Chem. Pharm. Bull.
32(3)1214—1218(1984)

Application of *o*-Hydroxyhydroquinonephthalein–Uranium (VI) Complex to Determination of Neomycin and Toburamycin[1)]

YOSHIKAZU FUJITA, ITSUO MORI,* and SHOKO KITANO

Osaka College of Pharmacy, 2–10–65, Kawai, Matsubara, Osaka 580, Japan

(Received June 9, 1983)

An application of *o*-hydroxyhydroquinonephthalein (Qn.Ph)–uranium (VI) [U(VI)] complex to the determination of neomycin (NM) and toburamycin (TOB) was developed. The spectrophotometric method is based on the fact that the absorption maximum of Qn.Ph.–U(VI)–NM or Qn.Ph.–U(VI)–TOB complex shows a red shift compared with that of Qn.Ph.–U(VI) complex and the magnitude of the increase in absorbance is proportional to the concentration of NM or TOB. This method could be used to determine 3—50 μg/10 ml of NM and 2—40 μg/10 ml of TOB; the apparent molar absorptivities were 1.28×10^5 l mol^{-1} cm^{-1} for NM and 1.17×10^5 l mol^{-1} cm^{-1} for TOB at 545 nm. Recoveries of NM and TOB added to human urine and saliva were examined.

Keywords——spectrophotometry; neomycin; toburamycin; *o*-hydroxyhydroquinonephthalein; uranium (VI); methyl cellulose; association complex

Though many spectrophotometric and colorimetric methods for the determination of aminoglycoside antibiotics have been reported,[2)] most of the methods have disadvantages such as complexity of procedure and lack of reproducibility.

We have already reported simple and sensitive spectrophotometric methods[3)] for the determination of thiamine, papaverine and quinine utilizing the *o*-hydroxyhydroquinonephthalein (Qn. Ph.)–uranium(VI) [U(VI)] complex. It was found that aminoglycoside antibiotics such as neomycin (NM) and toburamycin (TOB) produced colored complexes in the Qn.Ph.–U(VI) complex system.

In this paper, a simple, sensitive and reproducible spectrophotometric determination of NM and TOB using the Qn.Ph.–U(VI) complex was investigated. The proposed method was applied to the determination of NM or TOB added to human urine and saliva.

Experimental

Materials and Reagents——Stock solutions (1.0×10^{-2} mol dm^{-3}) of NM and TOB were prepared by dissolving neomycin sulfate (Sigma Chemical Co., Ltd.) and toburamycin (Shionogi Co., Ltd.; manifested potency, 942 μg/mg) in water, respectively, and the working solutions were prepared by suitable dilution of these stock solutions as required. A 1.0×10^{-3} mol dm^{-3} solution of U(VI) was prepared by dissolving uranyl nitrate (Merck Co., Ltd.) in water. A 5.0×10^{-4} mol dm^{-3} solution of Qn.Ph. was prepared by dissolving Qn.Ph. synthesized as described previously[4)] in methanol. A 0.5% solution of methyl cellulose (MC) was prepared by dissolving MC (1500 cps) in cold water. The buffer was 2.0×10^{-1} mol dm^{-3} acetic acid–2.0×10^{-1} mol dm^{-3} sodium acetate buffer (pH 4.8). All other materials and reagents were of analytical reagent grade, unless otherwise specified. Double-distilled water was used.

Apparatus——Absorption spectra and absorbance measurements were taken with a Shimadzu UV-240 recording spectrophotomter, with 1.0-cm matched silica cells. pH measurements were made with a Hitachi-Horiba F-7 AD glass electrode pH meter.

Standard Procedure——The following components were mixed in a 10-ml calibrated flask; 0.5 ml of 1.0×10^{-3} mol dm^{-3} U(VI) solution, 1.0 ml of 0.5% MC solution, 3.0 ml of the buffer (pH 4.8) solution, 2.0 ml of 5.0×10^{-4} mol dm^{-3} Qn.Ph. solution and a sample solution containing 3—50 μg of NM or 2—40 μg of TOB. The mixture was diluted to 10 ml with water and kept at 20—25 °C for 20 min. The absorbance of the Qn.Ph.–U(VI)–NM

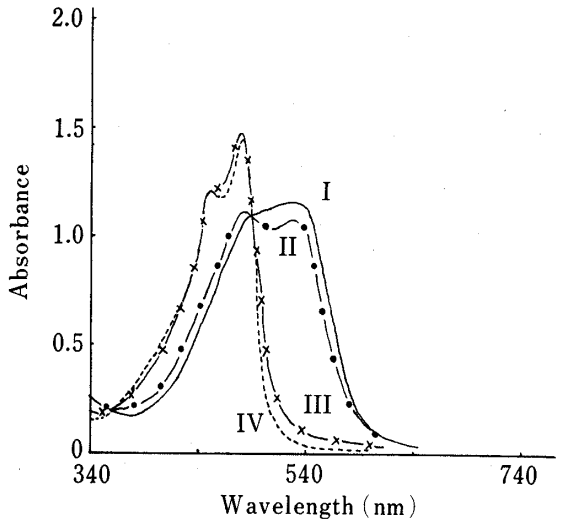

Fig. 1. Absorption Spectra of Qn.Ph., Qn.Ph.–U(VI), Qn.Ph.–U(VI)–NM and Qn.Ph.–U(VI)–TOB Solutions in the Presence of MC at pH 4.8

NM, TOB, U(VI) and Qn.Ph., 5.0×10^{-5} M; MC, 1.0 ml of 0.5% MC solution/10 ml; reference water. curve I (——), Qn.Ph.–U(VI)–NM; curve II (—●—), Qn.Ph.–U(VI)–TOB; curve III (—×—), Qn.Ph.–U(VI); curve IV (– – –), Qn.Ph.

or Qn.Ph.–U(VI)–TOB solution (solution A) was measured at 545 nm against the Qn.Ph.–U(VI) solution (solution B).

Procedure for the Determination in Human Urine and Saliva——Exactly 10 ml of human urine was taken and adjusted to pH 8—10 with 1 mol dm^{-3} sodium hydroxide solution. Next, 0.5—1 ml of 10^{-1} mol dm^{-3} calcium nitrate and 10^{-1} mol dm^{-3} barium chloride solutions were added as required, and the solution was diluted to 20 ml with water and centrifuged. A 1 ml aliquot of the centrifuged solution was taken and the NM or TOB content was determined by the standard procedure.

Exactly 5 ml of human saliva was taken, diluted to 10 ml with water and centrifuged. A 0.5 ml aliquot of the centrifuged solution was taken and the NM or TOB content was determined by the standard procedure.

Results and Discussion

The absorption spectra of Qn.Ph., Qn.Ph.–U(VI) (solution B), and Qn.Ph.–U(VI)–NM or Qn.Ph.–U(VI)–TOB (solution A) solutions in the presence of MC at pH 4.8 are shown in Fig. 1. The absorption maximum of solution A showed a red shift as compared with that of solution B and the magnitude of the increase in absorbance at 545 nm was proportional to the concentration of NM or TOB.

The maximum and constant absorbance was obtained between pH 4.6 to 5.2 by using 3.0 ml of 2.0×10^{-1} mol dm^{-3} acetic acid–2.0×10^{-1} mol dm^{-3} sodium acetate buffer solution among various buffer solution tested.

Among various nonionic and anionic surfactants such as MC, polyvinyl alcohol, Triton X-100 and sodium dodecyl sulfate, MC was the most effective dispersion agent; the maximum and constant absorbance was obtained with more than 0.5 ml of 0.5% MC solution in the final volume of 10 ml.

The molar ratio of U(VI) to Qn.Ph. in the presence of NM or TOB was found to be 1 : 2 by using the molar ratio and continuous variation methods. All further work was carried out with 0.5 ml of 1.0×10^{-3} mol dm^{-3} U(VI) solution and 2.0 ml of 5.0×10^{-4} mol dm^{-3} Qn.Ph. solution in the final volume of 10 ml.

It was found that the influence of foreign substances (especially phosphate ion) was decreased when the order of addition of the reagents was modified — when a solution containing NM or TOB was added to the solution obtained after color reaction between Qn.Ph. and U(VI) — as compared with the order of addition of the reagents used in the previous reports.[3)]

The maximum and constant absorbance of solution A at 545 nm against solution B was obtained when the solutions were kept at 20—25 °C for longer than 15 min. The absorbance was constant for at least 2 h.

Table I. Effect of Foreign Substances

Substance	Added as	Added (μg/10 ml)	Mole ratio (Substance/NM)	Absorbance at 545 nm
—	—	—	—	0.318
Fe(III)	Sulfate	1.4	1	0.352
Al(III)	Chloride	0.7	1	0.346
Cu(II)	Nitrate	7.9	5	0.335
Mo(VI)	Sodium	12.0	5	0.388
Co(II)	Nitrate	147.3	100	0.318
Zn(II)	Chloride	163.5	100	0.318
Ca(II)	Chloride	100.2	100	0.318
Oxalate	Sodium	22.1	10	0.250
Citrate	Acid	94.5	20	0.240
$H_2PO_4^-$	Potassium	242.5	100	0.310
Glycine	—	375.4	200	0.318
Glucosamine	Hydrochloride	895.9	200	0.318
Glucose	—	900.8	200	0.318
Uric acid	—	84.1	20	0.272
Human albumin	—	25.0	—	0.370
Cephalexin	—	434.2	50	0.318
Tetracycline	Hydrochloride	11.1	1	0.352
Thiamine	Hydrochloride	33.2	5	0.355
Diphenhydramine	Hydrochloride	63.8	10	0.362

NM, 15.4 μg/10 ml; U(VI), 5.0×10^{-5} M; Qn.Ph., 1.0×10^{-4} M; MC, 1.0 ml of 0.5% solution/10 ml; pH 4.8; reference, solution B.

Table II. Determination of NM and TOB Added to Human Urine (HU) and Human Saliva (HS)

		Added (μg)	Found[b] (μg)	Recovery[b] (%)	CV[a,c] (%)
HU[a]	NM	15.4	15.1	98.1	1.5
	TOB	11.7	11.1	95.0	1.1
HS[a]	NM	15.4	12.9	83.8	1.3
	TOB	11.7	9.6	82.4	1.7

a) HU (in 1 ml); HS (in 0.5 ml).
b) Average recovery from 5 determinations.
c) Coefficient of variation.

The calibration curves for the determination of NM and TOB were prepared by the standard procedure. Beer's laws were followed in the ranges of 3—50 μg of NM and 2—40 μg of TOB in the final volume of 10 ml. The sensitivities of this method, according to Sandell's scale,[5] were calculated to be 0.0048 μg/cm^2 for NM and 0.0040 μg/cm^2 for TOB, and the apparent molar absorptivities were 1.28×10^5 l mol^{-1} cm^{-1} and 1.17×10^5 l mol^{-1} cm^{-1}, respectively. The coefficients of variation were 0.88% for 15.4 μg/10 ml of NM ($n=8$) and 0.57% for 11.7 μg/10 ml of TOB ($n=8$).

Various substances were examined for interference. The results are summarized in Table I. Small amounts of some metal ions, such as iron(III), aluminum(III) and molybdenum(VI), interfered with the determination of NM because of the formation of colored complexes in weakly acidic media. Most of the other metal ions and anions tested, except oxalate and citrate ions, did not interfere in 100- to 200-fold excess. Amino acids, creatinine, hippuric acid and allantoin were tolerated in 100- to 200-fold excess, and ammonia, acetone, glucose,

TABLE III. Color Reaction between Qn.Ph.–U(VI) and Various Aminoglycoside Antibiotics

Substance	Added (M)	Absorbance at 545 nm
NM	2.5×10^{-6}	0.318
TOB	2.5×10^{-6}	0.296
Gentamycin	2.5×10^{-6}	0.412
Capreomycin	2.5×10^{-6}	0.282
Paromomycin	2.5×10^{-6}	0.220
Kanamycin	2.0×10^{-5}	0.530
Viomycin	2.0×10^{-5}	0.220
Streptomycin	2.0×10^{-5}	0.070
Clindamycin	5.0×10^{-5}	0
Lincomycin	5.0×10^{-5}	0

U(VI), 5.0×10^{-5} M; Qn.Ph., 1.0×10^{-4} M; MC, 1.0 ml of 0.5% MC solution/10 ml; pH 4.8; reference, solution B.

lactose, urea, *etc.* in 1000-fold excess over NM. Large amounts of uric acid could be conveniently eliminated by precipitation as the barium salt in weakly basic media or by uricase treatment. The influence of large amounts of phosphate ion could be effectively eliminated by precipitating phosphate as the calcium salt in weakly basic media. It was found that albumin was fairly well precipitated by addition of barium or calcium ion. Caffeine, phenacetin, antipyrine, ampicillin and salicylic acid did not interfere in 100- to 200-fold excess.

The molar ratio of NM to U(VI) in the complex was estimated to be 1 : 2 by using the molar ratio method. As the uranium ion exists as UO_2^{2+} in acidic media, it is concluded that the association complex may be expressed as $[(UO_2^{2+})\ (Qn.Ph.)_2]\ (NM)_2$.

Recoveries of NM and TOB added to human urine and saliva were examined. The results are shown in Table II. Recoveries of NM and TOB added to human urine were satisfactory, about 95—98%, but recoveries of NM and TOB added to human saliva were inadequate. The reason why the recoveries from human saliva are unsatisfactory is probably the influence of large amounts of glycosaminoglycan in saliva or insufficient centrifugation. Though the application of this method to other biological fluids such as human serum was not studied, the proposed method may also be applicable in other biological and clinical fields.

The color reaction between the Qn.Ph.–U(VI) complex and various aminoglycoside antibiotics was also studied by the standard procedure. The results are given in Table III.

Among aminoglycoside antibiotics, clindamycin and lincomycin did not produce colored complexes, and streptomycin scarcely did so. The proposed method should be useful for the determination of some aminoglycoside antibiotics fairly specifically.

Acknowledgement We are grateful to Dr. Y. Inamori and Dr. T. Nishino of this college for supplying materials.

References and Notes

1) This paper is part XXXIII of a series entitled "Application of Xanthene Derivatives for Analytical Chemistry," Part XXXII. *Bunseki Kagaku*, **32**, E327 (1983).
2) *a*) J. E. Fairbrother, *Pharm. J.*, **218**, 237, 509 (1977); *b*) D. W. Huges, A. Vilim, and W. L. Wilson, *Can. J. Pharm. Sci.*, **13**, 21 (1978); *c*) T. Uno, *Bunseki Kagaku*, **6**, 795 (1957); *d*) T. Sakaguchi, "Yakuhin Bunseki Kagaku," Nankodo, Tokyo, 1978, p. 530.
3) *a*) I. Mori, Y. Fujita, and K. Sakaguchi, *Bunseki Kagaku*, **30**, 261 (1981); *b*) I. Mori, Y. Fujita, and K.

Sakaguchi, *Bunseki Kagaku*, **31**, E 77 (1982); *c*) I. Mori, Y. Fujita, K. Sakaguchi, and S. Kitano, *Bunseki Kagaku*, **31**, 475 (1982).
4) Y. Fujita, I. Mori, and T. Enoki, *Bunseki Kagaku*, **24**, 253 (1975).
5) E. B. Sandell and H. Onishi, "Photometric Determination of Traces of Metals, General Aspects," 4th Ed., Wiley-Interscience, New York, 1978, p. 190.