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Fluorometric Determination of Magnesium with 3-Hydroxy-3′,4′-dimethoxyflavone

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In a previous paper,[2] we established a new method for the fluorometric determination of magnesium using 3,3′,4′-trihydroxyflavone. Later, 3-hydroxy-3′,4′-dimethoxyflavone was found to be more excellent for the fluorometric determination of micro amounts of magnesium. In the present paper, magnesium in urine and serum was assayed with good accuracy by using a strongly acid exchange resin to remove the interference of foreign ions.

Apparatu and Reagent

The fluorescence spectra and fluorescence intensity were measured with Shimadzu RF-501 spectrofluorometer. The slits were arranged to have an excitation beam of 15 mμ and a fluorescence beam of 2 mμ. Absorption spectra were obtained with Shimadzu MPS-50L spectrophotometer.

Cation Exchange Column——Dowex 50W X8 stuffed in 0.5 cm(ϕ) and 5 cm high for urine sample, and 0.5 cm high for serum sample.

Magnesium nitrate solution, 3-hydroxy-3′,4′-dimethoxyflavone solution, and various buffer solution were prepared in the similar manner as described in the previous paper.[2]

Procedure

Determination of Magnesium in Urine——Five ml of diluted urine (1→10) is poured into a column of Dowex 50W ×8 and the resin is washed with about 60 ml of water. Then the magnesium ion is eluted with 3N HCl. The first 15 ml of the eluted solution is placed in a 20 ml volumetric flask and diluted to 20 ml with water. Five ml of the solution is evaporated by rotary evaporator and the residue is dried in a cabinet dryer for 30 min at about 70°. After cooling, the residue is dissolved with 5 ml of water by shaking for about 3 min and then 5 ml of 1.0M ammonium buffer solution of pH 10.70 is added to it, and mixed. One half ml of the mixture and 1 ml of the reagent solution are added into 10 ml volumetric flask and the mixture is diluted to 10 ml with DMF. After mixing, the fluorescence intensity is determined at 497 mμ with excitation at 445 mμ.

Determination of Magnesium in Serum——One ml of diluted serum (1→10) is poured into a column of Dowex 50W ×8 and the resin is washed with about 40 ml of water. Then the magnesium ion is eluted with 3N HCl. The first 7 ml of the eluted solution is placed in a 10 ml peach like flask and evaporated by rotary evaporator. And the residue is dried in a cabinet dryer for 30 min at about 70°. After cooling, the residue is dissolved with 1 ml of water by shaking for about 30 min and then 1 ml of 1.0M ammonium buffer solution of pH 10.70 is added to it, and mixed. Using 0.5 ml of the mixture, magnesium is assayed in the same way as described in "Determination of Magnesium in Urine."

Result and Discussion

Conditions for Fluorometric Determination of Magnesium

Stability of the reagent solution, spectral characteristics of the magnesium chelate, effects of buffer content, pH of buffer solution and buffer concentration on fluorescence intensity, and variation of fluoroscence intensity with time were studied in the similar manner as described in the previous paper.[2]

1) Location: *Mitahora, Gifu.*
2) T. Hayashi, S. Kawai, and T. Ohno, *Chem. Pharm. Bull.* (Tokyo), **18**, 2407 (1970).

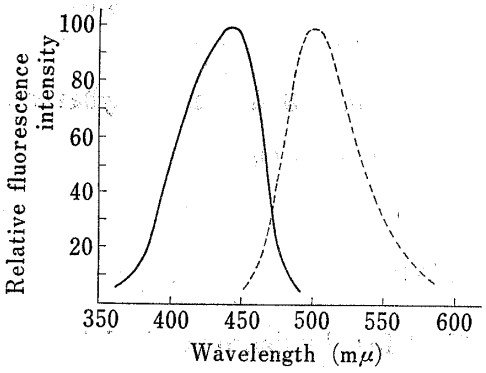

Fig. 1. Fluorescence and Excitation Spectra of Magnesium Chelate of 3-Hydroxy-3′,4′-dimethoxyflavone

-----: fluorescence spectrum
——: excitation spectrum

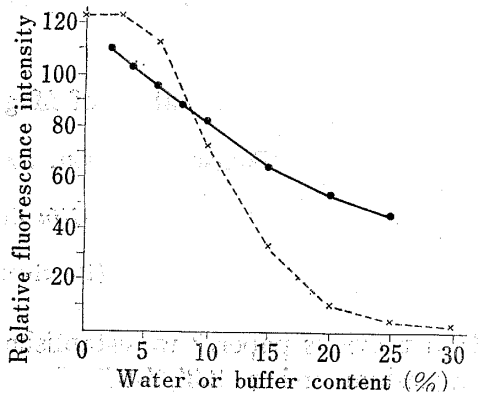

Fig. 2. Effect of Water and Buffer Content on Fluorescence Intensity

---×---: water
—●—: 0.5M ammonium buffer (pH 10.70)

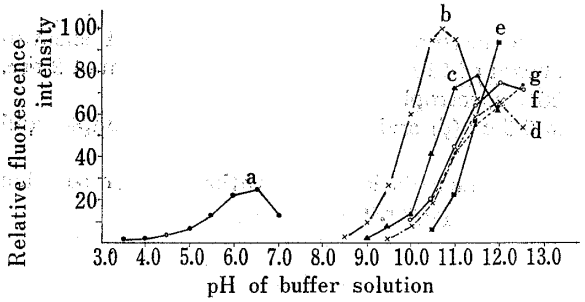

Fig. 3. Effect of pH of Buffer Solution on Fluorescence Intensity

a: 0.5M acetate buffer, b: 0.5M ammonium buffer, c: 0.5M monomethylamine buffer, d: 0.5M dimethylamine buffer, e: 0.5M trimethylamine buffer, f: 0.5M diethylamine buffer, g: 0.5M piperidine buffer
buffer content: 5.0%

The solution of 2.5×10^{-4}M 3-hydroxy-3′,4′-dimethoxyflavone in DMF was stable at least for 2 weeks under usual laboratory conditions. Its stability was superior to that of 3,3′,4′-trihydroxyflavone which should be freshly prepared every two days.

Fig. 1 shows the uncorrected excitation and fluorescence spectra of the magnesium chelate, which has a maximum at 445 mμ and 497 mμ respectively.

Fig. 2 shows effect of water or of buffer content on fluorescence intensity of the magnesium chelate. The slope of the decrease curve of the fluorescence intensity caused by the increase of buffer content is less than that in the case of water and is also remarkably less than that in

Table I. Spectrofluorometric Determination of Magnesium in the Presence of Various Ions

Ion	Amount added	Scale reading	Ion	Amount added	Scale reading
none		50.0	Zn^{2+}	5.0	50.8
Ni^{2+}	5.0 μg	50.3	KF	10.0	0
Cd^{2+}	25.0	51.0	NaH_2PO_4	10.0	0
Mn^{2+}	5.0	48.0	$(NH_4)_2\cdot C_2O_4$	10.0	0
Cu^{2+}	25.0	50.3	NaOAc	10.0	50.3
Al^{3+}	0.5	48.5	Na_2SO_4	10 0	49.8
Zr^{4+}	0.5	48.9	Na_2SO_3	10.0	50,8
Sr^{2+}	0.5	49.9	Na_2CO_3	100.0	50.2
Ca^{2+}	5.0	50.2	NaCl	1.0 mg	49.9
Fe^{3+}	5.0	48.5	KBr	1.0	49.9
Pb^{2+}	5.0	5.02	KI	1.0	49.9
Th^{4+}	0.5	48.9	$Na_2B_4O_7$	1.0	50.7
Be^{2+}	0.5	41.9			

Mg^{2+} taken: 1.0 μg

3,3′,4′-trihydroxyflavone used in the previous paper. Effect of pH of various buffer solutions are shown in Fig. 3. Each maximum fluorescence is found at different pH in various buffer solutions used. In the previous paper, we concluded that 3-hydroxy-3′,4′-dimethoxyflavone-magnesium chelate had a ligand to metal ratio of 1: 1, which suggests that one or two amine molecules, used as buffer solution, coordinate to magnesium and contribute to fluorescence emission. Therefore, there seems to be a settled correlation between the p*K*a value of the amine used and the difference in pH at which shows maximum fluorescence.

The fluorescence intensity was constant at a concentration ranging from 0.3 to 0.7 M of ammonium buffer and was constant for 1 hr after mixing.

When based on the results described above, a linear relationship was found between the fluorescence intensity and concentration of magnesium in the range from 0 to 2.0 μg.

Interference by Foreign Ion

Effect of several ions on the fluorescence intensity of the magnesium complex was tested with a solution containing 1.0 μg of magnesium, and the results are summarized in Table I. The strong interference by some kinds of anions is presumed to be due to the formation of their magnesium complexes.

TABLE II. Recovery of Magnesium from Urine

Added (μg)	Found (μg)	Recovery (%)
100	100	100
100	101	101
100	101	101
100	99	99
100	102	102
100	100	100
100	98	98

coefficient of variation: 1.24%

Determination of Magnesium in Urine and Serum

As shown in Table I, this method is seriously disturbed by phosphate which is contained in urine or serum, so a prior removal of interfering anions must be effected. In order to separate magnesium from interfering anions, an investigation on using a cation exchange column was performed. Six ml of aqueous magnesium nitrate solution (100 μg Mg^{2+}/ml) was poured into the column in which Dowex 50 W ×8 was stuffed in 0.5 cm(ϕ) and 5 cm high, and the resin was washed with water. No eluting of magnesium was observed in 60 ml of the washing.

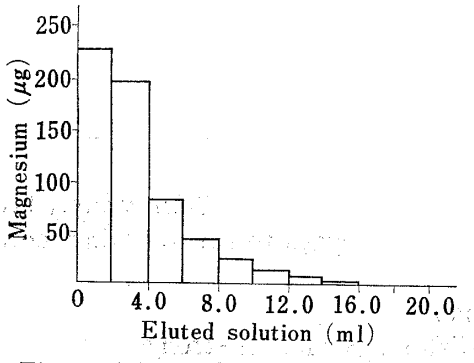

Fig. 4. Elution Curve of Magnesium from Dowex 50W ×8 (0.5×0.5 cm) with 3N HCl

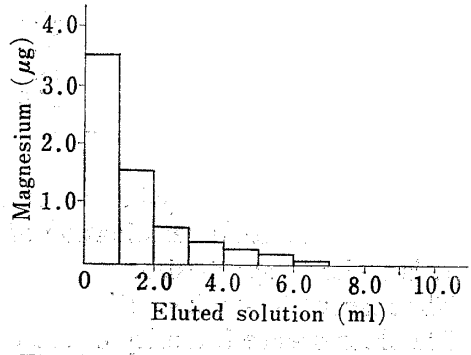

Fig. 5. Elution Curve of Magnesium from Dowex 50 W ×8 (0.5×0.5 cm) with 3N HCl

Table III. Recovery of Magnesium from Serum

Added (μg)	Found (μg)	Recovery (%)
2.50	1.90	76.0
2.50	1.90	76.0
2.50	1.82	72.8
2.50	1.90	76.0
2.50	1.86	74.4
2.50	1.98	79.2

coefficient of variation: 2.80%

Then, the magnesium adsorbed on the resin was eluted with 3 N HCl, and the magnesium in each 2 ml-portion of the eluate was determined by fluorometric procedure. As shown in Fig. 4, the whole magnesium was found in initial 15 ml of eluate. An aliquot of an aqueous magnesium nitrate solution was added to urine and recovery tests were carried out according to the method described in "Determination of Magnesium in Urine." The results shown in Table II were very satisfactory in precision.

Then, for the determination of magnesium in serum, a similar investigation using a cation exchange column was carried out. One ml of aqueous magnesium nitrate solution (6.0 μg Mg^{2+}/ml) was poured into the column in which Dowex 50W $\times$8 cation exchange resin was stuffed in 0.5 cm(ϕ) and 0.5 cm high, and the resin was washed with water. No eluting of magnesium was observed in 40 ml of the washing. Then, the magnesium adsorbed on the resin was eluted with 3 N HCl, and the elution curve for magnesium was similarly plotted shown in Fig. 5. The result shows that the whole magnesium is eluted in the initial 7 ml of the eluate. Recovery tests were carried out according to the method described in "Determination of Magnesium in Serum" and the reproducibility determined by carrying out five identical analyses is shown in Table III. The reason is not evident why the recovery values are low. Table IV shows the amount of magnesium in serum determined by the present procedure and atomic absorption spectrophotometry,[3] where the fluorometric values are corrected by the recovery factor (76%) in Table III. The results show a good agreement between the both methods.

Table IV. Comparison of Magnesium Analysis carried out by Fluorometry (a) and Atomic Absorption Spectrophotometry

Sample	(a)	(b)
A	27.5 μg/ml	27.7 μg/ml
	27.0	27.5
B	21.8	22.4
	22.2	22.0
C	28.1	27.8
	27.5	28.0

3-Hydroxy-3′,4′-dimethoxyflavone is more excellent than 3,3′,4′-trihydroxyflavone as a reagent for fluorometric determination of magnesium in regard to the following merits:

1) It is easier to synthesize.
3) The reagent in stock solution is more stable.
3) The fluorescence intensity of the chelate with magnesium is stronger.

3) J.B. Dawson and F.W. Heaton, *Biochem. J.* (London), **80**, 99 (1961).

4) Influence of buffer content on the fluorescence intensity is less.
5) Existence of excess magnesium does not cause a decrease of fluorescence intensity as shown in the previous paper.[2]

This method is as high sensitive as atomic absorption spectrophotometry and is remarkably more excellent than the Oxin Method used previously in regard to sensitivity and precision.

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Platelet Aggregation Inhibitors. V.[1] Pyrimidine Derivatives, Indole Derivatives, Benzothiophenes, and Benzoquinolizine Derivative

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Predominance of platelets in the white clot of blood in arteries has focused attention of their importance in arterial occlusion and has suggested that the inhibitor of platelet aggregation may be more useful than standard anticoagulant therapy.[3] Several organic compounds that powerfully inhibit adenosine 5'-diphosphate- and/or collagen-induced platelet aggregation have been investigated,[4] and heterocyclic compounds including pyrimidopyrimidines,[5] thieno compounds,[6,7] thiazolo compounds,[8,9] benzonaphthyridine,[10] pyrimidines,[11] fluoren compound,[12] aniline derivative,[13] quinazoline,[14] and others[15] are known as strong inhibitors

1) Part IV: K. Kikugawa, K. Iizuka, and M. Ichino, *J. Med. Chem.*, **16**, 358 (1973).
2) Location: *Komiya-chō 51, Hachiōji-cyty, Tokyo.*
3) *a*) J.F. Mustard, M.F. Glynn, F. Hovig, L. Jorgensen, M.A. Packham, F. Nishizawa, and H.C. Rowsell, "Physiology of Hemostasis and Thrombosis," S.A. Johnson, W.H. Seegers, ed., Thomas Springfield, I11, 1966, p. 288; *b*) G.W. Schnetzer, *Amer. Heart J.*, **83**, 552 (1972).
4) J.F. Mustard and M.A. Packham, *Pharmacol. Rev.*, **22**, 97 (1970).
5) *a*) P.R. Emmons, M.J.G. Harrison, A.J. Honour, and J.G.R. Mitchell, *Nature* (London), **208**, 255 (1965); *b*) A.A. Hassanein, A.G.G. Turpie, G.P. McNicol, and A.S. Dorglas, *Brit. Med. J.*, **5**, 83 (1970).
6) M. Nakanishi, H. Imamura, and K. Goto, *Yakugaku Zasshi*, **90**, 564 (1970).
7) *a*) German Patent, 1940572 (Dr. Karl Thomae, G.m.b.H.); *b*) German Patent, 2032686 (Dr. Karl Thomae, G.m.b.H.); *c*) U.S. Patent, 3475429 (Boehringer Ingelheim); *d*) J.J. Sixma and A.M.C. Treeschnigg, *Scand. J. Haemat.*, **8**, 417 (1971).
8) E.F. Elslager, N.F. Haley, J.R. McLean, S.C. Perricone, D. Potoczak, H. Veloso, D. Worth, and R.H. Wheelock, *J. Med. Chem.*, **14**, 397 (1971).
9) *a*) Belgian Patent, 755404 (Sandoz, S.A.); *b*) Japanese Patent, Kokai, 784 (1972).
10) H. Ott and G.M. Smith, *Brit. J. Pharmacol.*, **43**, 460 (1971).
11) German Patent, 2149249 (Pfizer Inc.).
12) *a*) R.D. Mackenzie, T.R. Blohm, E.M. Auxier, J.G. Henderson, and J.M. Steinbach, *Proc. Soc. Exp. Biol. Med.*, **137**, 662 (1971); *b*) G.P. Claxton, J.M. Grisar, E.M. Roberts, and R.W. Fleming, *J. Med. Chem.*, **15**, 500 (1972).
13) E. Deutsh, K. Lechner, K. Moser, and L. Stockinger, *Thromb. Diath. Hemorrh.*, **26**, 145 (1971).
14) German Patent, 2152521 (Bristol Myers Co.).
15) *a*) E.F. Elslager, N.F. Haley, J.R. McLean, S.C. Perricone, D. Potoczak, H. Veloso, D.F. Worth, and R.H. Wheelock, *J. Med. Chem.*, **14**, 782 (1971); *b*) E.F. Elslager, N.F. Haley, J.R. McLean, D. Potoczak, H. Veloso, and R.H. Wheelock, *J. Med. Chem.*, **15**, 61 (1972).