A Simple Flow Injection Assay of Ca(II) in Mineral Supplement Using Mg(II)-EDTA

Kwuanjit Manee-on[1], Somchai Lapanantnoppakhun[1,2], Jaroon Jakmunee[1,2] and Kate Grudpan[1,2]*

[1] Department of Chemistry, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand.
[2] Institute for Science and Technology Research and Development, Chiang Mai University, Chiang Mai 50200, Thailand.

Abstract

A new concept for a simple FI assay of Ca(II) employing a replacement reaction of Mg(II)-EDTA by Ca(II) in the present of Eriochrome Black T (EBT) is proposed. The released magnesium reacts with EBT. FI peak due to absorbance change of Mg(II)-EBT is proportional to Ca(II) concentration. Even with such a very simple FI set up, a through-put of 200 injections/h can be achieved. The procedure was applied to assay Ca(II) in mineral supplement samples. The results obtained agreed well with that of the British Pharmacopoeia titration method.

Key words Calcium, Magnesium, EDTA, Eriochrome Black T, mineral supplement

1. Introduction

Calcium is an important mineral for human body especially women. It reduces the risk of osteoporosis, the increased porosity or softening of bone, which usually occur in mature and postmenopausal women. However, too much of calcium consumption can cause problems. The effects of calcium overdose include renal damage and deposition of calcium in other areas of the body besides the bones. It can contribute to stone formation for people who are at risk of developing kidney stones.

Various analytical procedures have recently been developed for the determination of Ca(II) such as spectrophotometry [1], atomic spectroscopic techniques[2], electrochemical techniques [3] and complexometric titration [4-6]. The last ones are also employed as a standard method [4-6]. Flow based procedures [3, 7, 8] have also been reported for Ca(II) determination.

In this work, a new concept for a simple FI set-up with procedure employing a replacement reaction of Mg(II)-EDTA by Ca(II) in a present of Eriochrome Black T (EBT) is proposed. The released Mg (II) reacts with EBT in ammonia – ammonium chloride buffer of pH 9.5. Absorbance change due to Mg-EBT (at 530 nm) is followed. Peak height is proportional to the concentration of Ca(II). The procedure is demonstrated to be an alternative high sample through-put assay for Ca(II) in mineral supplement.

2. Experimental

2.1 Flow Manifold

FI manifold is schematically depicted in Fig. 1. It consists of simple components including a filter colorimeter.

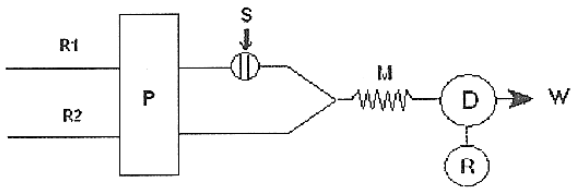

Fig. 1 Flow injection system for the determination of Ca(II) : R1= Mg(II)-EDTA reagent, R2 = EBT , P = peristaltic pump (Ismatec), S = injection valve (Rheodyne 6- Port valve), M = mixing coil, D = colorimeter (Cole Parmer 5564-15 with a green filter), R = recorder (Philips PM 8251) and W = waste.

2.2 Chemicals and Reagents

Stock standard Ca(II) solution (0.10 M) was prepared by dissolving 1.0010 g $CaCO_3$ (Fluka) in 1 ml of 1 M HCl. The solution was boiled to remove carbon dioxide before making the volume up to 100 ml with deionized water. Standard Ca(II) solutions of appropriate concentrations were obtained by further dilutions.

A solution containing Mg(II)-EDTA was prepared by dissolving 20.3300 g Na_2EDTA ($Na_2C_{10}H_{14}N_2O_8.H_2O$, Merck) and 37.2240 g $MgCl_2.6H_20$ (Fluka) in ammonia-ammonium chloride buffer (pH 9.5, 0.1 M) in a total volume of 1000 ml. The solution should have a Mg(II) : EDTA mole ratio of 1:1.5. A portion (0.01 g) of Eriochrome Black T (Aldrich) was dissolved in 100 ml of the ammonia-ammonium chloride buffer to become 0.01 % (w/v) solution.

2.3 Sample preparation

To prepare sample solutions from calcium carbonate tablets, the standard procedure was followed [6]. Not less than 20 tablets were weighed and finely powdered. A portion of the powder was weighed to obtain calcium equivalent to 100 mg and dissolved with 10 ml of 1 M HCl. The solution was boiled until clear solution was observed. After that it was diluted to 250 ml with deionized water. The treated sample was analyzed by the

*Corresponding author
E-mail: kate@chiangmai.ac.th

proposed method and the British Pharmacopoeia titration method [6].

For the capsule sample, 20 capsules were weighed. The accurate sample powder weight was obtained by the difference of the full and empty capsule.

3. Results and discussion

A set of conditions as summarized in Table 1 was selected. Fig. 2 illustrates the FI-gram.

Table 1 The conditions of the FI procedure for the determination of Ca(II).

R1	$[Mg^{2+}]$ in 0.1 M NH_3/NH_4Cl buffer	0.008 M
	[EDTA] in 0.1 M NH_3/NH_4Cl buffer	0.012 M
	Flow rate	5.1 ml/min
R2	Eriochrome Black T (EBT) in 0.1 M NH_3/NH_4Cl buffer	0.006 % w/v
	Flow rate	2.7 ml/min
S	Injection volume	50 µl
M	Mixing coil	75 cm
D	Detection wavelength	green filter (530 nm)

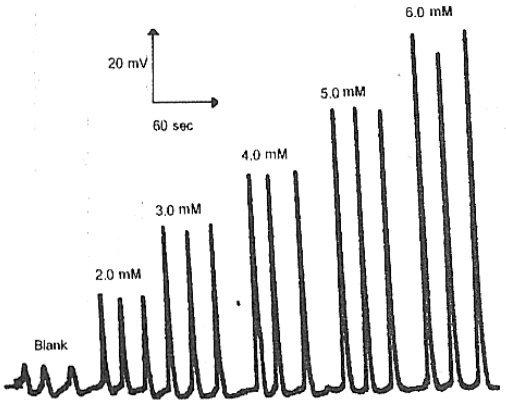

Fig. 2 FI-gram of Ca(II) 2.0-6.0 mM.

A linear calibration graph was obtained for 2.0-6.0 mM Ca(II) : $y = 22.094x - 14$, $r^2 = 0.9972$ with a detection limit (3σ) [9] of 0.6 mM, and 2% RSD (5 mM, n=11). A through-put of 200 injections per hour was achieved.

Effect of pH of solution injected was studied. According to the preliminary study, the standard solutions were chosen to be prepared in 0.01 M HCl (pH 2). Solutions adjusted to various pH values (2, 3 and 5) containing 5 mM Ca(II), were prepared. The solutions were injected into the FI system and evaluated for their analysis results by using a calibration graph prepared by employing standard solutions of pH 2. The analysis results were found to be the same as those of the standard Ca(II) prepared in pH 2 solution. This indicates that pH of the injected solution could be in the range of 2-5.

Although the procedure is not specific for Ca(II) but with its instrument simplicity and a high sample through-put, it should be useful to some certain types of samples which contain no interferents.

The results of the assay for Ca(II) in mineral supplement samples by the proposed procedure are summarized in Table 2 and agree well with the values obtained by the British Pharmacopoeia titration method [6]. This also indicates that no interference found in such assay of the samples.

Table 2 The determination results of Ca(II) mineral supplement samples by the proposed FI procedure and the British Pharmacopoeia titration method (average of triplicate results).

Sample[a]	Label	This work		BP Titration	
	mg/unit	mg/unit	%Label	mg/unit	%Label
No.1	600	573	96	610	102
No.2	250	243	97	246	99

[a] carbonate form, No.1 : tablet, No.2 : capsule

4. Conclusion

A simple FI determination of Ca(II) is proposed. It is based on the replacement of Mg(II)-EDTA by Ca(II). The released Mg(II) forms colored Mg(II)-Eriochrome Black T complex. The FI peak due to absorbance (at 530 nm) of the complex is proportional to Ca(II) concentration. The proposed procedure with very simple detection and high sample through-put is an alternative cost-effective assay of Ca(II) in mineral supplement.

5. Acknowledgements

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