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Fluorometric Determination of Magnesium with 3,3′4′-Trihydroxyflavone

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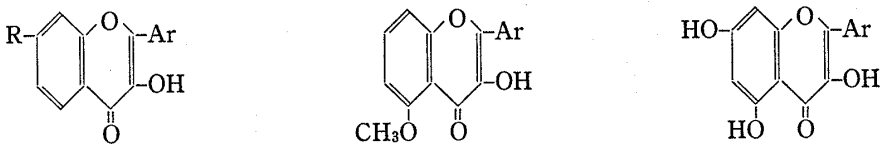
3,3′,4′-Trihydroxyflavone combines with magnesium ion in N,N-dimethylformamide to form a strongly fluorescent chelate which is useful for the determination of micro amounts of magnesium. A linear relationship between the fluorescence intensity and concentration of magnesium was found in the range from 0 to 5.0 μg of magnesium. The chelate which shows excitation maximum at 450 mμ and a fluorescence maximum at 503 mμ has a metal to ligand ratio of 1:1.

Fluorometric methods for the quantitative determination of magnesium with various reagents have been proposed,[2–20] but there seems to be no report on the fluorometric determination of magnesium with flavone derivatives. In a previous paper,[21] we reported that the types I, II, and III compounds in Chart 1 form fluorescent chelates with beryllium, magnesium, aluminum, thorium, and yttrium in 90% ethanol, and noted in preliminary experiments that some of the type I derivatives particularly produce very strong fluorescence emission with magnesium. This paper concerns a study on the fluorometric determination of magnesium with 3,3′,4′-trihydroxyflavone which belongs to type I compounds.



I (R=H, OH, OCH_3) II III

Chart 1. Types of Flavone Derivatives

1) Location: *Mitahora, Gifu.*
2) C.E. White and F. Cuttita, *Anal. Chem.*, **31**, 2083 (1959).
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21) T. Hayashi, K. Hara, S. Kawai, and T. Ohno, *Chem. Pharm. Bull.* (Tokyo), **18**, 1112 (1970).

Apparatus and Reagents

The fluorescence spectra and fluorescence intensity were measured with a Simadzu GSF-16 spectrofluorometer. The slits were arranged to have an excitation beam of 10 mμ and a fluorescence beam of 10 mμ. Absorption spectra were obtained with Simadzu MPS-50L spectrophotometer. Measurements of pH were made with a Hitachi-Horiba M-5 pH-meter.

Magnesium Nitrate Stock Solution——Magnesium nitrate stock solution was prepared by dissolving 128.2 mg of $Mg(NO_3)_2 \cdot 6H_2O$ (reagent grade) in 100 ml of N,N-dimethylformamide (DMF; reagent grade). Working solution was prepared by diluting it with DMF for use.

3,3′,4′-Trihydroxyflavone Solution——3,3′,4′-Trihydroxyflavone was synthesized as described in a previous paper.[21] 3,3′,4′-Trihydroxyflavone solution of 0.5×10^{-3}M was prepared by dissolving 13.5 mg of the solid material in 100 ml of DMF.

Buffer Solution——0.1 M acetate buffer and 0.1 M ammonium buffer of various pH and pH 10.70 ammonium buffer of various concentrations were prepared in the usual way. Sodium acetate, acetic acid, ammonium chloride, and ammonia water used for preparing buffer solutions were of reagent grade. Redistilled water was used in all the experiments.

Result and Discussion

Stability of 3,3′,4′-Trihydroxyflavone Solution

The solution of 0.5×10^{-3}M 3,3′,4′-trihydroxyflavone in DMF was allowed to stand in a brown bottle and after various periods of time, absorbance of the solution diluted (1→10) with DMF was measured at 365 mμ to determine whether the reagent fades with time. The results showed that the reagent solution was stable at least for 3 days under usual laboratory conditions. Therefore, 0.5×10^{-3}M solution of 3,3′,4′-trihydroxyflavone in DMF was freshly prepared every two days.

Solvents

From the preliminary experiments DMF was found to be the most suitable among several solvents tested, such as methanol, ethanol, propanol, carbon tetrachloride, and tetrahydrofuran. To each of twelve 10 ml volumetric flasks were added 1 ml of 0.5×10^{-3}M solution of 3,3′,4′-trihydroxyflavone in DMF, 1 ml of 0.25×10^{-3}M solution of magnesium nitrate in DMF, and various amounts of 0.5 M ammonium buffer solution of pH 10.70, and the mixture was diluted to 10 ml with DMF. After 30 min, the fluorescence intensity was determined at 503 mμ with excitation at 450 mμ. As shown in Fig. 1, the fluorescence intensity decreased with increasing buffer solution content. Fluorescence intensity in the presence of 30% of buffer solution was approximately one-sixth weaker than that at 10%. Therefore, buffer solution content was fixed exactly at 10%.

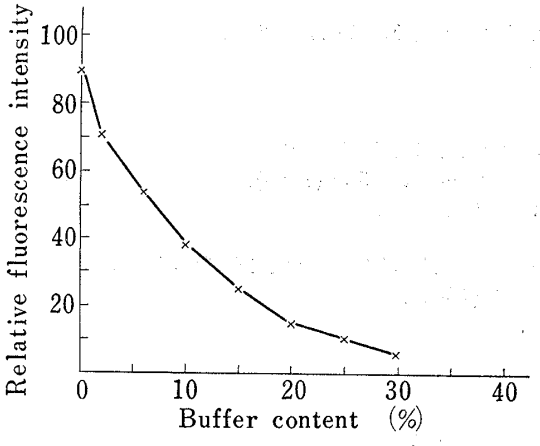

Fig. 1. Effect of Buffer (0.5M, pH 10.70) Content on Fluorescence Intensity

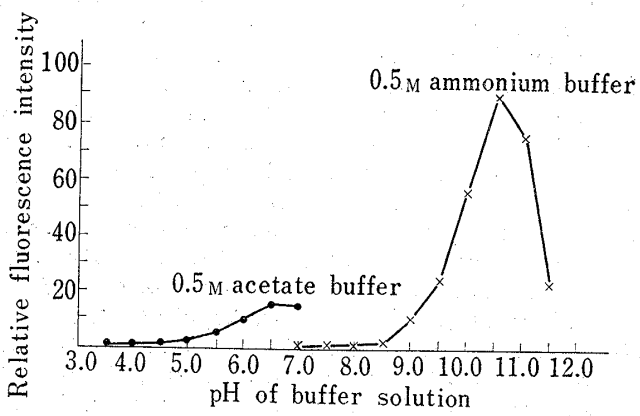

Fig. 2. Effect of pH of Buffer Solution on Fluorescence Intensity

Influence of pH

One ml of 0.5×10^{-3}M solution of 3,3′,4′-trihydroxyflavone, 0.25×10^{-3}M solution of magnesium nitrate, and 0.5M buffer solution of various pH were added to each of seventeen 10 ml volumetric flasks and the mixture was diluted to 10 ml with DMF. After 30 min, the fluorescence intensity was measured at optimum excitation and emission settings. Correlation of fluorescence intensity to pH of buffer solution is shown in Fig. 2. In the presence of 0.5M sodium acetate buffer, the maximum fluorescence intensity was found at pH 6.50, while in the presence of 0.5M ammonium chloride buffer, it was found in a range from pH 10.50 to 11.00 and the value at pH 10.50 is about eight times that at pH 6.50. From these results, 10.70 was selected as a suitable pH of the buffer solution.

Buffer Concentration

Effect of buffer concentration on fluorescence intensity was tested with a series of 10 solutions of 10 ml volume containing 1 ml of 0.5×10^{-3}M solution of 3,3′,4′-trihydroxyflavone, 0.25×10^{-3}M solution of magnesium nitrate, and various concentrations of ammonium buffers of pH 10.70. As shown in Fig. 3, the fluorescence intensity was constant at a concentration ranging from 0.3 to 0.7M. In the recommended procedure, 0.5M buffer of pH 10.70 was used.

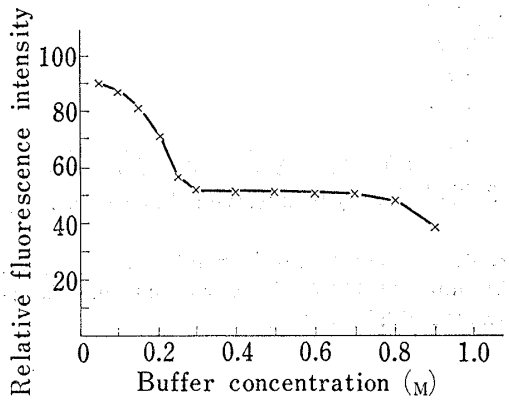

Fig. 3. Effect of Buffer Concentration (pH 10.70) on Fluorescence Intensity

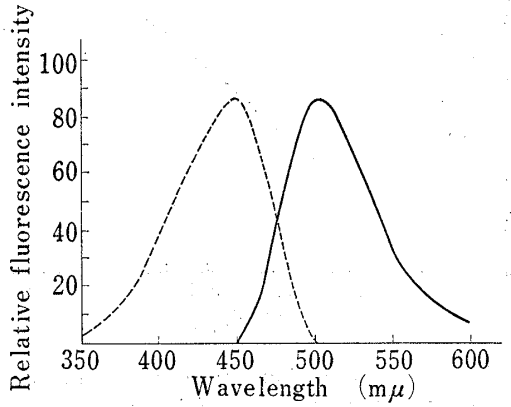

Fig. 4. Fluorescence and Excitation Spectra of Magnesium Chelate of 3,3′,4′-Trihydroxyflavone

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

Standing Time

A series of 12 identical solutions were prepared by adding 1 ml of 0.5×10^{-3}M solution of 3,3′,4′-trihydroxyflavone, 0.25×10^{-3}M solution of magnesium nitrate, and 0.5M ammonium buffer of pH 10.70 to each of 10 ml volumetric flasks and diluting to 10 ml with DMF. Then variation of fluorescence intensity with time was measured. The fluorescence intensity was nearly constant between 15 and 40 min after mixing.

Spectral Characteristics

Fig. 4 shows the uncorrected excitation and fluorescence spectra of a solution which contains 1 ml of 0.5×10^{-3}M solution of 3,3′,4′-trihydroxyflavone, 0.25×10^{-3}M solution of magnesium nitrate, 0.5M buffer solution of pH 10.70, and DMF to make 10 ml. Excitation spectrum of the chelate has a maximum at 450 mμ, while the maximum fluorescence emission occurs at 503 mμ.

Calibration Curve

A series of 11 solutions of 10 ml volume were prepared, containing 1 ml of 0.5×10^{-3}M solution of 3,3′,4′-trihydroxyflavone, 1 ml of 0.5M buffer solution of pH 10.70, various amounts

of magnesium nitrate solution, and DMF to make 10 ml. In 20 min after mixing, the fluorometer was set so that reading of the blank was 0 and reading of 5.0 μg standard was 100. The remaining solutions were then measured. As shown in Fig. 5, there was a linear relationship between the fluorescence intensity and concentration of magnesium in the range of 0 to 5.0 μg of magnesium.

Procedure

From the results described above, the following optimal conditions were adopted as the standard procedure for the fluorometric determination of magnesium in model solutions. A mixture of 1 ml each of an aqueous sample solution and 1.0M buffer solution is shaken vigorously in a glass-stoppered test tube. One ml of the mixture and 1 ml of 0.5×10^{-3}M solution of 3,3′,4′-trihydroxyflavone are placed in a 10 ml volumetric flask and the mixture is diluted to 10 ml with DMF. After 20 min, the fluorescence intensity is determined at 503 mμ with excitation at 450 mμ.

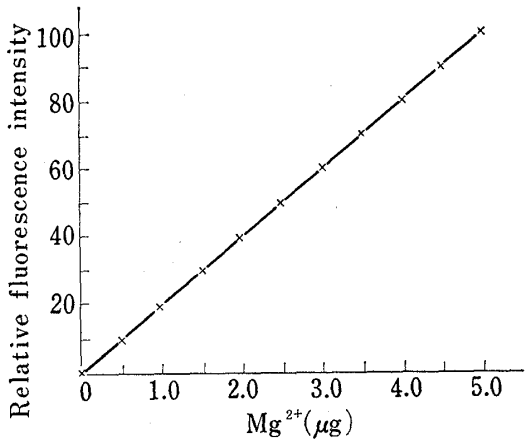

Fig. 5. Calibration Curve for Fluorometric Determination of Magnesium

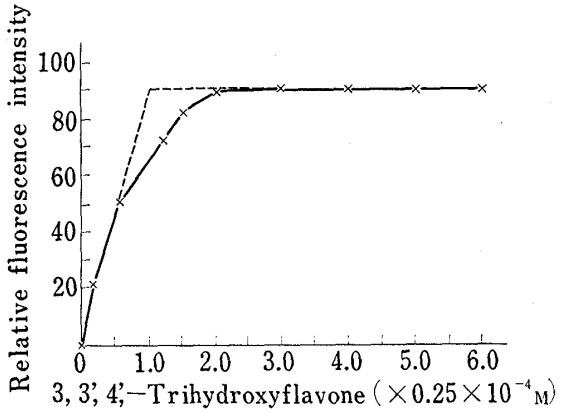

Fig. 6. Molar Ratio Method with Varying Amounts of 3,3′,4′-Trihydroxyflavone (Magnesium: 0.25×10^{-4}M)

Interferences by Foreign Ions

Effect of several ions on the fluorescence intensity of the magnesium complex was tested with a solution containing 3.0 μg of magnesium, and the results are summarized in Table I. The strong interference by phosphate or fluoride ion is presumed to be due to the formation of ammonium magnesium phosphate or magnesium fluoride complex. Such a quenching effect on the fluorescence of the magnesium-3,3′,4′-trihydroxyflavone complex may be applied to micro-fluorometric determination of phosphate and fluoride ions.

Structure of the Chelate

A spectrofluorometric study of complex formation by the molar ratio method, varying the amount of 3,3′,4′-trihydroxyflavone, indicated a magnesium:ligand ratio of 1:1 (Fig. 6).

The proposed structure is also supported by Job's method as shown in Fig. 7.

However, when excess magnesium was added to the reagent, a remarkable quenching was observed (Fig. 8).

To investigate this phenomenon, similar experiments were carried out on 3′,4′-dimethoxy-3-hydroxyflavone which has methoxyl groups instead of hydroxyl groups in 3′- and 4′-position. 3′,4′-Dimethoxy-3-hydroxyflavone showed some similar properties in the following points: it produced a very strong fluorescence emission with magnesium in DMF, the fluorescence intensity decreased with increasing buffer solution content, the results of molar ratio method (Fig. 9) and Job's method (Fig. 10) indicated that a chelate having a metal to ligand ratio of

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

Ion	Amount added	Scale reading
none		60.0
Ca^{2+}	5.0 μg	58.8
Zn^{2+}	0.5 μg	58.8
Be^{2+}	0.01 μg	60.6
Sr^{2+}	0.2 μg	60.6
Mn^{2+}	0.04 μg	58.8
Y^{3+}	0.01 μg	56.4
Cu^{2+}	0.01 μg	56.0
Ni^{2+}	0.5 μg	59.4
Zr^{4+}	0.2 μg	58.8
Al^{3+}	0.1 μg	58.2
Cd^{2+}	0.8 μg	58.5
NaCl	1.0 mg	59.4
KCl	1.0 mg	58.8
KBr	1.0 mg	59.4
$Na_2B_4O_7 \cdot 10H_2O$	1.0 mg	60.0
Na_2CO_3	0.05 mg	58.2
AcONa	0.1 mg	57.6
Na_2SO_4	0.1 mg	55.2
KH_2PO_4	0.01 mg	1.0
KF	0.01 mg	3.8

Mg^{2+} taken: 3 μg

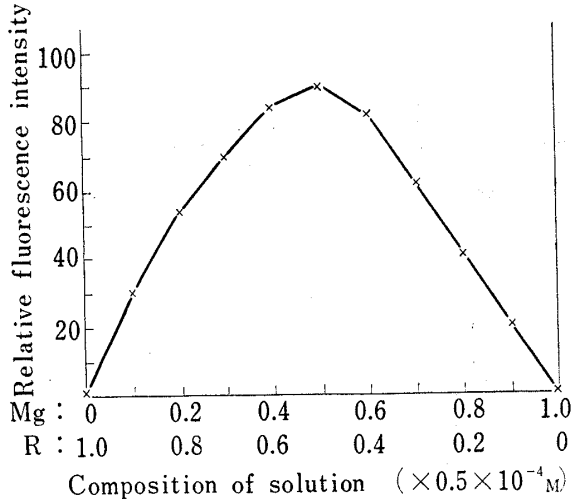

Fig. 7. Determination of Composition of Magnesium Chelate of 3,3′,4′-Trihydroxyflavone by Job's Method

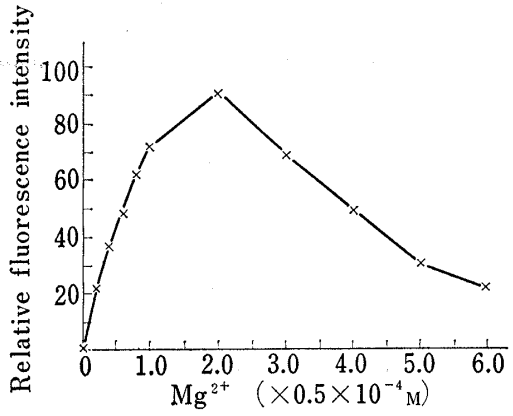

Fig. 8. Molar Ratio Method with Varying Amounts of Magnesium (3,3′,4′-Trihydroxyflavone: 0.5×10^{-4}M)

1:1 was formed, but, in this case, no quenching was observed by an excess of magnesium (Fig. 9).

These evidences suggest that 3,3′,4′-trihydroxyflavone forms a strongly fluorescent chelate with magnesium between 3-hydroxyl and 4-carbonyl group, and it converts to nonfluorescent chelate with excess magnesium as shown in Chart 2.

Further examination of their absorption spectra also seems to support these speculation. Two series of solutions were prepared. In the first series, each contained 1 ml of 0.5×10^{-3}M solution of 3′,4′-dimethoxy-3-hydroxyflavone, 1 ml of 0.5M buffer solution of pH 10.70, various

amounts of 0.5×10^{-3}M solution of magnesium nitrate, and DMF to make 10 ml. Fig. 11 shows the absorption spectra of 3′,4′-dimethoxy-3-hydroxyflavone and of its magnesium chelate, where curve A corresponds to the absorption spectrum of the reagent with a maximum at 363 mμ, and curves B to F are for various levels of magnesium. A new absorption maxi-

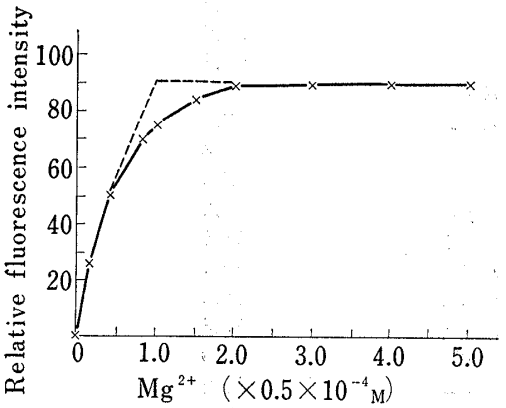

Fig. 9. Molar Ratio Method with Varying Amounts of Magnesium (3′,4′-Dimethoxy-3-hydroxyflavone: 0.5×10^{-4}M)

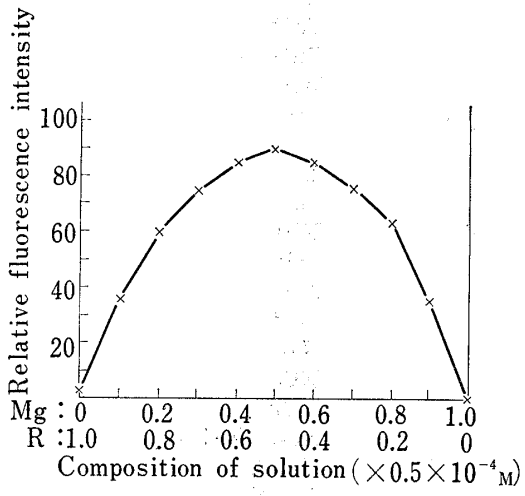

Fig. 10. Determimation of Composition of Magnesium Chelate of 3′,4′-Dimetoxy-3-hydroxyflavone by Job's Method

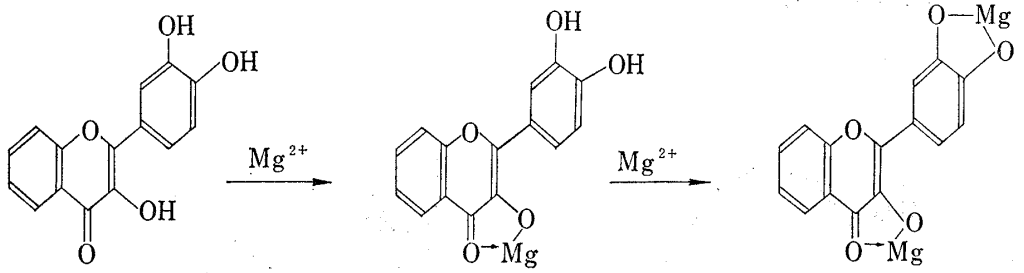

Chart 2

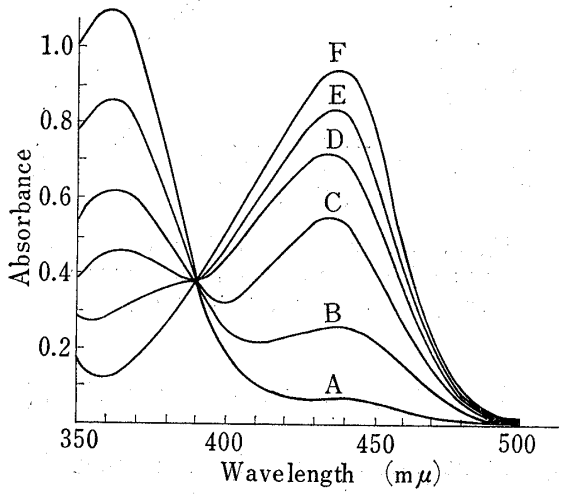

Fig. 11. Absorption Spectra of 3′,4′-Dimethoxy-3-hydroxyflavone and Its Magnesium Chelate

magnesium concentration: B 1.0×10^{-5}M, C 3.0×10^{-5}M, D 5.0×10^{-5}M, E 2.0×10^{-4}M, F 5.0×10^{-4}M

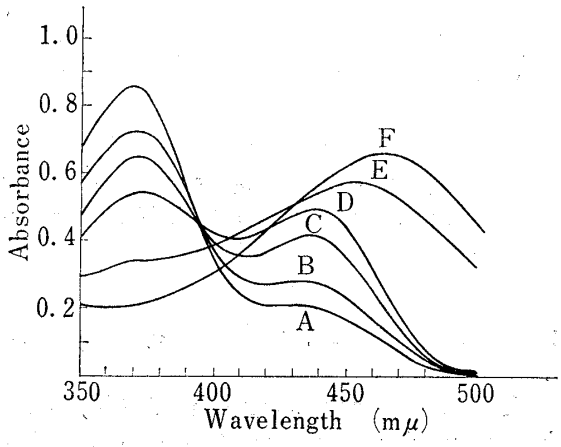

Fig. 12. Absorption Spectra of 3,3′,4′-Trihydroxyflavone and Its Magnesium Chelate

magnesium concentration: B 1.0×10^{-5}M, C 3.0×10^{-5}M, D 5.0×10^{-5}M, E 2.0×10^{-4}M, F 5.0×10^{-4}M

mum and an isobestic point are observed at 439 and 391 mμ, respectively. The latter indicates that there is only one equilibrium between magnesium chelate and the reagent. In the second series, similar examination was carried out by using 3,3′,4′-trihydroxyflavone. As shown in Fig. 12, where curve A having a maximum at 370 mμ is the absorption spectrum of the reagent and curves B to F are for various levels of magnesium, in which a new absorption band (λ_{max} 439 mμ) and the isobestic point (395 mμ) are observed below 0.5×10^{-4}M of magnesium concentration in the solution. However, when magnesium concentration is above 0.5×10^{-4}M, the new absorption band shows a bathochromic shift and the isobestic point is not observed. From the difference observed between Fig. 11 and 12, it is considered that 3,3′,4′-trihydroxyflavone forms a chelate of a different structure with excess of magnesium. 3,3′,4′-Trihydroxyflavone is therefore recommended as one of stable fluorometric reagents for the determination of magnesium. This method is rapid, sensitive, and useful for the spectrofluorometric determination of microamounts of magnesium in biological samples containing calcium, such as urine and blood plasma.