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New and Selective Spectrophotometric Determination of Streptomycin using *o*-Hydroxyhydroquinonephthalein and Manganese(II)[1]

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A new, simple, sensitive and selective spectrophotometric method for the determination of streptomycin (SM) using *o*-hydroxyhydroquinonephthalein (Qn.Ph.) and manganese (II) [Mn(II)] in 40% methanolic media was established. This method is based on the fact that the SM-Mn(II)-Qn.Ph. complex shows a red shift in weakly basic media as compared with the Mn(II)-Qn.Ph. complex. This method could be used to determine up to ~25 μg/10 ml of SM; the apparent molar absorptivity was estimated to be 2.4×10^5 l mol^{-1} cm^{-1} at 570 nm. Guanidino compounds appear to be involved in this color reaction. Recovery of SM added to human urine was good.

Keywords——spectrophotometry; streptomycin; *o*-hydroxyhydroquinonephthalein; manganese(II) poly(*N*-vinylpyrrolidone); aqueous-methanolic media; guanidino compound

Streptomycin(SM), an aminoglycoside antibiotic,[2] is widely used in clinical chemotherapy. Many methods for the determination of SM have been reported, and there have been several related reviews and commentaries.[3] There are various spectrophotometric and colorimetric procedures for the assay of SM.

These methods are based on[3e]: (1) the use of the guanidino group (*e.g.*, Sakaguchi reaction),[4] (2) the use of the amino group or glucosamine (*e.g.*, Elson–Morgan reaction),[5] (3) the use of the aldehyde group,[6] (4) the use of the maltol reaction.[7]

Most of these methods are subject to interference from foreign substances and have disadvantages such as complexity of procedure, or lack of sensitivity and reproducibility. Lately, Alykov[8] reported a sensitive fluorometric method for the determination of aminoglycoside antibiotics by using a multivalent metal ion and organic reagent, for example praseodymium (III) and fluorescein-complexone.

On the other hand, we noticed that the manganese(II)[Mn(II)]-*o*-hydroxyhydroquinonephthalein(Qn.Ph.) complex exhibited a considerable red shift in the presence of SM in weakly basic aqueous-methanol, and found that the color reaction was fairly selective and sensitive.

In this paper, a new, simple, sensitive and selective spectrophotometric determination of SM using Qn.Ph. and Mn(II) has been established. The proposed method was applied to the determination of SM added to human urine.

Experimental

Reagents and Solutions——Standard SM Solution: A stock solution (1.0×10^{-3} M, M = mol dm^{-3}) of SM was prepared by dissolving streptomycin sulfate (Banyu Pharmaceutical Co., Ltd., Tokyo) in water, and the working solution was prepared by suitable dilution of this stock solution; the solution was stored in an amber glass bottle.

Mn(II) and Qn.Ph. Solutions: A 1.0×10^{-3} M aqueous solution of Mn(II) and a 1.0×10^{-3} M methanol solution of Qn.Ph. were prepared as described in a previous report.[9]

Poly(*N*-vinylpyrrolidone) (PVP, K-90) Solution: A 1.0% methanol solution of PVP (Tokyo Kasei Kogyo Co., Tokyo) was prepared.

Buffer Solution: A 0.1 M glycine and sodium chloride–0.1 M sodium hydroxide buffer (Sørensen buffer) solution was used.

All other chemicals were of analytical reagent grade, unless otherwise specified. Double-distilled water was used.

Apparatus——Absorption spectra and absorbance measurements were made with a Shimadzu Model 240 recording spectroph tometer, with 1.0-cm matched silica cells. pH measurements were made a Hitachi-Horiba M(del F-7 AD glass electrode pH meter.

Standard Procedure——The following components were mixed in a 10-ml amber calibrated flask; 0.5 ml of 1.0×10^{-3} M Mn(II) solution, 3.0 ml of 1.0% PVP solution and 1.0 ml of 1.0×10^{-3} M Qn.Ph. solution. The pH of the final solution was adjusted to pH 10.0—10.3 with 2.5 ml of 0.1 M glycine and sodium chloride–0.1 M sodium hydroxide buffer solution, and a solution containing up to ~25 μg of SM was added. The mixture was diluted to 10 ml with water, immediately transferred to an amber test tube with a stopper, and mixed thoroughly by repeatedly inverting and shaking the tube. The solution was kept at 25°C for 25 min and the absorbance of the SM-Mn(II)-Qn.Ph. solution (solution A) was measured at 570 nm against the Mn(II)-Qn.Ph. solution (solution B).

Results and Discussion

Absorption Spectra

In Fig. 1, curves I, II and III show the absorption spectra of Qn.Ph., Mn(II)–Qn.Ph. and SM–Mn(II)–Qn.Ph. solutions, respectively, in the presence of PVP in weakly basic media. On the addition of SM to solution B, it was evident that the SM–Mn(II)–Qn.Ph. complex exhibited a considerable red shift in aqueous-methanolic media; the complex showed maximum absorbance at 570 nm against water. On the other hand, the SM–Mn(II) solution showed no absorption in this wavelength range.

Therefore, the absorbance of solution A at 570 nm against solution B was used for the determination of SM.

Effect of pH and Surfactants

The effect of pH on the reaction was examined in the pH range of 9.5 to 11.0. The maximum and constant absorbance was obtained between pH 10.0 to 10.3 when the final solution (40% methanolic solution) was adjusted with 2.5 ml of 0.1 M glycine and sodium chloride–0.1 M sodium hydroxide buffer solution among various buffer solutions tested, such as 0.1 M glycine and sodium chloride–0.1 M sodium hydroxide, 0.05 M sodium tetraborate–0.1 M sodium hydro-

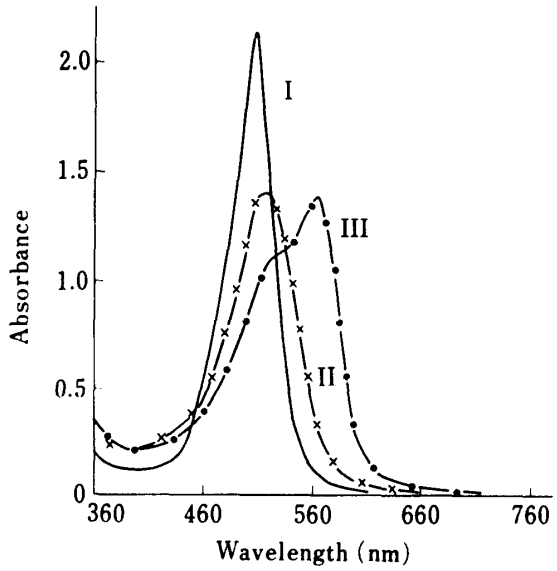

Fig. 1. Absorption Spectra of Qn.Ph., Qn.-Ph.-Mn(II) and Qn.Ph.-Mn(II)–SM Solutions in 40% Methanolic Media at pH 10.1

SM, Mn(II) and Qn.Ph., 5.0×10^{-5} M; PVP, 3.0 ml of 1.0% PVP solution/10 ml; reference, water; curve I, Qn.-Ph.; curve II, Qn.Ph.–Mn(II); curve III, Qn.Ph.–Mn(II)–SM.

TABLE I. Effect of Surfactant

Surfactant	Absorbance at λ max	
None	0.124	560
PVP (K-90)	0.620	570
PVP (K-30)	0.482	570
PVP (K-15)	0.298	570
PVA (n:2000)[a]	0.438	565
LT-221[a]	0.156	560
SDS[a]	0.136	560
CTAC[a]	0	—

SM, 14.5 μg/10 ml; Mn(II), 5.0×10^{-5}M; Qn.Ph., 1.0×10^{-4}M;suy-factant, 1.0 ml of 1.0 ˚ surfactant solution/10 ml; pH 10.1; reference, solution B.

a) PVP, polyvinyl alcohol; LT-221, polyoxyethylene sorbitan monolaurate; SDS, sodium dodecyl sulfate; CTAC, cetyltrimethylammonium chloride.

xide, 0.1 M ammonium chloride–0.1 M ammonia, 0.1 M sodium carbonate–0.1 M sodium hydrogen carbonate, *etc.*

Among various surfactants, PVP(K-90) was the most effective dispersion agent. The maximum and constant absorbance could be obtained with 1.0 ml of 1.0% PVP solution in the final volume of 10 ml. The results are given in Table I.

Effect of Qn.Ph. and Mn(II) Concentrations

The effect of dyes was studied. Qn.Ph. was superior to the other dyes examined in terms of sensitivity. The results are shown in Table II.

TABLE II. Effect of Dyes

Dye	Absorbance at λ max	
Qn. Ph.	0.620	570
Ph. fl.[a)]	0.080	605
Salicyl. fl.[a)]	0.048	580
PR[a)]	0.116[b)]	545
Gall.[a)]	0	—
CAS[a)]	0	—
MTB[a)]	0	—
PAR[a)]	0	—

SM, 145 μg/10 ml; Mn(II), 5.0×10^{-5} M; dye, $10\times10^{-4.5}$ M; PVP, 3.0 ml of 1.0% PVP solution/10 ml; pH 10.1; reference, solution B.

a) Ph.fl., phenylfluorone; saliccyl.fl., salicylfluorone, PR, pyrogallol red; Gall., gallein; CAS, chromazurol S; MTB, methylthymol blue; PAR, 4-(2-pyridylazo)resorcinol.

b) Very unstable.

The effect of metal ions was also studied. In this reaction system, only Mn(II) was effective among the various metal ions tested: Mn(II), zinc(II), copper(II), praseodymium(III), cobalt(II), cadmium(II), *etc.* The results are shown in Table III.

The effect of the amount of Qn.Ph. on absorbance was examined by varying the molar

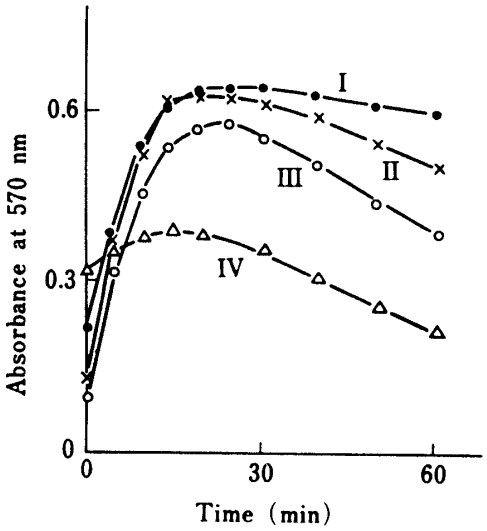

Fig. 2. Relationship between Standing Time and Absorbance at Various Qn. Ph. Concentrations

SM, 14.5 μg/10 ml; Mn(II), 5.0×10^{-6} M; PVP, 3.0 ml of 1.0% PVP solution/10 ml; pH 10.1; reference, solution B. Qn.Ph. concentrations [Mn (II): Qn.Ph.]: curve I, 1.0×10^{-4} M (1: 2); curve II, 1.5×10^{-4} M (1: 3); curve III, 2.0×10^{-4} M (1: 4) ;curve IV, 5.0×10^{-5} M (1: 1).

TABLE III. Effect of Metal Ions

Metal ion	Absorbance at λ max	
Mn(II)	0.620	570
Zn(II)	0.032	570
Cu(II)	−0.043	535
Pr(III)	−0.022	535
Cd(II)	0	—
Co(II)	0	—
Pd(II)	0	—
Ca(II)	0	—
Fe(III)	0	—
Th(IV)	0	—

SM, 14.5 μg/10 ml; Qn.Ph., 1.0×10^{-4} M; Metal ions, 5.0×10^{-5} M; PVP, 3.0 ml of 1.0 PVP solution/10 ml;pH 10.1; reference, solution B.

ratio of Qn.Ph. to Mn(II), the amounts of Mn(II) and SM being kept constant. The molar ratio of Mn(II) 1: Qn.Ph. 2 was most effective. The results are shown in Fig. 2. Further, the molar ratio of Mn(II) to Qn.Ph. in the complex was found to be 1: 2 in the presence of SM by using the molar ratio method (absorption maximum, 570 nm).

Accordingly, all further work was carried out with 5.0×10^{-5} M Mn(II) and 1.0×10^{-4} M Qn.Ph. in the final volume of 10 ml.

Optimal Concentration of Methanol

The effect of various water-miscible organic solvents was studied by measuring the absorbance of solution A at 570 nm against solution B. The most suitable solvent was methanol among various water-miscible organic solvents tested: methanol, ethanol, acetone, acetonitrile, 1,4-dioxane, *N*,*N*-dimethylformamide and dimethylsulfoxide(DMSO). The results are summarized in Fig. 3.

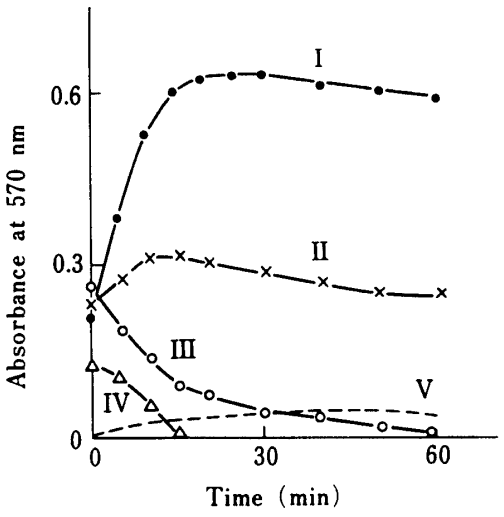

Fig. 3. Relationship between Standing Time and Absorbance in Various Water-Micible Organic Solvents (40% Organic Solvent) at 25°C

SM, 14.5 μg/10 ml; Mn(II), 5.0×10^{-5} M; Qn.Ph., 1.0×10^{-4} M; PVP, 3.0 ml of 1.0% PVP solution/10 ml; pH 10.1; reference, solution B; curve I, methanol; curve II, ethanol; curve III, acetone; curve IV, acetonitrile; curve V, DMSO.

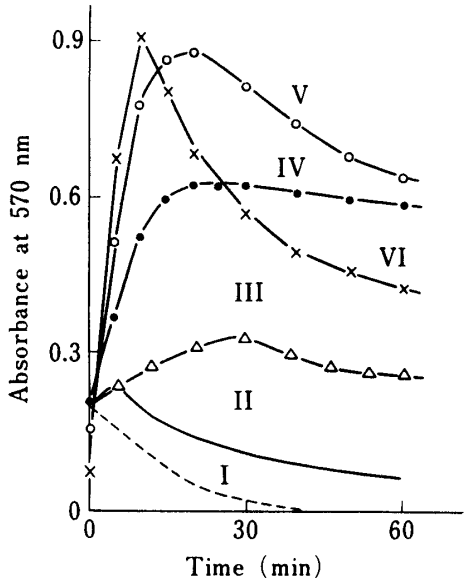

Fig. 4. Relationship between Standing Time and Absorbance at Various Methanol Concentrations at 25°C

SM, 14.5 μg/10 ml; Mn(II), 5.0×10^{-5} M; Qn.Ph., 1.0×10^{-4} M; PVP, 3.0 ml of 1.0% PVP solution/10 ml; pH 10.1; reference, solution B. Methanol concentrations: curve I, 10%; curve II, 20%; curve III, 30%; curve IV, 40%; curve V, 50%; curve VI, 60%.

The effect of methanol concentration was also studied. The absorbance of solution A at 570 nm against solution B gradually increased with increasing amount of methanol. However, in this work 40% methanolic solution was used from the viewpoints of stability at 25°C, reproducibility and simplicity of analytical procedure. The results are given in Fig. 4.

Sequence of Addition and Stability

The same results were obtained in this reaction system even when the order of addition of the reagents was varied. However, the influence of foreign substances was greatly decreased when a solution containing SM was added to the solution obtained after the color reaction between Mn(II) and Qn.Ph. It was found that solution A of SM–Mn(II)–Qn.Ph. was extremely unstable and was decolorized by ambient light. Under the standard conditions, the maximum and constant absorbance of solution A at 570 nm against solution B was obtained when solutions A and B were both kept at 25°C for 20—30 min in amber test tubes.

Calibration Curve and Reproducibility

The calibration curve for the determination of SM was obtained by the standard procedure. Beer's law was followed up to ~25 μg of SM in the final volume of 10 ml. The sensitivity of this method, according to Sandell's scale,[10] was 0.0023 μg/cm^2 for SM at 570 nm, and the apparent molar absorptivity was calculated to be 2.4×10^5 l mol^{-1} cm^{-1}. The reproducibility for 14.5 μg of SM (5 experiments) was found to be 2.3%.

Influence of Foreign Substances

Various substances were examined for interference. Most of the substances tested did not interfere when present in an equimolar amount with respect to SM. Zinc(II), cobalt(II), amino acids, organic acids and sulfide ion did not interfere in 5- to 10-fold excess. Taurine, creatinine, uric acid, hippuric acid and various anions were tolerated in 50- to 100-fold excess, and urea, ammonia, acetone, glucose in 1000-fold excess. Human albumin did not interfere in large amounts, and salicylic acid, caffeine, phenacetin, antipyrine, diphenhydramine and isoniazid did not interfere in 100-fold excess. These results are summarized in Table IV.

TABLE IV. Effect of Foreign Substances

Substance	Added (μg/10 ml)	Mole ratio (Substance/SM)	Absorbance at 570 nm
—	—	—	0.620
Fe(III) (sulfate)	7.0	5	0.745
Ca(II) (chloride)	5.0	5	1.405
$S_2O_3^{2}$ (sodium)	28.0	10	0.440
Glutamine	36.6	10	0.580
Histidine	38.8	10	0.620
Human albumin	500.0	—	0.620
Hippuric acid	448.0	100	0.620
Creatinine	282.8	100	0.620
Glucose	450.0	100	0.620
Caffeine	485.5	100	0.620
Salicylic acid	345.3	100	0.620
Urea	1501.5	1000	0.620

SM, 14.5 μg/10 ml; Mn(II), 5.0×10^{-5} M; Qn.Ph., 1.0×10^{-4} M; PVP, 3.0 ml of 1.0% PVP solution/10 ml; pH 10.1; reference, solution B.

Moreover, any interference could be significantly overcome by measuring the difference of absorbance at 570 nm between solution A and solution B containing the same ingredients except for SM. That is to say, SM could be determined by measuring absorbance at 570 nm according to the standard addition method in the presence of interfering substances.

Recovery Study

Recovery of SM added to human urine was examined. Satisfactory results were obtained in the following way. Exactly 10 ml of human urine containing up to ~25 μg of SM was adjusted to about pH 10 with 1 M sodium hydroxide solution. Then the solution was diluted

TABLE V. Determination of SM added to Human Urine

SM		Recovery[a] (%)	CV[b] (%)
Added (μg)	Found[a] (μg)		
7.3	7.1	96.6	3.8
14.5	14.4	99.2	2.5
21.8	21.7	98.5	2.9

a) Average recovery from 5 experiments.
b) Coefficient of variation.

to 20 ml with water and centrifuged if necessary. A requisite volume (less than 1 ml) of the sample solution was taken and the SM content was determined according to the standard procedure using the standard addition method. The results are shown in Table V.

Reaction between Mn(II)-Qn.Ph. and Other Substances

The effects of other aminoglycoside antibiotics and other guanidino compounds were also studied by the procedure described above. Of the aminoglycoside antibiotics tested, only dihydrostreptomycin(containing guanidino groups) gave the same result as SM. Guanidino compounds increased the absorbance of solution B. The results are shown in Table VI.

TABLE VI. Reaction of other Guanidine Compounds and Aminoglycoside Antibiotics

Substance	Added (M)	Absorbance at 570 nm
SM	2.5×10^{-6}	0.620
Dihydrostreptomycin (sulfate)	2.5×10^{-6}	0.620
Guanidine (nitrate)	2.0×10^{-4}	0.062
Canavanine (sulfate)	2.0×10^{-4}	0.072
Creatine	2.0×10^{-4}	0.036
1, 3-Diphenylguanidine	2.0×10^{-4}	0.180
Arginine	2.0×10^{-4}	0.185
Guanethidine (sulfate)	5.0×10^{-5}	0.266
Angiotensin III[a]	1.0×10^{-5}	0.060
Bradykinin[b]	1.0×10^{-5}	0.350
Guanosine	2.0×10^{-4}	0
Kanamycin (sulfate)	2.0×10^{-4}	0
Neomycin (sulfate)	2.0×10^{-4}	0
Paromomycin (sulfate)	2.0×10^{-4}	0
Toburamycin (sulfate)	2.0×10^{-4}	0
Gentamicin (sulfate)	2.0×10^{-4}	0

a) Angiotensin III, Arg-Val-Tyr-Ile-His-Pro-Phe.
b) Bradykinin, Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg.

The molar ratio of Mn(II) to Qn.Ph. in the complex was estimated to be 1 : 2 by using the molar ratio and the continuous variation methods. The composition of the SM–Mn(II)–Qn.Ph. complex could not be determined by molar ratio method, and further investigation is still necessary.

Conclusion

A simple, sensitive and selective spectrophotometric determination of SM based on the formation of the SM–Mn(II)–Qn.Ph. complex in 40% methanolic solution and in the presence of PVP was developed. Beer's law was obeyed at up to ~25 μg of SM in the final volume of 10 ml. The apparent molar absorptivity in this procedure was estimated to be 2.4×10^5 l mol^{-1} cm^{-1} for SM at 570 nm. This method is inferior to the fluorometric methods[8,11] in sensitivity, but is about 20 times and 2 times more sensitive than the method[12] utilizing the Sakaguchi reaction and the periodate-thiobarbituric acid method,[13] respectively. The coefficient of variation for 5 determinations (for 14.5 μg of SM) was 2.3% and many foreign substances scarcely affected the determination of SM. Recovery of SM added to human urine was good, so this method may be applicable to biological fluids, as well as for assay of SM in pharmaceutical preparations, *etc.*

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References and Notes

1) This paper is Part XXVIII of a series entitled "Application of Xanthene Derivatives for Analytical Chemistry," Part XXVII, *Chem. Pharm. Bull.*, **31**, 1398 (1983). This work will be presented at 103rd Annual Meeting of the Pharmaceutical Society of Japan in Tokyo, April 1983.
2) *a*) H. Umezawa, "Advances in Carbohydrate Chemistry and Biochemistry," Vol. 30, ed. by R.S. Tipson and D. Horton, Academic Press, 1974, p. 183; *b*) Proceedings of the 5th Anniversary Symposium of Institute of Bioorganic Chemistry on Recent Advances in the Chemistry of Aminoglycosides, *Jap. J. of Antibiotics*, **32**, Supplement 1—236 (1979); *c*) M. Ookoshi, *Saishin Igaku*, **34**, 1487 (1979); *d*) S. Umezawa, *Kagaku*, **33**, 183, 283 (1978).
3) *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. Ya Goncharskya, aud V.G. Koroleva, *Antibiotiki* (Moscow), **24**, 859 (1979); *d*) H.W. Unterman and S. Weissbuch, *Pharmazie*, **29**, 752 (1974); *e*) T. Uno, *Bunseki Kagaku*, **6**, 795 (1957); *f*) T. Sakaguchi, "Yakuhin Bunseki Kagaku," Nankodo, Tokyo, 1978, p. 530; *g*) The Japan Society for Analytical Chemistry, "Bunseki Kagaku Binran," Maruzen Ltd., Tokyo, 1981, p. 1199.
4) S. Sakaguchi, *J. Biochem.* (Tokyo), **5**, 13 (1925).
5) L.A. Elson and W.T. Morgan, *Biochem. J.*, **27**, 1824 (1933).
6) E.K. Marshall, K.C. Jr. Blanchard, and E.L. Buhle, *J. Pharmacol. Exp. Ther.*, **90**, 367 (1947).
7) J.R. Scheck and M.A. Spielman, *J. Am. Chem. Soc.*, **67**, 2276 (1945).
8) N.M. Alykov, *Zh. Anal. Khim.*, **36**, 1387, 1606 (1981).
9) I. Mori, Y. Fujita, and K. Sakaguchi, *Bunseki Kagaku*, **31**, E239 (1982).
10) E.B. Sandell and H. Onishi, "Photometric Determination of Traces of Metals, General Aspects," 4th Ed., Wiley-Interscience, New York, 1978, p. 190.
11) *a*) R.B. Conn and R.B. Davis, *Nature* (London), **183**, 1053 (1959); *b*) S. Yamada and H.A. Itano, *Biochim. Biophys. Acta*, **130**, 538 (1966); *c*) Y. Ohkura and M. Kai, *Anal. Chim. Acta*, **120**, 411 (1979).
12) Y. Kanazawa, *J. Antibiot.*, **4**, 552 (1951).
13) *a*) E. Duda, *Anal. Chem.*, **51**, 651 (1973); *b*) H. Fukumoto, *Eisei Kagaku*, **22**, 380 (1976).