, OPLX and TP in pharmaceutical preparations (tablet, capsule and injection). The content of a tablet or capsule was accurately weighed and ground in a mortar to a fine powder. The required amount of the powder was weighed, transferred into a 100-ml volumetric flask, diluted to the mark with water and filtered. An injection without pretreatment was transferred into a 100-ml volumetric flask and diluted to the mark with water. An appropriate amount of the sample solution was taken and assayed according to the standard procedure. The results obtained by the proposed method were in good agreement with those results of the other procedures, and recoveries were satisfactory (about 98—101%). The results are presented in Table III.

Study of the Fluorometry

As the formation of this ternary complex reduced the fluorescence of solution B, a fluorescence quenching method for the determination of these drugs was also investigated. The uncorrected fluorescence emission spectra of solutions A and B are shown in Fig. 1. On

TABLE II. Effect of Foreign Substances

Substance	Added ^{a)}	Recovery of LCM (%)	Added ^{a)}	Recovery of CP (%)
—	—	100.0	—	100.0
$\text{Fe}(\text{III})$, alum	2	115.6	2	127.8
$\text{Ca}(\text{II})$, chloride	100	100.0	100	100.0
I^- , potassium	2	88.0	2	88.7
H_2PO_4^- , potassium	100	100.0	100	100.0
Citric acid	2	86.5	5	91.0
Ascorbic acid	2	118.7	2	115.5
Glycine	5	83.5	10	91.5
Caffeine	2	116.3	2	127.6
Glucose	100	100.0	100	100.0
Pyridoxine	2	117.8	1	108.3
Diphenhydramine	2	116.3	1	107.6
Calcium pantothenate	2	97.3	5	90.0
Human albumin	(20 μg)	117.4	(10 μg)	105.2

LCM and CP, $1.0 \times 10^{-5} \text{ M}$; eosin and $\text{Pd}(\text{II})$, $1.5 \times 10^{-4} \text{ M}$; MC, 0.075%; reference, solution B.
a) Molar ratio (substance/LCM or CP).

TABLE III. Determination of Some Drugs in Pharmaceutical Preparations

Sample	Content (mg) ^{a, b)}			Recovery ^{b)} (%)
	Nominal amount	Present method	Other method	
CP·HCl tablet	10.0	9.90	9.89 ^{d)}	100.8
TA·HCl injection	1.0	1.02	1.01 ^{e)}	100.5
LCM capsule	250.0	253.4		99.1
OFLX tablet	100.0	103.5	102.8 ^{f)}	99.9
TP injection ^{c)}	201.3	195.7	197.4 ^{e)}	98.4

a) Drug content, mg per 1 tablet, capsule or injection. b) Mean of 5 determinations. c) As aminophylline injection. d) Qnph-Pd(II) method, ref. 10). e) JPX method, ref. 11). f) UV method, ref. 11).

Fig. 1. Emission Spectra of Eosin-Pd(II) and Eosin-Pd(II)-CP Solutions under the Recommended Conditions

CP, 7.5×10^{-6} M; eosin and Pd(II), 1.0×10^{-4} M; MC, 0.075%; pH, 4.3; excitation wavelength, 462 nm.
—●—, CP-eosin-Pd(II); —○—, eosin-Pd(II).

the addition of CP to solution B, the relative fluorescence intensity (R. fl. int.) of solution B fell significantly, and the magnitude of the decrease was proportional to the concentration of CP.

In the development of the recommended procedure for the fluorometry, only the amounts of eosin and Pd(II) were studied, and the same conditions as for spectrophotometry were adopted for other reaction variables. The optimal amounts of eosin and Pd(II) were 1.0×10^{-4} M in the final volume of 10 ml. The calibration curves were linear in the range up to *ca.* 7.5×10^{-6} M of these drugs. The quenching effects decreased in the order TA > CP, LCM > TP > OFLX.

In conclusion, color reactions among various drugs, eosin and Pd(II) were examined, and spectrophotometric and fluorometric determinations of some drugs (CP, TA, LCM, OFLX and TP) were established by using ternary complex formation. The ternary complex formed in this reaction system was regarded as the ion-association complex between $(\text{Pd}^{\text{II}}(\text{drug})_n)$ cation and eosin anion. Compared with the conventional methods using organic dyes,^{2, 12)} or metal ions¹³⁾ alone, the present method, which does not require an extraction procedure, has the advantages of simplicity, sensitivity and reproducibility. The present method should be useful and convenient for the simple determination and detection of various drugs for quality control and /or for screening tests, as well as for assay of pharmaceutical preparations.

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References and Notes

- 1) This paper is Part LXIII of a series entitled "Application of Xanthene Derivatives for Analytical Chemistry," Part LXII: I. Mori, Y. Fujita, K. Fujita, Y. Koshiyama and Y. Nakahashi, *Anal. Lett.*, accepted (1987). This work was presented at 106th and 107th Annual Meetings of the Pharmaceutical Society of Japan in Chiba, April 1986 and in Kyoto, April 1987.
- 2) a) M. Pesez and J. Bortos, "Colorimetric and Fluorimetric Analysis of Organic Compounds and Drugs," Marcel Dekker, Inc., New York, 1974; b) The Japan Society for Analytical Chemistry, "Bunseki Kagaku Binran," Maruzen Ltd., Tokyo, 1976; c) T. Inoue, "Iyakuhin Seizai No Bunseki," Chizinshoin, Ltd., Tokyo, 1978; d) T. Sakaguchi, "Yakuhin Bunseki Kagaku," Nankodo, Tokyo, 1978; e) W. Sadée and G. C. M. Beelen (M. Saito and Z. Tamura), "Drug Level Monitoring," Hirokawa Shoten, Tokyo, 1982; f) T. Sakai, *Dojin News*, **30**, 1 (1984); g) T. Mitsui, *Bunseki*, **1985**, 103.
- 3) a) Y. Fujita, I. Mori, S. Kitano, H. Kawabe and Y. Kamada, *Bull. Chem. Soc. Jpn.*, **57**, 1828 (1984); b) I. Mori, Y. Fujita, H. Kawabe and K. Fujita, *Bunseki Kagaku*, **34**, 648 (1985); c) I. Mori, Y. Fujita, H. Kawabe, K. Fujita, T. Tanaka and A. Kishimoto, *Analyst* (London), **111**, 1409 (1986); d) I. Mori, Y. Fujita and K. Sakaguchi, *Bunseki Kagaku*, **30**, 2599 (1982).
- 4) A. Kitahara, Y. Tamai, S. Hayano and I. Hara, "Surface Active Agents," Kodansha Scientific, Tokyo, 1981.
- 5) a) A. Albert and E. P. Sejesnt (T. Matsuura), "The Ionic Constants," Maruzen Co., Tokyo (1963); b) M. Shibata, M. Nakamizo and H. Kakiyama, *Nippon Kagaku Kaishi*, **1972**, 681.
- 6) H. Freiser and Q. Fernando (T. Fujinaga and E. Sekido), "Ionic Equilibria in Analytical Chemistry," Kagakudojin Ltd., Tokyo, 1977.
- 7) The Society of Synthetic Organic Chemistry, Japan, "The Pocketbook of Solvents," Ohme-sha, Tokyo, 1984.
- 8) D. P. Shcherbov, D. N. Lisitsina and I. D. Vredenskaya, *Org. Reagently Anal. Khim., Tezisy Dokl. Vses. Konf., 4th*, **2**, 129 (1976) [*Chem. Abstr.*, **88**, 15447s (1978)].
- 9) P. R. Haddad, *Talanta*, **24**, 1 (1977).
- 10) I. Mori, Y. Fujita and S. Kitano, *Bunseki Kagaku*, **32**, E1 (1983).
- 11) "Commentary of the Japanese Pharmacopoeia, Ed. X," Nankodo, Tokyo, 1981.
- 12) a) J. Lelijveld and H. Kortmann, *Bull. World Health*, **42**, 447 (1970); b) L. A. Roberts, *J. Pharm. Sci.*, **58**, 1015 (1969); c) S. Ogawa, M. Morita, K. Nishiura and K. Fujisawa, *Yakugaku Zasshi*, **85**, 650 (1965); d) U. R. Cieri, *J. Assoc. Off. Anal. Chem.*, **61**, 937 (1978); e) A. I. Zhebentyaev, *Khim.-Farm. Zh.*, **19**, 111 (1985) [*Chem. Abstr.*, **102**, 13791 (1986)].
- 13) a) M. Sawada, I. Motoyama, S. Yamada and Y. Kanaya, *Bunseki Kagaku*, **35**, T16 (1986); b) S. Tanabe and H. Taniguchi, *Bunseki*, **1985**, 879.