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Spectrophotometric Determination of Calcium-Complexing Ability

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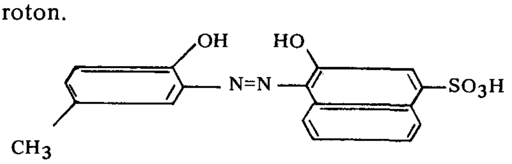
ABSTRACT

A convenient and reliable spectrophotometric technique for the quantitative determination of calcium-complexing constants is presented. A calcium-ion sensitive dye, Calmagite, at a pH between 10 and 10.6, was found to be suitable for these determinations. The theoretical and practical bases for the use of Calmagite are described.

Convenient and reliable techniques for the quantitative determination of complexing abilities of chelating agents for divalent ions especially Ca^{++} are always in demand. Because of the low concentrations usually involved in these determinations coupled with the fact that the complexed and free divalent ions are both in solution, a sensitive *in situ* indicator is required at equilibrium conditions. A number of techniques have been developed on this basis using the onset of precipitation (1), ion exchange with radioactive Ca^{++} (2), and calcium-ion selective electrodes (3) among others. Another possible approach is the use of a spectrophotometric technique using a calcium sensitive dye. In 1960, Lindstrom and Diehl (4) introduced a material, 1-(1-hydroxy-4-methyl-2-phenylazo)-2-naphthol-4-sulfonic acid, called Calmagite, which under specific conditions changes color from blue to red upon the addition of calcium or magnesium ions. Although Calmagite is used primarily as an end point indicator for the titration of Ca^{++} solutions (4), it appears to have the characteristics necessary for determining calcium-complexing constants of chelating agents. This paper describes the theoretical and practical bases for the use of Calmagite for this purpose.

THEORY

Calgamite is a tribasic acid as shown below. It has two weakly dissociating phenolic protons and a strong sulfonic acid proton.



Thus, the acidic equilibria of interest are:

$$H_2D^- \overset{K_1'}{\rightleftharpoons} HD^= + H^+ \quad (1)$$

$$HD^= \overset{K_2'}{\rightleftharpoons} D^{\equiv} + H^+ \quad (2)$$

Of the three dye species shown in equations 1 and 2, two, H_2D^- and $D^{\equiv}$, are red (absorption maxima at 520 nm), but the divalent anion $HD^=$ is blue (absorption maximum at 610 nm). Since the calcium salt of Calmagite, CaD^-, is also red, the blue-colored anion is essential to the usefulness of this dye system. Hence, maximum sensitivity in such a system will occur when $HD^=$ is at its maximum concentration.

The concentration of $HD^=$ is pH dependent as implied by equations 1 and 2. The acid concentration dissociation constants, K_1' and K_2', are given by the expressions,

$$K_1' = \frac{[HD^=]\,[H^+]}{[H_2D^-]} \quad (3)$$

$$K_2' = \frac{[D^{\equiv}]\,[H^+]}{[HD^=]} \quad (4)$$

where the bracketed terms represent concentrations not activities. Combining these equations with the material balance equation,

$$D_o = [D^{\equiv}] + [HD^=] + [H_2D^-] \quad (5)$$

we obtain for [HD=],

$$\frac{[HD^=]}{D_o} = \frac{1}{1 + [H^+]/K_1' + K_2'/[H^+]} \quad (6)$$

Differentiating with respect to $[H^+]$ and setting the derivative equal to zero, the hydrogen ion concentration at which the fraction of $[HD^=]$ is at its maximum is given by,

$$[H^+]_{max} = \sqrt{K_1' K_2'} \quad (7)$$

Substituting back into equation 6, the maximum $[HD^=]$ fraction is

$$\left(\frac{[HD^=]}{D_o}\right)_{max} = \frac{1}{1 + 2\sqrt{K_1'/K_2'}} \quad (8)$$

Evaluation of these maximum quantities requires only a knowledge of the concentration dissociation constants of the two acidic anions of Calmagite.

Lindstrom and Diehl (4) determined constants for the Calgamite system at a constant ionic strength, designated K_1 and K_2, from the pH values at which the concentrations of the acid and conjugate base were equal. These constants are neither concentration equilibrium constants nor activity equilibrium constants. Nevertheless, being readily determinable and eminently usable, they are widely employed in spectrophotometric investigations. It can be readily shown, if we assume the pH meter measures hydrogen-ion activity, $[H^+]f_{H^+}$, where f_{H^+} is the activity coefficient that $K_1 = K_1' f_{H^+}$ and $K_2 = K_2' f_{H^+}$. Since at constant ionic strength f_{H^+} is constant, equation 7 can be rewritten as,

$$(pH)_{max} = \frac{1}{2}(pK_1 + pK_2) \quad (9)$$

Equation 8, however, remains unaltered since (K_2'/K_1') is equal to K_2/K_1. From pK_1 and pK_2 values of 8.14 and 12.35, respectively, at an ionic strength of 0.10, (4), we find a $(pH)_{max}$ of 10.25 and a $([HD^=]/D_o)_{max}$ value of 0.984. Furthermore, the flatness of the maximum as determined by equation 6 shows that from a pH of 9.9 to 10.6, the dianionic form remains above 98%. These calculations indicate that Calmagite can be used with high sensitivity in the pH 10 region by observing spectrophotometrically the blue-colored $HD^{=}$ species, with minimal interference from the anionic forms H_2D^- and $D^{\equiv}$.

When a divalent cation, such as Ca^{++}, is added to a Calmagite solution, a wine-colored complex, CaD^-, is formed. The dissociation of this species can be represented by,

$$CaD^- \overset{K_D'}{\rightleftharpoons} Ca^{++} + D^{\equiv} \tag{10}$$

and the expression for K_D'

$$K_D' = \frac{[Ca^{++}]\,[D^{\equiv}]}{[CaD^-]} \tag{11}$$

Since $HD^{=}$ is the component to be measured spectrophotometrically, eliminating $[D^{\equiv}]$ from equations 4 and 11 we get,

$$\frac{K_D'[H^+]}{K_2'} = \frac{[Ca^{++}]\,[HD^{=}]}{[CaD^-]} = K \tag{12}$$

If measurements are conducted at constant pH and constant ionic strength, the left hand side of the expression will be a constant which, for convenience, will be designated K. The determination of K is equivalent to calibrating the calcium-dye system. K, of course, will vary with both the hydrogen-ion concentration and the ionic strength of the solution.

The expression for the absorbance of a Calmagite-calcium solution at a pH of ca. 10 and a wavelength close to the absorption maximum of $HD^{=}$ can be written as,

$$A = \frac{A_0[HD^{=}]}{\alpha D_0} + \frac{A_\infty[CaD^-]}{D_0} \tag{13}$$

where A is the observed absorbance, A_0 is the absorbance of $HD^{=}$ when the concentration of this species is αD_0, A_∞ is the absorbance of CaD^- when its concentration is D_0, and α is the fraction of the dye in the divalent anionic form as calculated by equation 6. Contributions from H_2D^- and $D^{\equiv}$, although insignificant by virtue of their very low concentrations and small absorbances at the stipulated conditions, are essentially corrected for by the use of this form of expression. In addition to equations 12 and 13 the material balance equations

$$C_0 = [Ca^{++}] + [CaD^-] \tag{14}$$

$$D_0 = [HD^{=}]/\alpha + [CaD^-] \tag{15}$$

are necessary to obtain the expression for K as a function of absorbance. Suitable combination of these equations gives

$$[HD^{=}] = \alpha D_0 \left(\frac{A-A_\infty}{A_0-A_\infty}\right) \tag{16}$$

$$[CaD^-] = D_0 \left(\frac{A-A}{A_0-A_\infty}\right) \tag{17}$$

$$[Ca^{++}] = \frac{K}{\alpha}\left(\frac{A_0-A}{A-A_\infty}\right) \tag{18}$$

and for K,

$$K = \alpha C_0 \left(\frac{A-A_\infty}{A_0-A}\right) \cdot \alpha D_0 \left(\frac{A-A_\infty}{A_0-A_\infty}\right) \tag{19}$$

If a constant quantity of dye, D_0. and varying calcium content, C_0, are used in a series of absorbance measurements, K can be determined graphically or by linear regression from the rearranged equation,

$$\frac{C_0}{A_0-A} = \frac{K}{\alpha(A-A_\infty)} + \frac{D_0}{A_0-A_\infty} \tag{20}$$

By plotting the left hand side of equation 20 as the ordinate against the reciprocal of $(A-A_\infty)$, a straight line should result with the slope equal to K/α and the intercept equal to $D_0/(A_0-A_\infty)$. It is important to note that the Calmagite concentration D_0 is not required to obtain K. Indeed, D_0, if not known, can be determined in this manner. Having determined the nature of the relationships for the Calmagite-calcium system, their application to systems involving a calcium-complexing agent can now be examined.

If a colorless material, which complexes Ca^{++} and does not interact with Calmagite, is added to a Calmagite-calcium solution, the concentrations of the various species will be altered, but the spectrophotometric expressions 16, 17 and 18 remain valid for calculating the new concentrations. The complexing agent simply reduces the quantity of calcium available for interaction with the Calmagite. The concentration of calcium complexed by the chelating agent is given by the material balance equation

$$[Ca]_B = C_0 - [Ca^{++}] - [CaD^-] \tag{21}$$

in which $[Ca^{++}]$ and $[CaD^-]$ are determined from the absorbance of the solution using equations 17 and 18, respectively. Since the concentration of the free chelating compound is obtained by subtracting $[Ca]_B$ from the total chelate added, it becomes a relatively simple matter to calculate the stability or dissociation constant for the complexing agent.

It thus appears from theory and background information that the Calmagite-calcium system should be suitable for determining quantitatively the calcium-complexing ability of materials in the pH region of 10.

EXPERIMENTAL

Materials

Calmagite was obtained from Aldrich Chemical Company. The material, which was rather impure based upon absorbance measurements, was purified as follows: five grams of Calmagite was slurried in 100 ml of 5% HCl for one hour and filtered. The solid was dried and dissolved in 50 ml of methanol. To the filtered methanolic solution, 500 ml of absolute ether is added with stirring. After 1 hr the solution is filtered. The solid obtained is generally a few tenths of a gram and is insoluble in water. The filtrate is again diluted with 500 ml of ether and the resulting precipitated solid is removed. This is repeated 5 or 6 times. The solid material after the fifth or sixth addition generally has a purity of 80% to 90% and weighs ca. 1 g. This material was used for the spectrophotometric determination.

Calmagite stock solutions contained ca. 50 mg of Calmagite and 1.4 ml of 0.10 N NaOH, corresponding to the monosodium salt, diluted to 250 ml. In all the experimental work, 5 ml of the stock in 50 ml test solutions was used.

Monoethanolamine (Matheson Coleman Bell) was redistilled, and the middle fraction was used to prepare a buffer solution at a pH of ca. 10. The stock solution contained 6.02 g of ethanolamine in 200 ml of 0.10 N HCl. Five ml of the stock was used in 50 ml of test solution.

Calcium chloride dihydrate (Mallinckrodt, Analytical Reagent), sodium citrate dihydrate (Mallinckrodt, Analytical Reagent) and sodium chloride (J.T. Baker, Analyzed Reagent) were used without further purification to prepare the appropriate solutions.

Deionized redistilled water was used for all the solutions.

Procedure

Test solutions were prepared with the appropriate quantities of stock solutions and made up to 50 ml. Absorbances were read with a Shimadzu QV50 spectrophotometer at 630 nm. The pH of each solution was measured with a Coleman 38A pH meter.

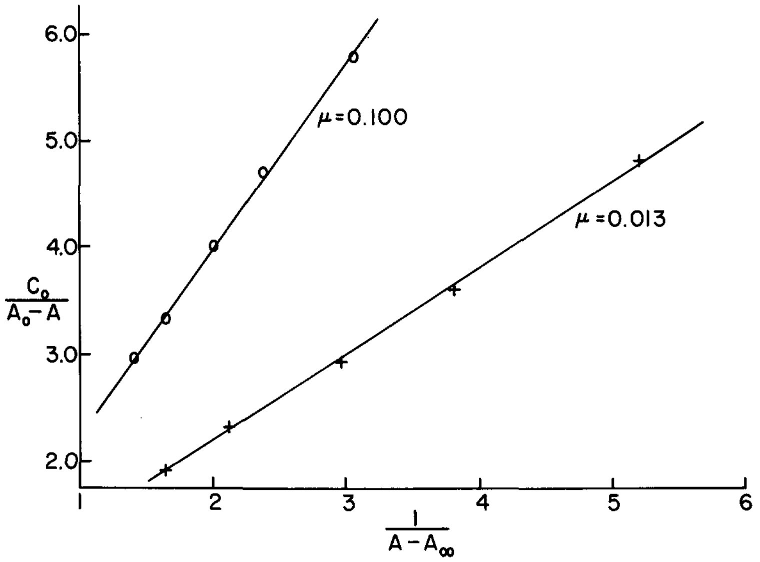


FIG. 1. Calibration for the Calmagite-Calcium System according to Equation (20).

RESULTS AND DISCUSSION

Calmagite-Calcium System

The experimental behavior of the Calmagite-calcium system, obtained with calcium concentrations from 0.4 x 10^{-4} to 3 x 10^{-4} M, is shown in Figure 1 to be in accord with the predictive linear plot of equation 20. From a least squares analysis of the data values for D_o, the dye concentrations, of 0.45 x 10^{-4} and 0.46 x 10^{-4} M and for K, 1.69 x 10^{-4} and 0.79 x 10^{-4}, are obtained at ionic strengths of 0.100 and 0.013, respectively. These results show a constancy of dye concentration and a dissociation constant that is strongly dependent on ionic strength. Examination of these observations will serve to test the theoretical concepts in addition to developing the information necessary for the use of Calmagite in calcium-complexing determinations.

The dye concentration obtained from the weight of dye used to prepare the solution is different than that determined using equation 20. The former, as indicated in the experimental section, is ca. 0.56 x 10^{-4} M. Since Calmagite is known to be notoriously difficult to purify, this difference can be an indication of the purity of the Calmagite used in these experiments. A comparison of the two values indicates a purity of ca. 81%. The purity can also be estimated from the A_o values of the dye solutions. From an average absorbance of 0.855 and the interpolated literature value of 1.88 x 10^{-4} for the molar absorptivity at 630 nm (4), the concentration of dye is found to be 0.455 x 10^{-4} M. Although the exact coincidence of this value with the above is fortuitous, a reasonable agreement between the two values is sufficient to support the validity of equation 20.

The value of K, obtained at an ionic strength of 0.100, can be compared with a similar constant evaluated by Lindstrom and Diehl for a relatively pure sample of Calmagite at the same ionic strength (4). K, defined in equation 12, is the reciprocal of their "apparent stability constant," which (in their notation) is $\log K_{10.00} = 3.67$. Converting and treating the corresponding quantity obtained from equation 20 in the same manner, we find a value of 3.63. The agreement is within the combined experimental errors. This comparison of equilibrium constants gives added confidence in the reliability of the results especially as the Calmagite used in this study is impure. The results strongly suggest that the impurity is inert spectrally and does not interact with Ca^{++}.

The various equilibria are described in this paper by concentration equilibrium constants and as such are functions of the activity coefficients of the species involved in the equilibria. The relationship is given in equation 22:

$$K = K^\circ \frac{f_{CaD^-}}{f_{Ca^{++}} f_{HD^=}} \qquad (22)$$

where K° is the equilibrium constant at zero ionic strength, i.e., the activity equilibrium constant. Since the activity coefficients are dependent upon ionic strength, K will reflect this variation. The experimental values of K, given above, differ significantly; whether this difference conforms to expectations can be tested.

A generally accepted relationship between activity coefficients and ionic strength beyond the Debye-Huckel limiting law range (1) is

$$-\log f_i = 0.5\, Z_i^2 \left(\frac{\sqrt{\mu}}{1 + \sqrt{\mu}} - 0.2\mu \right) \qquad (23)$$

where Z_i is the ionic charge. Substituting this expression into logarithmic form of equation 22 gives

$$pK = pK^\circ - 0.5 \left(Z^2_{Ca^{++}} + Z^2_{HD^=} - Z_{CaD^-} \right) \left(\frac{\sqrt{\mu}}{1 + \sqrt{\mu}} - 0.2\mu \right) \qquad (24)$$

This expression can be used to test the experimental equilibrium constants by verifying the coefficient of the ionic strength term. However, since K is a function of the hydrogen-ion concentration as defined in equation 12, the values used in equation 24 must be at the same $[H^+]$. The experimental values can be corrected to a $[H^+]$ of 10^{-10} M using the expression

$$K_{corr} = \frac{(K_{exp} \times 10^{-10}) f_{H^+}}{[H^+] f_{H^+}} \qquad (25)$$

in which $f_H{}^+$ in the numerator can be determined using equation 23 and the denominator, being the hydrogen-ion activity, from the pH at the test conditions. Thus, we find at $\mu = 0.100$, pH = 10.14 and a $pK_{exp} = 3.77$ that $pK_{corr} = 3.74$, and similarly at $\mu = 0.013$, pH = 10.01 and a $pK_{exp} = 4.10$, $pK_{corr} = 4.14$. Using the corrected values and the corresponding ionic strengths, a coefficient of 3.3 is obtained for the ionic strength term in equation 24. This favorable comparison with the value of 3.5, obtained from

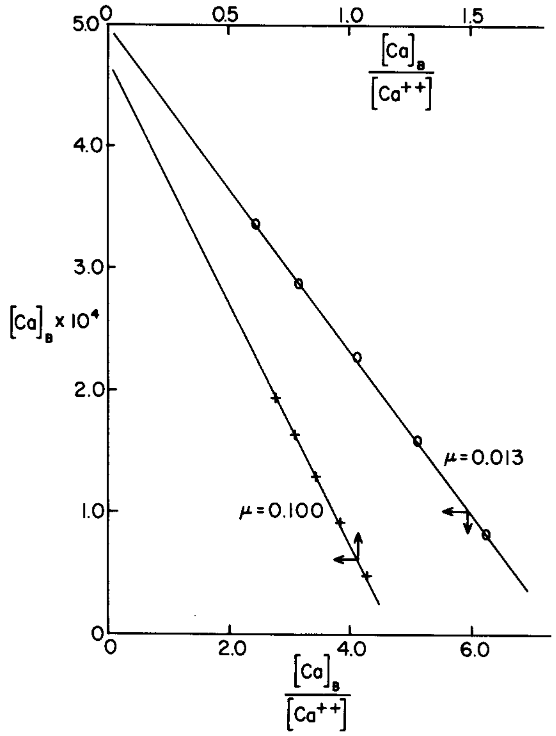


FIG. 2. Dissociation Constant Determination for the Calmagite-Calcium-Citrate System according to Equation (27).

the ionic charges of the species involved in the equilibrium, gives further support for the consistency of the behavior of the Calmagite-calcium system.

Calmagite-Calcium-Citrate System

The applicability of the Calmagite-calcium system in determining the complexing constants of chelating agents was investigated using the citrate anion. At the required pH of ca. 10, the trivalent citrate ion is not encumbered by proton association, allowing for a relatively uncomplicated citrate-calcium system. Thus, the dissociation constant, K_B, of the calcium citrate complex, $CaCit^-$, is given by

$$K_B = \frac{[Ca^{++}]\,[Cit^{\equiv}]}{[CaCit^-]} = \frac{[Ca^{++}]\,(B_o = [Ca]_B)}{[Ca]_B} \qquad (26)$$

in which B_o is the total citrate concentration, and $[Ca]_B$ is defined by equation 21. Both $[Ca^{++}]$ and $[Ca]_B$ can be obtained from the absorbance data using equations 17, 18 and 21.

Rewriting equation 26 in the form

$$[Ca]_B = Bo\text{-}K_B \frac{[Ca]_B}{[Ca^{++}]} \qquad (27)$$

yields an expression that can be used to obtain the dissociation constant of $CaCit^-$ for systems in which the total citrate concentration is kept constant from a plot or linear regression computation using $[Ca]_B$ and $[Ca]_B/[Ca^{++}]$ as variables. It is interesting to note, as in a similar situation revealed earlier, that it is not necessary to use, indeed even to know the citrate concentration, B_o, to determine K_B. However, since B_o is known, a comparison with the intercept of the above plot can be used as a check of the method.

From the absorbance data obtained at a constant citrate concentration of 5.00 x 10^{-4} M and calcium concentrations ranging from 1.00 x 10^{-4} M to 5.00 x 10^{-4} M, the graphs in Figure 2 were constructed according to equation 27. The slopes, obtained by the least square method, give for K_B values of 4.04 x 10^{-4} and 0.67 x 10^{-4}, or pK_B values of 3.39 and 4.17 at ionic strengths of 0.100 and 0.013, respectively; and correspondingly, intercepts of 4.75 x 10^{-4} M and 4.98 x 10^{-4} M were obtained. The latter two values, on the basis of linear regression analysis, compare reasonably well with the actual citrate concentration of 5.00 x 10^{-4} M.

The concentration dissociation constants for $CaCit^-$ are very dependent upon ionic strength. As before, equations 23 and 24 can be used to compare the experimental and the semi-empirical ionic strength dependence of this calcium complex. The coefficient of the ionic strength term calculated from the ionic charges is 6; the experimentally determined value is 6.4. Again, reasonably good agreement is obtained. The internal consistency and literature comparisons (1), (2), (3) of the results for the calcium citrate complex strongly supports the validity of the Calmagite method for determining calcium-complexing ability.

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