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Spectrophotometric Determination of Gentamicin by Using a Membrane Filter Preconcentration Technique with *o*-Hydroxyhydroquinonephthalein and Uranium(VI)

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Color reactions among amino glycoside antibiotics such as gentamicin (GM), a metal ion such as uranium(VI), and *o*-hydroxyhydroquinonephthalein (Qnph) as a xanthene dye were studied by using a preconcentration technique based on membrane filtration. A simple, new, and highly sensitive spectrophotometric determination of GM has been established by using a preconcentration to collect Qnph-uranium(VI)-GM ternary complex. Beer's law is obeyed over the range 0—5.4 μg of GM in 5 cm^3 of dimethyl sulfoxide at 565 nm, and the apparent molar absorptivity for GM is 4.2×10^5 dm^3 cm^{-1} mol^{-1} with a Sandell sensitivity of 0.0013 μg cm^{-2} GM at 565 nm. The procedure is about 2.5-fold as sensitive as the method employing aqueous medium, and is applicable to determination of GM in calf serum.

We have already reported a simple and highly sensitive spectrophotometric determination of antibiotics such as neomycin, toburamycin (TOB),[1] streptomycin (SM),[2] cephalexin, ampicillin,[3] and minocycline[4] which uses *o*-hydroxyhydroquinonephthalein (Qnph) and uranium(VI), manganese(II), palladium(II), or zirconium(IV) in the presence of surfactants without solvent extraction. Alykov[5] reported a determination of amino glycoside antibiotics which uses Pyrocatechol Violet (PV), aluminium(III), and amino glycoside antibiotics ternary complex in water–ethanol (7:3) medium. But, most of these methods are somewhat unsatisfactory for gentamicin (GM) in respect of sensitivity and selectivity, and thus their application to biological samples has scarcely been carried out.

On the other hand, we have already reported[6] a new and sensitive spectrophotometry of micro quantities of papaverine hydrochloride as organic bases which resorts to a preconcentration technique based on membrane filtration of the 2,4,5,7-tetrachlorofluorescein (T.Cl.fl.)-palladium(II)-papaverine hydrochloride ternary complex.

In this research, a color reaction was studied of amino glycoside antibiotics by utilizing the Qnph–metal ion–amino glycoside colored formation with a metal ion and Qnph, and suitable conditions were established for the spectrophotometric determination of microquantities of GM by using a preconcentration technique based on the use of membrane filter to collect the Qnph-uranium(VI)-GM ternary complex. The proposed method was applied to an assay of GM in calf serum.

Experimental

Reagents and Materials. GM (Shionogi Co., Ltd.; manifested potency 612 μg mg^{-1}) and uranium(VI) solutions (5.0×10^{-4} mol dm^{-3}) were prepared as described in our previous report.[1] Qnph methanol solution (5.0×10^{-4}mol dm^{-3}) and Walpole buffer solution (sodium acetate-acetic acid) were prepared according to our previous reports,[1,6] and 0.05% methyl cellulose (MC, 1500 cps, Kishida Chemical Co., Ltd.) solution was prepared by dissolving MC in water. All the other materials and reagents were of analytical grade and were used without further purification. Doubly deionized water was used.

Apparatus. A Shimadzu model UV-160 or 240 recording spectrophotometer with 1.0 cm silica cells was used to record absorption spectra and for absorbance measurements. A Hitachi-Horiba F-7AD glass electrode pH meter was used for pH measurements. A Toyo TM 4 membrane filter (nitrocellulose membrane, 25 mm in diameter and 0.2 μm in pore size) and a Toyo KGS-25 filter holder were used.

Standard Procedure. A GM solution containing 0—5.4 μg of GM was placed in a 10 cm^3 volumetric flask; to this were added 0.5 cm^3 of 0.05% MC solution, 2.0 cm^3 of acetate buffer solution (pH 5.0), 2.0 cm^3 of 5.0×10^{-4} mol dm^{-3} uranium(VI) solution, and 1.0 cm^3 of 5.0×10^{-4} mol dm^{-3} Qnph solution. The mixture was diluted to the mark with water, agitated well (aqueous solution A), and kept at 20—25 °C for 10 min together with a reference solution (Qnph–uranium(VI) solution, aqueous solution B). Aqueous solutions A and B were each filtered with a membrane filter, and then the filtered substances were washed with about 10 cm^3 of water. The membrane filters were dissolved in 5 cm^3 of dimethyl sulfoxide (DMSO), and then the absorbance of DMSO solution A was measured at 565 nm against DMSO solution B as a reference. The concentration of GM was determined by using a calibration curve.

Results and Discussion

Color Reaction, Absorption Spectra, and Effects of Dyes and Metal Ions. The absorption spectra of Qnph solution (DMSO solution C), Qnph–uranium(VI) binary complex solution (DMSO solution B), and Qnph–uranium(VI)–GM ternary complex solution (DMSO solution A) are shown in Fig. 1. The absorption maximum of DMSO solution A is around 565 nm, and the magnitude of absorbance at 565 nm is proportional to the concentration of GM.

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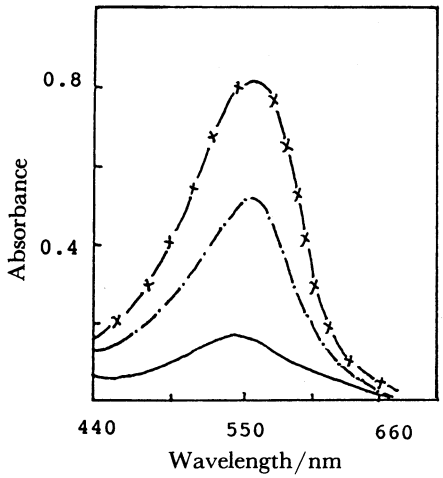

Fig. 1. Absorption spectra of Quph-uranium(VI)-GM DMSO solution (DMSO solution A), Quph-uranium(VI) DMSO solution (DMSO solution B) and Qnph DMSO solution (DMSO solution C). Uranium(VI): 1.0×10^{-4} mol dm^{-3}; Qnph: 5.0×10^{-5} mol dm^{-3}; Reference: DMSO.
——: DMSO solution C, —·—·—: DMSO solution B, —×—: DMSO solution A.

The effects of xanthene dyes and metal ions in this reaction system were also examined by measuring the absorbance of xanthene dye-metal ion-GM DMSO solution against the xanthene dye-metal ion DMSO solution. In respect of sensitivity, stability, and selectivity, Qnph as a xanthene dye and uranium(VI) as a metal ion are superior to the other xanthene dyes-salicylfluorone (Sal.fl), phenylfluorone (Phfl), *o*-hydroxyhydroquinonesulfophthalein (Qn.sul.ph), Gallein (Gall), Xylenol Orange (XO), and PV- and the other metal ions such as molybdenum(VI), tungstic acid (W(VI)), palladium(II), and lanthanum(III). These results are given in Tables 1 and 2. Next, color reactions between the Qnph-uranium(VI) solution and various amino glycoside antibiotics were compared by measuring the absorbance at 565 nm against DMSO solution B. Of the various amino glycoside antibiotics tested, the color reactions for GM and TOB are most sensitive. The results are given in Table 3.

On the basis of these results, GM was chosen for the purpose of fundamental investigations on the determination of amino glycoside antibiotics using Qnph and uranium(VI).

Effect of pH. A constant and maximum difference of absorbance between DMSO solutions A and B was obtained in the pH range from 4.8 to 5.2 when the final solution was adjusted with 2.0 cm^3 of 2.0×10^{-1} mol dm^{-3} sodium acetate-acetic acid buffer solution.

Effect of Surfactants. The effect of surfactant on the preconcentration technique using a membrane filter was examined for various surfactants. Although DMSO solutions A and B in the absence of surfactant are lacking in reproducibility and sensitivity, the presence of a small amount of MC (1500 cps), a nonionic surfactant, is most effective of the various surfactants: poly(*N*-vinylpyrrolidone) (PVP, K-15), poly(vinyl alcohol) (PVA, n=2000), MC, and sodium dodecyl sulfate (SDS). A maximum and constant absorbance of DMSO solution A was observed addition of 0.4—0.6 cm^3 of 0.05% MC solution in a final volume of 10 cm^3.

Table 1. Effect of Dyes[a)]

Dye	λ_{max}/nm	Absorbance
Qnph	565	0.420
Sal.fl	565	0.357
Phfl	570	0.129
Qn.sul.ph	550	0.079
Gall	560	0.068
PV	555	0.004
XO	—	—

a) GM taken: 2.7 μg/5 cm^3; uranium(VI): 1.0×10^{-4} mol dm^{-3}; dyes: 5.0×10^{-5} mol dm^{-3}; MC: 0.5 cm^3 of 0.05% MC solution/10 cm^3; pH: 5.0; reference: dye-uranium(VI) DMSO solution.

Table 2. Effect of Metal Ions[a)]

Metal ion	λ_{max}/nm	Absorbance
U(VI) (UO_2^{2+})	565	0.420
Mo(VI) (MoO_4^{2-})	565	0.156
W(VI) (WO_4^{2-})	495	0.039
V(V) (VO_3^-)	530	0.111
Pd(II)	545	0.379
La(III)	530	0.063

a) GM taken: 2.7 μg/5 cm^3; metal ions: 1.0×10^{-4} mol dm^{-3}; Qnph: 5.0×10^{-5} mol dm^{-3}; MC: 0.5 cm^3 of 0.05% MC solution/10 cm^3; pH: 5.0; reference: Quph-metal ion DMSO solution.

Table 3. The Apparent Molar Absorptivities of Amino Glycoside Antibiotics Obtained by the Present Method[a)]

Compound	Apparent molar absorptivity at 565 nm ε/dm^3 mol^{-1} cm^{-1}
Gentamicin (GM)	4.2×10^5
Toburamycin (TOB)	2.6×10^5
Kanamycin	1.0×10^5
Viomycin	0.5×10^5
Streptomycin (SM)	4.9×10^3

a) Uranium(VI): 1.0×10^{-4} mol dm^{-3}; Qnph: 5.0×10^{-5} mol dm^{-3}; MC: 0.5 cm^3 of 0.05% MC solution; pH: 5.0; reference: Qnph-uranium(VI) DMSO solution.

Table 4. Effect of Foreign Substances[a)]

Substance	Added		Absorbance at 565 nm	Recovery
	μg	mole ratio		%
—	—	—	0.420	100.0
Iron(III) (Sulfate)	4.2	15	0.503	119.8
Magnesium (Nitrate)	48.6	400	0.429	102.1
Calcium (Chloride)	80.2	400	0.400	95.2
Phosphate (Sodium)	0.5	1	0.359	85.5
Oxalic Acid	6.3	10	0.386	91.9
Oleic Acid	211.8	150	0.543	129.3
Citric Acid	28.8	30	0.247	58.8
Uric Acid	126.0	150	0.242	57.6
Glutamic Acid	220.0	300	0.420	100.0
Deoxyribonucleic Acid	3.0	—	0.320	76.2
Trichloroacetic Acid	122.5	150	0.934	222.4
D-Glucose	2702.4	3000	0.420	100.0
Glycine	1126.1	3000	0.420	100.0
Thiamine (Hydrochloride)	13.3	10	0.688	163.8
Human albumin	10.0	—	0.565	134.5
Caffeine	29.1	30	0.336	80.0
Minocycline (Hydrochloride)	6.9	3	0.770	183.3
Ampicillin	610.0	350	0.420	100.0
Adenosine Triphosphate	380.0	150	0.163	38.8
Chondroitin Sulfate (Sodium)	500.0	—	0.488	116.2
N-Hexadecylpyridinium (Chloride)	3.0	2	0.580	138.1
Sulfisomidin	139.2	100	0.450	107.1

a) GM taken: 2.7 μg/5 cm^3; uranium(VI): 1.0×10^{-4} mol dm^{-3}; Qnph: 5.0×10^{-5} mol dm^{-3}; MC: 0.5 cm^3 of 0.05% MC solution/10 cm^3; pH: 5.0; reference: Qnph–uranium(VI) DMSO solution (DMSO solution B).

Effects of Qnph and Uranium(VI) Concentrations. The effect of Qnph and uranium(VI) concentrations on the standard procedure was examined by varying the molar ratio of Qnph to uranium(VI) and measuring the absorbance at 565 nm with the amounts of uranium(VI) and GM kept constant. The molar ratio of uranium(VI) 2 to Qnph 1 is most effective for the determination of GM. Accordingly, all further works were carried out with 1.0×10^{-4} mol dm^{-3} uranium(VI) and 5.0×10^{-5} mol dm^{-3} Qnph in the final volume of 10 cm^3.

Effects of Membrane Filters and Organic Solvents. Effects of organic solvent-soluble membrane filters and water miscible organic solvents in this reaction system were examined according to our previous report.[6)] Nitrocellulose of 0.20 μm pore size is most effective of the pore sizes tested: 0.10, 0.20, 0.30, 0.45, 0.65, and 0.80 μm. DMSO is the best in respect of sensitivity and reproducibility.

Calibration Curve and Reproducibilty. Beer's law holds in the concentration range up to 5.4 μg of GM in the final volume of 5 cm^3 DMSO. The sensitivity, according to Sandell's scale, is 0.0013 μg cm^{-2} for GM at 565 nm, and the apparent molar absorptivity was calculated to be 4.2×10^5 dm^3 mol^{-1} cm^{-1}. The coefficient of variation (CV) for 5 replicate determinations was 1.6% for 2.7 μg of GM.

Interference of Foreign Substances. The interference of various ions and substances was examined for the determination of 2.7 μg 5 cm^{-3} of GM. Coexistence of a metal ion such as iron(III) or copper(II) causes positive errors, though the interference can be suppressed by addition of nitrilotriacetic acid (NTA) as a masking agent. Coexistence of oxalate, or phosphate, or fluoride ion, or protein interferes with carrying out the reaction. Glycine, D-glucose, glutamic acid, salicylic acid, or ampicillin does not interfere when present in 300- to 3000-fold excess over GM. These results are summarized in Table 4.

The effect of various agents was investigated to suppress the interference of protein in this proposed method using membrane filter. Sulfosalicylic acid is most suitable, because pH adjustment of sample solution is easy and because the effect of metal ions is partially masked by its addition, of the agents tested: methanol, trichloroacetic acid, sulfosalicylic acid, tungstic acid, perchloric acid, etc.

Composition of Complex. The molar ratio of GM to uranium(VI) in the Qnph–uranium(VI)–GM ternary complex was estimated to be 1:2 by the molar ratio method. The molar ratio of the Qnph–uranium(VI) complex mixture in the presence of GM was 1:2 as determined by the continuous variation and molar ratio methods. Thus, though further investigation is necessary, the colored ternary complex formed in this reaction system appears to be a ternary complex with a ratio Qnph 1: uranium(VI) 2: GM 1 in DMSO solution.

Recovery of GM Added to Calf Serum. Recovery of GM added to calf serum (Nakarai Chemical Co., Ltd.) was examined by the proposed method. A satisfactory result was obtained in the following way: Exactly 0.5 cm^3 of calf serum containing up to 0—54 μg of GM was taken, 2.0 cm^3 of 3.0% sulfosalicylic acid

Table 5. Analytical Results of GM or TOB Added to Calf Serum[a]

	Amounts of antibiotics		Recovery/%[b]
	Added/μg	Found/μg[b]	
Gentamicin	1.36	1.44	105.6
(GM)	2.72	2.90	106.6
Toburamycin	1.46	1.48	101.4
(TOB)	2.92	3.04	104.1

a) Calf serum taken: 0.5 cm³. b) Average from 5 determinations.

solution was added to this solution, and the mixture was filtered by using a membrane filter. The filtrate was diluted to 10 cm³ with water, and 1.0 cm³ of this diluted solution was taken, and then the GM content was determined according to the standard procedure. The results are given in Table 5. The experimental results were in good agreement with these given in our previous report[4] and those obtained according to other methods.[7,8]

In conclusion, this proposed spectrophotometric determination of GM by the membrane filter preconcentration technique is more than 2.5-fold as sensitive as the method employing aqueous medium,[3] and 30-fold as sensitive as the method using PV–aluminium(III) complex.[5] The proposed method is simple, rapid, sensitive, and reproducible as compared with the Qnph–uranium(VI) aqueous method,[1] the PV–aluminium(III) method,[5] and other methods.[7,8]

Although further investigation is necessary, this preconcentration method using membrane filter may be available as a new, highly sensitive, and simple analytical procedure for various pharmaceutical and biological samples.

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