The Selective Chelatometry of Calcium in the Presence of Magnesium with Ethylene Glycol-bis(β-aminoethyl ether)-N, N, N′, N′-tetraacetic Acid

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A difference in the stability constants of ethylene glycol-bis(β-aminoethyl ether)-*N*, *N*,-*N*′, *N*′-tetraacetic acid (EGTA) chelates of calcium and magnesium ($K_{Ca\text{-}EGTA}/K_{Mg\text{-}EGTA}=10^{5.8}$)[1-3] allows the selective chelatometry of calcium in the presence of magnesium if an appropriate method is available to indicate the equivalence point. Several methods have been reported for this purpose: potentiometric titration using a mercury electrode[2], photometric titration using murexide as an indicator[4], visual titration using Zn-EGTA-zincon system as an indicator[3,5], and visual titration using calcon as an indicator and tartrate to prevent the precipitation of magnesium[6].

In our own previous investigation[7], some theoretical considerations were made of the color change in the vicinity of the equivalence point of chelatometry, using the NY-HA system as an indicator, and more recently a method for the selective chelatometry of calcium has been worked out using the Zn-EGTA-PAN system as an indicator[8]. The choice of an indicator system, the conditions for the titration of calcium with EGTA, and the experimental results from the titration of calcium using the Zn-EGTA-PAN system as an indicator constitute the present paper.

The desirable properties of the metal indicator for the chelatometry of calcium in the

1) G. Schwarzenbach, "Die Komplexometrische Titration", Dritte Auflage, Ferdinand Enke Verlag, Stuttgart (1957), p. 97.
2) R. W. Schmid and C. N. Reilley, *Anal. Chem.*, **29**, 264 (1957).
3) A. Ringbom, G. Pensar and E. Wänninen, *Anal. Chim. Acta*, **19**, 525 (1958).
4) H. Flaschka and J. Ganchoff, *Talanta*, **8**, 720 (1961).
5) F. S. Sadek, R. W. Schmid and C. N. Reilley, ibid., **2**, 38 (1959).
6) R. A. Burg and H. F. Conaghan, *Chemist-Analyst*, **49**, 100 (1960).
7) G. Nakagawa and M. Tanaka, *Talanta*, **9**, 917 (1962).
8) G. Nakagawa, H. Wada and M. Tanaka, ibid., in press.

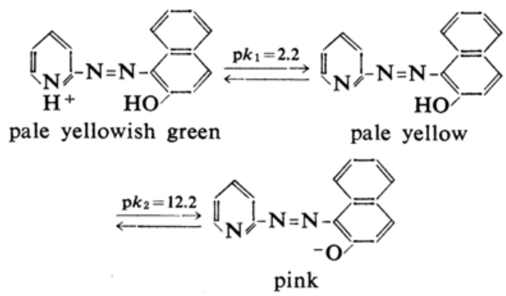
presence of magnesium may be summarized as follows: the indicator should react with calcium to give a sensitive coloration but should not react with magnesium under the condition where the precipitation of magnesium does not occur, e. g., at pH$<$10 unless a masking agent such as tartrate is used. No metal indicator having such properties has been reported. Thus an indicator system such as the NY-HA system ought to be used for a successful visual titration of calcium in the presence of magnesium. Even in this case, HA must not react with magnesium; hence, the use of the *o, o′*-dihydroxyazo compounds often utilized in chelatometry has to be given up for the present purpose. On the other hand, *o*-hydroxyazo compounds having a pyridine ring[9,10], such as 1-(2-pyridylazo)-2-naphthol (PAN) and 4-(2-pyridylazo)-resorcinol (PAR), or a thiazol ring[11,12], such as 1-(2-thiazolylazo)-2-naphthol, react with neither calcium nor magnesium, but they are known to exhibit a sensitive coloration with copper, zinc, cadmium, nickel, etc. and they are of current use in the chelatometry of these metals[9,10]. Therefore, an appropriate combination of such dyes and these metals would serve as a useful indicator in the chelatometry of calcium in the presence of magnesium. Some theoretical consideration will be given below to the use of the Zn-EGTA-PAN system as an indicator.

The indicator transition in the vicinity of the equivalence point in the chelatometry of M using the NY-HA system as an indicator can be expressed by Eq. 1 according to our previous paper[8]:

$$[Y]_t''/[M]_t = 1 - \frac{[NA_n]}{[A]^n}\,\frac{1}{K_1[NY]} + \frac{[A]^n}{[NA_n]}\,\frac{K_2[NY]}{[M]_t} \qquad (1)$$

where $K_1 = K_{MY}K_{NA_n}/K_{NY}\beta_{(M)}$, where $K_2 = K_{NA_n}\alpha_{H(Y)}/K_{NY}$[13], and where $[M]_t$ and $[Y]_t''$ denote the total concentrations of M and of the complexan used as a titrant respectively.

The acid-base properties of PAN may be described as follows[14,15];



Thus, an aqueous solution of PAN is pale yellow in the pH value range of 3$\sim$10.

Since PAN forms a 1:2 chelate with zinc at pH values higher than 6 when its quantity exceeds that of the metal[15], it is certainly $Zn(PAN)_2$ that participates in the indicator transition in the vicinity of the equivalence point of the chelatometry of zinc at pH value higher than 6, using PAN as an indicator. In the absence of the third chelating agent (an auxiliary chelating agent), $\beta_{(Ca)}=1$, $K_{ZnA_2}/(\alpha_{H(A)})^2 = K_{ZnA_2}'$ and $K_{ZnY}/\alpha_{H(Y)} = K_{ZnY'}$. Designating the undissociated PAN as HA, Eq. 1 is transfomed into Eq. 2:

$$[Y]_t''/[Ca]_t = 1 - \frac{[ZnA_2]}{[HA]^2}\cdot\frac{K_{ZnY}}{K_{CaY}K_{ZnA_2}'[ZnY]} + \frac{[HA]^2}{[ZnA_2]}\cdot\frac{K_{ZnA_2}'[ZnY]}{K_{ZnY'}[Ca]_t} \qquad (2)$$

where $[ZnA_2]/[HA]$ is a function of the percentage of color change. The second and third terms on the right side of Eq. 2 mainly concern the color change before and after the equivalence point respectively. From Eq. 2 it may easily be seen that the color change is affected by the pH value, by [ZnY] and, when a 1:2 complex such as ZnA_2 participates, by [HA]. Let

$$K_{ZnY}/K_{CaY}K_{ZnA_2}'[ZnY][HA] = f_1$$

and

$$K_{ZnA_2}'[ZnY][HA]/K_{ZnY'}[Ca]_t = f_2$$

It then follows that the color change in the vicinity of the equivalence point becomes sharper the lower the f_1 and f_2 values. However, it should be remembered that higher values of $K_{CaY'}$ and $[Ca]_t$ are always desirable since $f_1f_2 = 1/K_{CaY'}\cdot[Ca]_t$. When dealing with a $10^{-2}\sim10^{-3}$ M solution of calcium, the titration has to be carried out at a pH value where $K_{CaY'}$ is higher than 10^8 (see Fig. 1). As is well known, $\alpha_{H(Y)}$ is given by formula 3, in which $K_1,\ldots\ldots,K_4$ are the formation constants of the proton complexes of the fully dissociated species of EGTA:

$$\alpha_{H(Y)} = 1 + K_1[H] + K_1K_2[H]^2 + K_1K_2K_3[H]^3 + K_1K_2K_3K_4[H]^4 \qquad (3)$$

The conditional stability constants, $K_{CaY'}$, can

9) K. L. Cheng and R. H. Bray, *Anal. Chem.*, **27**, 782 (1955).

10) P. Wehber, *Z. anal. Chem.*, **158**, 10 (1957).

11) B. S. Jensen, *Acta Chem. Scand.*, **14**, 927 (1960).

12) G. Nakagawa and H. Wada, *J. Chem. Soc. Japan, Pure Chem. Sec.* (*Nippon Kagaku Zasshi*), **83**, 1185 (1962).

13) Substituting $\alpha_{H(Y)}$ by $\alpha_{H,M_{II}(Y)}$ the effect of a second metal (M_{II}) as well as pH can be quantitively accounted for by means of the concept "ligand buffer": cf. M. Tanaka, *Anal. Chim. Acta*, in press.

14) B. F. Pease and M. B. Williams, *Anal. Chem.*, 31, 1044 (1959).

15) G. Nakagawa and H. Wada, *J. Chem. Soc. Japan, Pure Chem. Sec.* (*Nippon Kagaku Zasshi*), submitted for publication.

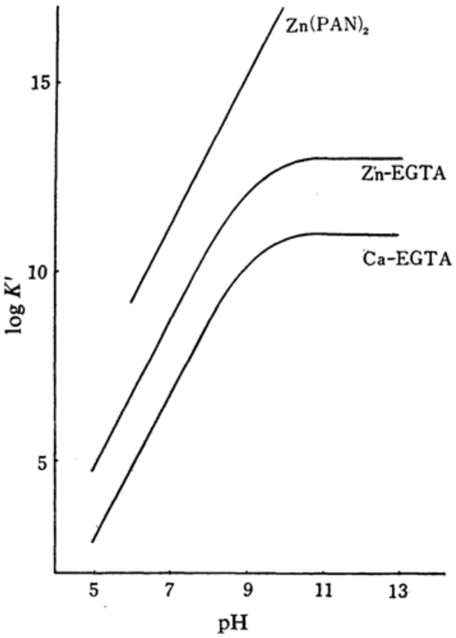


Fig. 1. Conditional stability constants of Zn(PAN)$_2$, Zn-EGTA and Ca-EGTA.

be calculated at various pH values by means of the relationships $K_{CaY'}=K_{CaY}/\alpha_{H(Y)}$ and $\log K_{CaY}=11.0$ (Fig. 1). Similarly, the conditional stability constants of the PAN chelate can be computed for various pH values using the value of $\log K_{ZnA_2}=21.7$ (Fig. 1)[15-17]. For an ordinary condition of chelatometry, viz. $[HA]=10^{-5}$ M and $[ZnY]=10^{-4}$ M, f_1 and f_2 have been computed for various pH values; the results are tabulated in Table I.

From these results it is anticipated that at the pH value of 7 decoloration will occur before the equivalence point, while at the pH value of 10 it will occur gradually after the equivalence point. Therefore, the titration might be successfully carried out in the intermediate pH value range, viz., pH 8∼9 (cf. Fig. 1 of Ref. 7).

In the presence of magnesium, the excess EGTA will be bound with magnesium to form MgY. We know that [Y] in equilibrium with Mg and MgY is given by Eq. 4 and that it is a function of $[Mg]_t/[MgY]$ over a considerable range of pH values, viz., pY after the equivalence point is buffered at a higher level by the excess magnesium[13].

$$[Y]=\frac{[MgY]}{([Mg]_t-[MgY])K_{MgY}} \tag{4}$$

Thus the change in pY in the vicinity of the equivalence point is smaller in the presence of magnesium than in its absence, and the indicator transition after the equivalence point becomes sluggish.

TABLE I. f_1 AND f_2 AT VARIOUS pH

Conditions: $[HA]=10^{-5}$ M, $[ZnY]=10^{-4}$ M, $[Ca]_t=10^{-3}$ M

pH	$\log f_1$	$\log f_2$
7.0	−0.3	−3.4
8.0	−2.4	−3.3
9.0	−4.4	−2.9
10.0	−6.4	−1.5

16) Hydrolysis of zinc ions is not taken into account.

17) A. Corsini, I. M. Yih, Q. Fernando and H. Freiser, *Anal. Chem.*, **34**, 1090 (1962).

Results and Discussion

All the results summarized in Figs. 2, 3 and 4 were obtained by the trial procedure given in the Experimental section below. Zn(PAN)$_2$ being sparingly soluble in water, it precipitates out near the equivalence point[18]. Therefore, the rate of reaction (5) to the right side is slow and the indicator transition is somewhat irreversible.

$$Zn(PAN)_2 + Y \rightleftharpoons ZnY + 2\,PAN \tag{5}$$

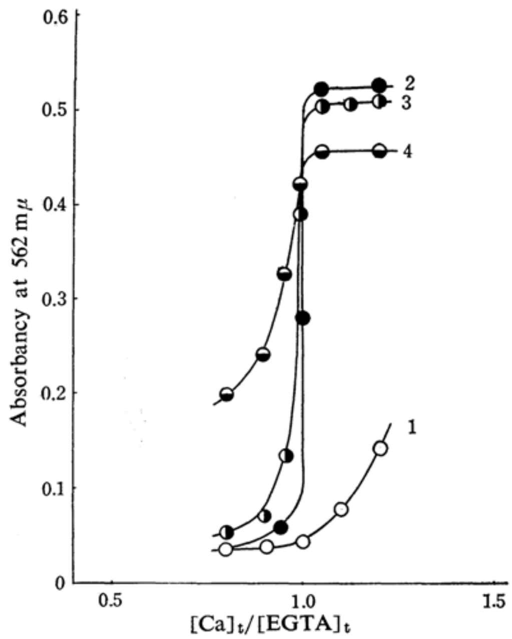


Fig. 2. Effect of pH on the titration of EGTA with calcium standard solution.
Conditions: $[EGTA]_t''=10^{-3}$ M
$[PAN]_t=2\times10^{-5}$ M
$[Zn\text{-}EGTA]_t=4\times10^{-5}$ M
Curve 1: pH 7.0 Curve 2: pH 8.0
Curve 3: pH 9.0 Curve 4: pH 10.0

18) Organic solvents such as dioxane and alcohol have been employed to solubilize Cu(PAN). However, since the solubility of Zn(PAN)$_2$ does not increase appreciably by the addition of such organic solvents, which hardly improves the present situation.

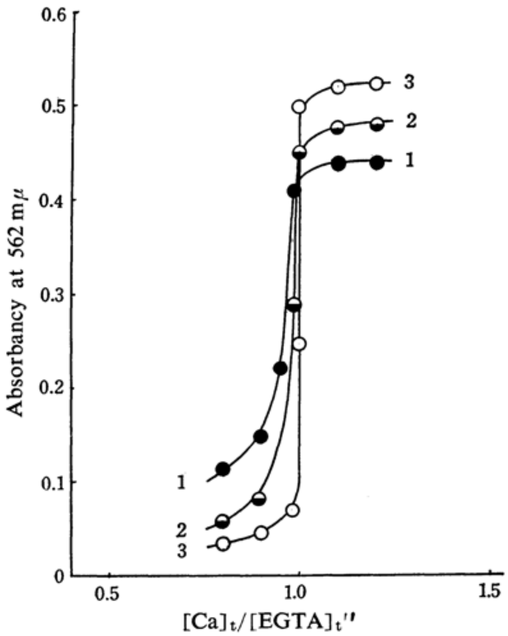


Fig. 3. Effect of Zn-EGTA concentration.
Conditions: $[EGTA]_t''=10^{-3}$ M
$[PAN]_t=2\times10^{-5}$ M, pH 8.0
$[Zn\text{-}EGTA]_t$: Curve 1: 4×10^{-4} M
Curve 2: 8×10^{-5} M
Curve 3: $(2.5\sim5)\times10^{-5}$ M

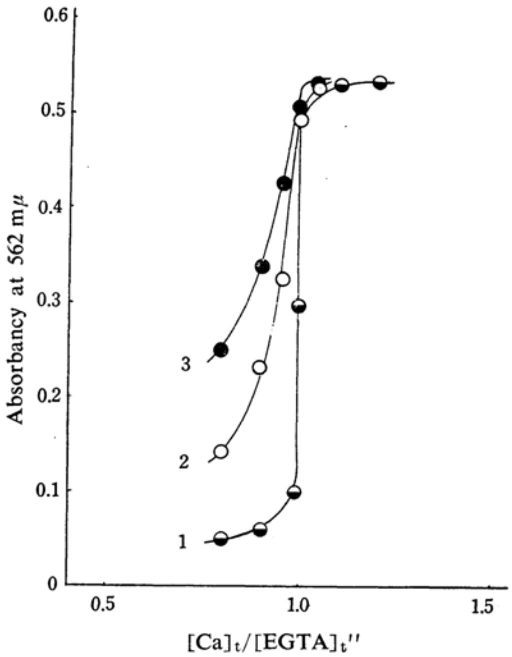


Fig. 4 (a). Effect of magnesium (I).
Conditions: $[EGTA]_t''=2.5\times10^{-3}$ M
$[PAN]_t=2\times10^{-5}$ M
$[Zn\text{-}EGTA]_t=5\times10^{-5}$ M, pH 8.0
Magnesium present: Curve 1: none
Curve 2: 2.5×10^{-3} M
Curve 3: 5×10^{-3} M

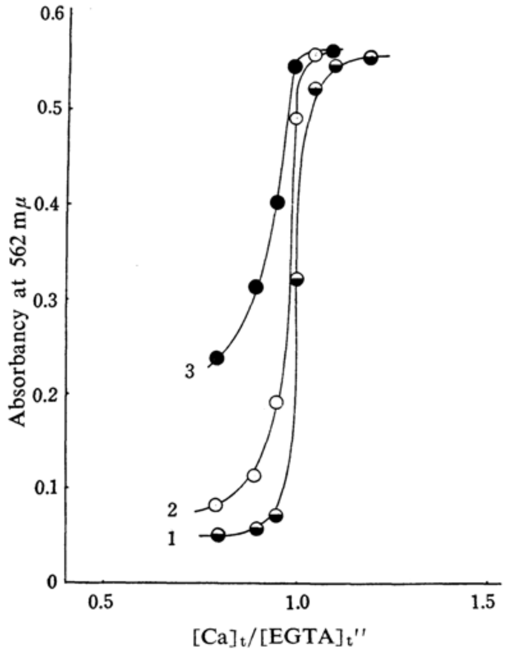


Fig. 4 (b). Effect of magnesium (II).
Conditions: $[EGTA]_t''=10^{-3}$ M
$[PAN]_t=2\times10^{-5}$ M
$[Zn\text{-}EGTA]_t=2.5\times10^{-5}$ M, pH 8.0
Magnesium present: Curve 1: none
Curve 2: 10^{-3} M
Curve 3: 10^{-2} M

Therefore, it is preferable to titrate the excess EGTA with the calcium standard solution in order to have the reaction 5 proceed from right to left near the equivalence point. Moreover, it is recommended that the solution be heated to about 50~60°C near the equivalence point and that the calcium solution be added drop by drop under vigorous stirring.

Influence of Various Factors.—*The Effect of the pH Value.*—Figure 2 shows the indicator transition in the vicinity of the equivalence point: at a pH value of 7 the color of $Zn(PAN)_2$ is not fully developed even after the equivalence point, while at a pH value of 10 a considerable coloration is observed before the equivalence point. In the intermediate pH range, viz. pH 7.5~9, a very neat color change from pale yellow to pink is realized. Thus within this pH value range the visual titration of calcium will be possible.

The Effect of the Zn-EGTA Concentration.—The effect of the Zn-EGTA concentration is illustrated in Fig. 3: a distinct indicator transition is observed at the optimum concentration of $(2.5\sim5)\times10^{-5}$ M Zn-EGTA.

The Effect of the PAN Concentration.—Theoretically the lower the PAN concentration, the sharper the indicator transition near the equivalence point. However, for the visual titration the PAN concentration of 2×10^{-5} M is the most satisfactory; a lower concentration of PAN makes the indicator transition obscure because of a faint coloration of $Zn(PAN)_2$.

The Effect of Magnesium. — The effect of magnesium on the calcium determination is shown in Fig. 4. The presence of magnesium gives rise to a pink shade of $Zn(PAN)_2$ before the equivalence point. Thus, in order to secure a distinct indicator transition at the equivalence point in the presence of magnesium, the concentrations of PAN and Zn-EGTA and the pH value of the solution should be kept as low as practical; the most distinct color change is observed under the following conditions: 2×10^{-5} M of PAN, 2.5×10^{-5} M of Zn-EGTA and a pH value of 8 (Fig. 4-b)[19].

In the chelatometry of calcium in the presence of magnesium, the back titration of the excess EGTA with a calcium standard solution is preferable. Denoting the total concentrations of calcium present in the sample and used as a titrant as $[Ca]_{sample}$ and $[Ca]_{titrant}$ respectively, the total concentration of EGTA, $[Y]_t''$, at the equivalence point is given by:

$$[Y]_t'' = [Ca]_t = [Ca]_{sample} + [Ca]_{titrant}$$

Hence, in such a back titration the effect of magnesium on the indicator transition is not determined by $[Mg]_t/[Ca]_{sample}$ but by $[Mg]_t/[Ca]_t$: the color change at the equivalence point becomes less distinct with an increasing $[Mg]_t/[Ca]_t$ ratio (Fig. 4). For a sample with a high $[Mg]_t/[Ca]_{sample}$ ratio, the indicator transition is much improved by the addition of a large excess of EGTA, viz., making the $[Mg]_t/[Ca]_t$ ratio low. As shown in Fig. 4-b, the visual titration of calcium ($[Ca]_t=10^{-3}$ M, $[Ca]_{sample}=0.5\times10^{-3}$ M) in the presence of ten times as much as magnesium ($[Mg]_t=5\times10^{-3}$ M) is possible by the procedure recommended below.

Recommended Procedure.—Fifty milliliters of a buffer solution (pH value: 8) and 5~20 ml. of a 10^{-2} M EGTA solution[20] are added to 25 ml. of a neutral sample solution containing 1~4 mg. of calcium and less than 10 mg. of magnesium[21]. After the addition of 2.5~3 ml. of a 10^{-3} M Zn-EGTA solution and 2 ml. of a 10^{-3} M PAN solution, the excess EGTA is titrated with a 10^{-2} M calcium standard solution. Near the equivalence point, the solution is heated to about 50~60°C and the titration is continued drop by drop while vigorously stirring the solution. A color change from pale yellow to pink is observed at the equivalence point. Titrate to a pink with no yellowish shade.

19) Though the effect of magnesium is less at pH 7.5, appropriate buffer solution of this pH is hardly available.

20) Total concentration of EGTA to be added is preferably more than 1.5 times calcium or 1/2 of magnesium in the sample solution.

21) By means of the photometric titration, presence of about 100 mg. of magnesium is tolerable.

Experimental

Solutions. — *10^{-2} M EGTA Standard Solution.* — 3.8036 g. of EGTA (Dojindo Chemical Co., Kumamoto, Japan) are dissolved in 20 ml. of 1 N sodium hydroxide and the whole is diluted to one liter. The solution is standardized chelatometrically with a 10^{-2} M zinc standard solution using Eriochrome black T as an indicator.

10^{-2} M Calcium Standard Solution.—1.000 g. of calcium carbonate is dissolved in a small amount of hydrochloric acid, the excess of which is expelled completely by evaporation to dryness on a steam bath. The residue is taken up in distilled water and diluted to one liter. The solution is standardized chelatometrically with an EDTA standard solution.

$(1\sim5)\times10^{-3}$ M Zn-EGTA Solution. — An equivolume mixture of a 10^{-2} M EGTA solution and a 10^{-2} M zinc solution is diluted to an appropriate concentration.

10^{-3} M PAN Solution.—Twenty-five milligrams of PAN (Dojindo Chemical Co., Kumamoto, Japan) are dissolved in 100 ml. of methyl alcohol.

Buffer Solution with a pH Value of 8.—1 N hydrochloric acid is added to a saturated solution of borax until the pH value of the solution is brought to 8.0.

Apparatus.—Filter photometer and conventional titration cell (Hirama Co., Tokyo, Japan).

Trial Procedure.—To a cell for the photometric titration are added 10 ml. of a buffer solution, 2~5 ml. of 10^{-2} M EGTA solution[22] and 0.1~2 ml. of a 5×10^{-3} M Zn-EGTA solution. The solution is then diluted to 20 ml. After the addition of 0.4 ml. of a 10^{-3} M PAN solution, the photometric titration is carried out, using a 10^{-2} M calcium standard solution as the titrant.

Summary

The optimum conditions for the chelatometry of calcium with EGTA have been deduced from the theoretical consideration of the equilibria involved. Some experimental results have been given on the chelatometry of calcium in the presence of magnesium using the Zn-EGTA-PAN system as an indicator. This proposed method seems to be better than earlier ones.

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22) Initial concentration of EGTA may be preferably of the order of 10^{-3} M.