

Spectrophotometric Determination of Magnesium, Calcium, Strontium and Barium Present in Pairs by Use of Tetramethylmurexide

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A spectrophotometric method is described for the determination of magnesium, calcium, strontium and barium coexisting in binary mixtures. The method employs tetramethylmurexide as an auxiliary ligand, and has been developed for pure two-component systems, such as are involved in investigations of cation-exchange equilibria on anionic polymers. The analysis is performed with calibration curves of the $\varphi = f[\text{Me(I)}, \text{Me(II)}]$, where φ is the ratio of two absorbances A_1 and A_2 measured at two suitable wavelengths, λ_1 and λ_2 . The wavelengths λ_1 and λ_2 are chosen from the absorption spectra of solutions containing the separate metals Me(I) or Me(II) . The absorption spectra of solutions containing the tetramethylmurexide complexes of Ca^{2+} , Sr^{2+} or Ba^{2+} ions are independent of the identity of the anions present in the system (Cl^- , NO_3^- and CH_3COO^-). The results show that two arbitrarily chosen elements of the alkaline earths can be determined with a good accuracy in the presence of each other over a large concentration range (0.001–0.05 M). Calcium, strontium and barium can be estimated with high accuracy in solutions containing a considerable excess of either magnesium or potassium, this exceeding the concentrations of the above alkaline-earth elements by factors of 100, 50 and 20, respectively. Calcium can also be estimated with barium or strontium present in a 30-fold or a 20-fold molar excess, respectively.

The use of murexide as an indicator for complexometric determination of calcium, copper and nickel is well known [1]. This compound (the ammonium salt of 5,5'-nitridibarbituric acid) forms coloured complexes with many bivalent and multivalent cations [1–4], and has been used for the spectrophotometric determination of calcium [5–7], strontium [8], zinc [9], and elements of the rare earths such as scandium, yttrium, lanthanum [10, 11] and ytterbium [12].

Raaflaub [5] used murexide as an auxiliary ligand for the spectrophotometric determination of Ca^{2+} ions in biological fluids (see also [13]). The formation of the coloured complex between calcium and murexide is, however, rather dependent upon the pH of the solution because of the presence of four imido groups in the murexide molecule. For this reason, this method requires the maintenance of a fixed pH value during the spectrophotometric determination. *Raaflaub* [14] overcame this disadvantage by using tetramethylmurexide instead of murexide. The *keto* groups neighbouring the methyl groups in a molecule of tetramethylmurexide cannot assume the enolic form in contrast to murexide, and the formation of the coloured complex of tetramethylmurexide with cations is independent of pH within the

range 4.8–8.1 [14]. Tetramethylmurexide has been successfully used for the study of the binding of Ca^{2+} ions to the carboxyl groups of pectin, monouronides and polyuronides [15–17].

In connection with the application of pectin as a natural prophylactic or remedy against poisoning with heavy metals or radioactive strontium, it is important to study the exchange of Ca^{2+} , Sr^{2+} and Ba^{2+} ions on this material. During work on these problems, it was necessary to have a simple but exact method for the determination of magnesium, calcium, strontium and barium in arbitrarily chosen binary mixtures.

Hitherto, murexide has been used only for the determination of calcium and magnesium in the presence of each other either by complexometric or by spectrophotometric methods [1, 7]. (The chelatometric determination of magnesium, strontium and barium in the presence of each other is unsatisfactory because the stability constants of the complexes formed between ethylenediaminetetraacetic acid and these metals are very similar [1].) We have therefore developed a spectrophotometric method for the determination of arbitrarily chosen binary mixtures of elements from the series magnesium, calcium, strontium and barium by using tetramethylmurexide as an auxiliary coloured ligand. The method permits the analysis of pure solutions containing the salts of these elements, and was developed, as stated primarily to facilitate the study of exchange equilibria on ion-exchangers. The principle of the method could, however, be applied to solutions containing more components. The method is rather simple and does not require expensive equipment, in contrast to the determination of these elements by atomic absorption spectrometry; in some combinations of elements it is also more exact.

Experimental

Chemicals

Tetramethylmurexide was synthesized from caffeine *via* tetramethylalloxantin [18, 19]. The molar absorptivities of the ammonium salt of tetramethylmurexide ($\epsilon_{530} = 13,800$) and of its Ca-complex ($\epsilon_{490} = 20,000$) were consistent with corresponding values of murexide cited in literature [2, 13].

The salts [CaCl_2 , SrCl_2 , BaCl_2 , MgCl_2 , KCl , $\text{Ba}(\text{NO}_3)_2$ and $\text{Ba}(\text{OAc})_2$] and the other chemicals used were of analytical grade. The calcium acetate solution was prepared by decomposing CaCO_3 with acetic acid, filtering off the undissolved carbonate residue, expelling the released CO_2 by heating on a boiling water bath, and adjusting the pH. The solutions of strontium nitrate and strontium acetate were prepared by neutralization of the corresponding acids to pH 7.0–7.5 with 0.1 N solutions of strontium hydroxide. The solutions of magnesium nitrate and magnesium acetate were prepared by neutralization of MgO with the corresponding acid. After filtering off the residual MgO, the pH of the filtrate was adjusted with the corresponding acid to 6.5–7.0. The specific conductivity of the distilled water was $3 \times 10^{-6} \text{ ohm}^{-1} \text{ cm}^{-1}$.

Analytical methods

The principle of quantitative determination of alkaline earth cations is fully described in the next section.

The absorbances of solutions were determined with a compensation spectrophotometer (UVISPEC-Hilger) with 0.1 mm slit width and 1 cm cells at 23–25°C. The wavelength

scale was calibrated with the hydrogen spectral lines at 486 and 656 nm and by use of a didymium glass filter within the wavelength region 400–650 nm [20]. The stock solution of 2×10^{-4} M tetramethylmurexide (NH_4^+ form) was prepared separately for each series of measurements just before use. All absorbance measurements were carried out with 4×10^{-5} M solutions of tetramethylmurexide.

The molar concentrations of metal chlorides in solution were determined by potentiometric titration with either 0.1 N or 0.01 N solutions of AgNO_3 , using a silver electrode. The molar concentrations of solutions of calcium and magnesium salts were determined by the usual chelatometric titration, murexide or oriochrome black being used as indicators. The concentrations of strontium nitrate and acetate solutions were computed from the consumption (to neutrality) of strontium hydroxide by the respective acids. The concentrations of barium nitrate and acetate solutions were calculated directly from the weights of the analytically pure preparations used.

Results and Discussion

Absorption spectra of tetramethylmurexide in solutions of calcium, strontium, barium and magnesium salts

As mentioned, murexide (NH_4^+ form) forms coloured complexes with Ca^{2+} , Sr^{2+} and Ba^{2+} ions. The stability constants (K) of these complexes and the wavelengths corresponding to maximum absorbance (λ_{max}), found by some authors, are summarized in Table 1. The stabilities of the complexes decrease in the order: $(\text{CaMur}^+) > (\text{SrMur}^+) > (\text{BaMur}^+)$.

Table 1

Stability constants (K) of murexide complexing with Ca^{2+} , Sr^{2+} , Ba^{2+} and Mg^{2+} ions and wavelengths corresponding to maximum absorbance (λ_{max})

Mo	K	λ_{max} [nm]	K	K	λ_{max} [nm]	K	λ_{max} [nm]
Ca^{2+}	500 ¹	487	210 ²	500 ³	483	398 ⁴	483
Sr^{2+}				280	—	100 ⁵	495
Ba^{2+}				138	513	100 ⁵	510
Mg^{2+}				0.3–0.5	487	< 1 ⁴	
Ref.	[2]		[13]		[3]		[4]

1. pH 7.0, μ 0.005–0.10; 2. pH 7.2, μ 0.15 (KCl); 3. neutral solutions; 4. μ 0.10 (KNO_3), 10°C; 5. 23°C.

Tetramethylmurexide shows the same stability constant for its Ca-complex as does murexide ($K = 500$). The absorption spectra of both indicators are practically identical [19]. We have determined the absorption spectra of tetramethylmurexide (4×10^{-5} mol l⁻¹) in 0.05 M (Fig. 1) as well as in 0.002 M (Fig. 2) solutions of CaCl_2 , SrCl_2 , BaCl_2 and MgCl_2 . Absorption spectra were also measured on 0.002 M solutions of the nitrates and acetates of these metals, but they were identical with the spectra obtained with the chlorides. This means that the spectra of the complexes of tetramethylmurexide with Ca^{2+} , Sr^{2+} and Ba^{2+} (and also Mg^{2+}) are independent of the presence of Cl^- , NO_3^- and CH_3COO^- ions.

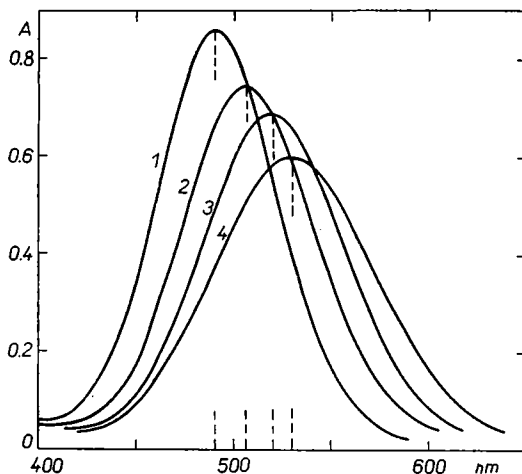


Fig. 1. Absorption spectrum of tetramethylmurexide in 0.05 M solutions of CaCl₂, SrCl₂, BaCl₂ and MgCl₂.

1. CaCl₂; 2. SrCl₂; 3. BaCl₂; 4. MgCl₂. Tetramethylmurexide; 4×10^{-5} mol l⁻¹.

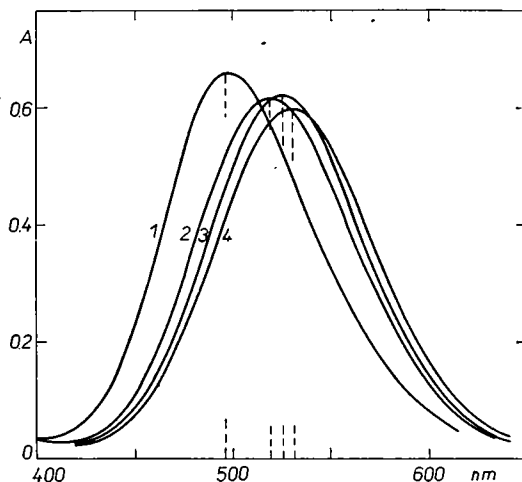


Fig. 2. Absorption spectrum of tetramethylmurexide in 0.002 M solutions of CaCl₂, SrCl₂, BaCl₂ and MgCl₂.

1. CaCl₂; 2. SrCl₂; 3. BaCl₂; 4. MgCl₂. Tetramethylmurexide; 4×10^{-5} mol l⁻¹.

The values of λ_{\max} given in Table 1 were measured with solutions in which a major part of the murexide was in the form of the complex, *i.e.* in solutions with high concentrations of alkaline-earth cations. In less concentrated solutions where the complex is formed only in part, the maximum absorbance shifts to slightly longer

wavelengths. Tetramethylmurexide complexes with Ca^{2+} , Sr^{2+} and Ba^{2+} ions behave in the same manner (Table 2). The Mg^{2+} and K^{+} ions do not form any complex with tetramethylmurexide. The absorption spectrum of tetramethylmurexide is the same in KCl solutions as in MgCl_2 solutions. The spectra are invariant within the concentration range $0.002\text{--}0.05\text{ mol l}^{-1}$.

Table 2

Dependence of λ_{max} on CaCl_2 , SrCl_2 , BaCl_2 , MgCl_2 and KCl concentration in solutions containing tetramethylmurexide ($4 \times 10^{-5}\text{ mol l}^{-1}$)

Me	λ_{max} [nm]	
	0.05 mol l^{-1}	0.002 mol l^{-1}
Ca^{2+}	490	497
Sr^{2+}	506	519
Ba^{2+}	519	526
Mg^{2+}	530	530
K^{+}	530	530

Schwarzenbach and Gysling [2] described a marked hypsochromic effect in murexide solutions caused by a high concentration of Mg^{2+} ions (2 mol l^{-1}); see also [3]. The value of λ_{max} shifts from 530 nm to 470 nm , approximately. This hypsochromic effect is not evident in tetramethylmurexide solutions containing Mg^{2+} in 0.05 M concentration. It begins to be effective only when the concentration of Mg^{2+} ions exceeds 1 mol l^{-1} .

Principle of the analytical method

The spectra of the various complexes of tetramethylmurexide overlap considerably, especially in highly dilute solutions (Figs. 1 and 2). In solutions containing an arbitrary pair of cations, Me(I) and Me(II) , from the series Ca^{2+} , Sr^{2+} , Ba^{2+} and Mg^{2+} (or K^{+} , respectively), the resulting curve $A = f(\lambda)$ is situated between the absorbance curves for the individual Me(I) or Me(II) ions.

The method requires first the determination of the total concentration of both cations in the solution under investigation. For a solution containing soluble chlorides, the total concentration of cations may be calculated on the basis of chloride-ion concentration, determined argentometrically. In the solutions of nitrates and acetates the cations must be exchanged by a cation-exchanger for H^{+} ions, the concentration of which is then determined alkalimetrically. When the total concentration of both cations in the solution is known, they can be determined separately with the help of calibration curves, drawn from the measured absorbances of various known mixtures of the two cations at a definite wavelength. The total concentration of cations in the solutions used for calibration has to be exactly equal to that of the solution under investigation. Absorbances have to be determined at a wavelength at which the greatest possible difference exists between the absorbances due to the separate cations. Moreover, the values of the absorbances must be great enough to give the required accuracy.

Quotient φ

Buddecke and *Drzeniek* [13] following the method of *Ettori* and *Scoggan* [22] constructed calibration curves for the system $\text{CaCl}_2\text{--KCl}$ by measuring absorbances at two wavelengths, corresponding to the maximum absorbances of murexide in the presence of CaCl_2 (λ_{Ca}) and KCl (λ_{K}), respectively. They computed the quotient $\varphi = A_{\lambda_{\text{Ca}}}/A_{\lambda_{\text{K}}}$ on the basis of measured absorbances. Then they drew the calibration curve as the relationship between the quotient φ and the concentration of Ca^{2+} ions in solution; $\varphi = f[\text{Ca}^{2+}]$.

This procedure [22, 13] has the following advantages, when the absorbance of a particular solution is measured at two wavelengths in the same cell: 1. The calculation of the quotient φ , which is given by the ratio of both absorbances, eliminates incidental small differences in the path-lengths of individual cells. 2. The stock solution of tetramethylmurexide need not to be measured especially carefully into the test solution. 3. The error due to slow decomposition of tetramethylmurexide in the course of measurement lessens. The tetramethylmurexide is subject to decomposition under the weakly alkaline conditions of the experiment. If a small amount decomposes, a small and approximately proportional decrease in the absorbances determined at two different wavelengths occurs, but the ratio of these absorbances (the quotient φ) does not change significantly [22].

We prefer, therefore, to construct the calibration curve by means of the quotient φ than to use absorbances measured only at one wavelength.

In highly dilute solutions (e.g. 0.002 M) the absorption spectra of SrCl_2 and BaCl_2 or BaCl_2 and MgCl_2 differ from each other by only about 6 nm (Fig. 2). It would therefore seem that strontium and barium or barium and magnesium in pairs cannot be determined in these solutions. Fig. 3 shows the absorbance spectra of tetramethylmurexide in 0.002 M solutions of SrCl_2 (curve 1) and BaCl_2 (curve 2). The absorbances measured at wavelengths corresponding to the maximum (V_1 , V_2) cannot be used

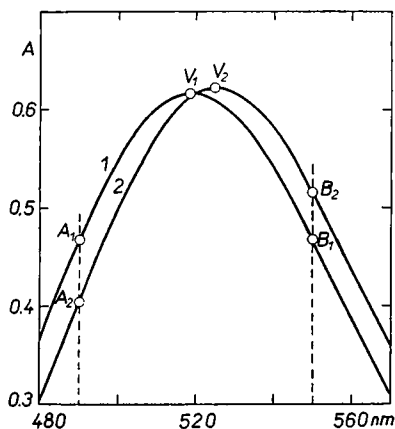


Fig. 3. Absorption spectrum of tetramethylmurexide in 0.002 M solutions of SrCl_2 and BaCl_2 . (Determination of quotient φ .)

1. SrCl_2 ; 2. BaCl_2 .

V_1 and V_2 — maximum absorbance for solution of SrCl_2 or BaCl_2 , respectively.

A_1 , A_2 — absorbances at 490 nm; B_1 , B_2 — absorbances at 550 nm.

for computing the quotient φ in the same manner as performed by *Buddecke* and *Drzeniek* [13], because the absorbance changes very little with varying concentration of both cations. The quotient φ must therefore be determined for wavelengths situated at both sides of the maxima of curves, e.g. at 490 and 550 nm. With increasing proportion of Sr^{2+} ions in the mixture, the absorbance A_{490} rises from the limiting value A_2 to a limiting value A_1 while the absorbance A_{550} falls from the limiting value B_2 to the limiting value B_1 . Thus, the quotient $\varphi = A_{490}/A_{550}$ grows with increasing proportions of Sr^{2+} ions in solution to such an extent that the concentrations of Sr^{2+} and Ba^{2+} ions can still be determined with sufficient accuracy. On the other hand, the small difference in absorption spectra of dilute solutions containing SrCl_2 and BaCl_2 , BaCl_2 and MgCl_2 (or BaCl_2 and KCl , respectively), requires a considerable accuracy in the determination of the quotient φ .

Conditions for exact determination of the quotient φ

Tetramethylmurexide has a great advantage over murexide in that the absorbances of solutions containing the complexes of tetramethylmurexide with metals are independent of pH within the range 4.8–8.1 [14]. We have tested experimentally this property of tetramethylmurexide. The values of φ , determined in the solutions of tetramethylmurexide (4×10^{-5} mol l⁻¹) containing 0–3 millimoles Ca/1000 ml with ionic strength $\mu = 0.15$ (KCl) have been constant and independent of pH within the range 4.7–8.7 [15].

Stability of tetramethylmurexide solution

The solution of tetramethylmurexide is not stable and decomposes slowly, especially in weakly alkaline solutions. It is, therefore, necessary to prepare the stock solution of tetramethylmurexide immediately before use. The stability of tetramethylmurexide solution (4×10^{-5} mol l⁻¹) in distilled water and in 0.025 M, unbuffered CaCl_2 , SrCl_2 and BaCl_2 (pH 5.8–6.2) was studied by measuring the changes in absorbance at 500 and 530 nm as a function of time. The values of the quotient φ for the salt solutions were constant within the limits of error for 4 hours, and thereafter decreased slowly. In distilled water without added salts, they were constant for two hours, and then decreased significantly, until after 4 hours, the total decrease was about 1%. If the analysis is, therefore, carried out immediately after the preparation of the solution of tetramethylmurexide, the time interval not exceeding 4 hours, the slow decomposition of tetramethylmurexide is not significant. The rate of decomposition is, however, much more significant in weakly alkaline solutions. At the limiting value of pH 8.7, the tetramethylmurexide decomposition is already rather fast. Correct values can be obtained in this case only when the analysis is carried out within one hour. From the point of view of accuracy, the pH of calibration solutions as well as analysed samples should be within the range 5.0–7.0. (Note: According to *Spare* [21], glycerol, methanol and propyleneglycol retard murexide decomposition in alkaline solutions at pH 12.)

Adjustment of the wavelength

In most cases, the absorbance measurements are performed at a wavelength where the absorbance curve is steep. A small alteration of wavelength (e.g. 1 nm) corresponds to a considerable change of absorbance which also causes a change in

the quotient φ (Fig. 3). For instance, the determination of the absorbance A_{490} with an error of ± 0.001 requires an adjustment of wavelength with a precision of about ± 0.1 nm. Such a precise adjustment of wavelength, cannot be achieved with the spectrophotometer, but it is easy to overcome this difficulty. Absorbances of both calibration solutions and analysed solutions must be measured first at the first wavelength, e.g. 490 nm (with only one adjustment of wavelength) and subsequently at the second wavelength, e.g. 550 nm. In this way, the absorbance of the analysed solutions are measured at exactly the same wavelengths as those of the calibration solutions. The absorbance measurements with a given solution must be carried out for both wavelengths with the same cell.

Accurate measurements require that the calibration curve be drawn afresh for each series of analysed samples. The values of the absorbances were read twice with an error of less than 0.001. Under the above conditions, the results are quite reproducible. It followed from a great number of analyses (more than 100) that the quotient φ may be determined with considerable accuracy, the error being less than ± 0.0025 .

Influence of ionic strength of solution

The absorbance of solutions containing tetramethylmurexide complexes is affected by the *activity* of ions present in solution in addition to the indicator concentration. (This topic has been dealt with in more detail in one of our preceding papers [15], in which it has been demonstrated that tetramethylmurexide can be used for the determination of Ca^{2+} ion activity in solutions containing biological substances.) It is, therefore, necessary for both calibration solutions and analysed solutions to have the same ionic strength.

Determination of two cations in the presence of each other

Accuracy of analysis

The calibration curves, shown later in this paper were determined by using solutions of the chlorides of the various metals. For two cations where absorption spectra were quite different (e.g. Ca^{2+} — Mg^{2+}), the absorbances were determined at wavelengths of maximum absorbance, whereas they were determined at wavelengths on either side of the absorbance maxima for the other systems. The wavelengths were chosen according to the type of cation pair and the concentration of the solution. The wavelengths, used for every particular absorbance measurement, are given in subsequent tables.

The estimates of analytical error for all cation pairs are based on the assumption that the overall cation concentration in solution is known. The correct relative error in the determination of the concentrations of the individual cations is then determined by the slope of the calibration curve for the cation-ratio, considered in relation to the error in the determination of the quotient φ . This error ($\Delta\varphi$) was assumed to be ± 0.0025 for all measurements. The application of the method has confirmed the correctness of the experimental error determined in this way.

Determination of calcium and magnesium in the presence of each other

The calibration curve for mixtures of solutions of calcium and magnesium chlorides having an overall concentration of 0.05 mol l^{-1} is shown in Fig. 4 (curve 1), while the corresponding curve for an overall concentration of 0.092 mol l^{-1} is given in

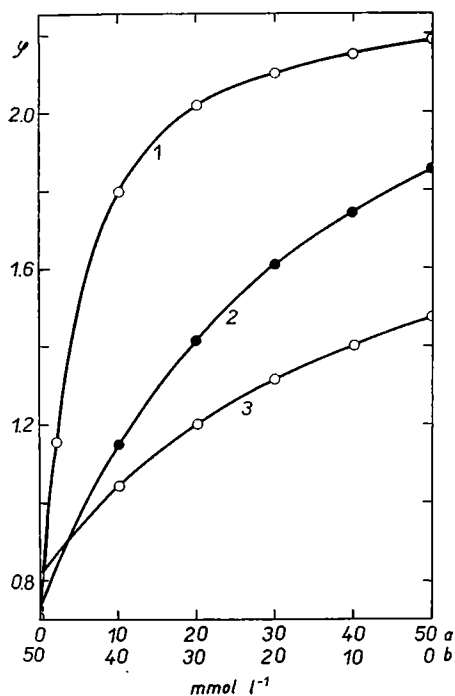


Fig. 4. Calibration curves for the determination of cation pairs Ca-Mg, Ca-Ba and Ca-Sr (0.05 M solutions).

1. Ca-Mg, $\varphi = A_{495}/A_{530}$;
a) [Ca], b) [Mg].
2. Ca-Ba, $\varphi = A_{490}/A_{525}$;
a) [Ca], b) [Ba].
3. Ca-Sr, $\varphi = A_{480}/A_{520}$;
a) [Ca], b) [Sr].

Fig. 5. Calibration curves for the determination of cation pairs Ca-Mg, Ca-Ba and Ca-Sr (0.002 M solutions).

1. Ca-Mg, $\varphi = A_{495}/A_{530}$;
a) [Ca], b) [Mg].
2. Ca-Ba, $\varphi = A_{495}/A_{525}$;
a) [Ca], b) [Ba].
3. Ca-Sr, $\varphi = A_{485}/A_{530}$;
a) [Ca], b) [Sr].

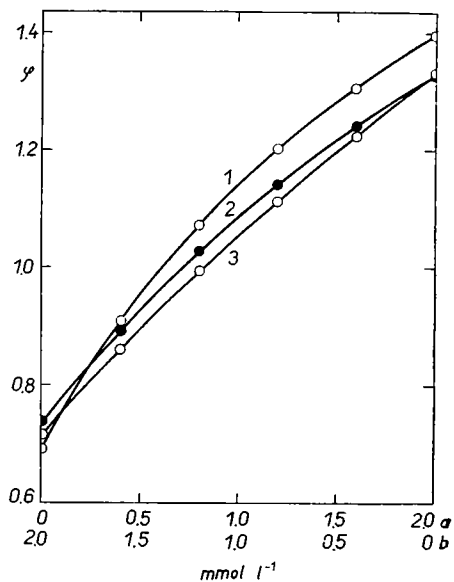


Fig. 5 (curve 1). The considerable curvature of the calibration curve at higher calcium content (Fig. 4) is due to a relatively high value of the stability constant of the tetramethylurexide complex of calcium.

As magnesium does not form any complex with tetramethylurexide, and the solutions of both elements show a considerable difference in absorption spectra (Figs. 1 and 2), calcium and magnesium may be determined in the presence of each other within a broad range of concentrations (0.002–0.05 M). Calcium alone can be determined in the presence of a considerable excess of magnesium (ratio 1 : 100).

Table 3
Determination of calcium and magnesium in the presence of each other

Ca [mmol l ⁻¹]	Mg [mmol l ⁻¹]	Ca : Mg	± ΔCa [%]	± ΔMg [%]	φ
0.40	1.60	1 : 4	1.4	0.4	
1.00	1.00	1 : 1	0.8	0.8	<i>A</i> ₄₉₅ / <i>A</i> ₅₃₀
1.60	0.40	4 : 1	0.6	2.5	
0.50	50.0	1 : 100	1.9		
1.00	50.0	1 : 50	1.1		
1.50	50.0	1 : 33	0.9		<i>A</i> ₄₉₅ / <i>A</i> ₅₃₀
2.00	50.0	1 : 25	0.7		
5.0	45.0	1 : 9	0.6	0.1	
10.0	40.0	1 : 4	0.7	0.2	
25.0	25.0	1 : 1	1.3	1.3	<i>A</i> ₄₉₅ / <i>A</i> ₅₃₀
40.0	10.0	4 : 1	1.5	5.9	

The errors of analyses for various calcium and magnesium concentrations are given in Table 3. Magnesium determination in an excess of calcium (ratio 1 : 4) in 0.05 M solution is already less accurate owing to the low slope of the calibration curve in the region of high calcium concentration (ΔMg = ±5.9%).

Determination of calcium and barium in the presence of each other

Likewise, calcium and barium can be determined in the presence of each other with great accuracy. The calibration curve for 0.05 M solutions is shown in Fig. 4 (curve 2), while the calibration curve for 0.002 M solutions is in Fig. 5 (curve 2).

Table 4
Determination of calcium and barium in the presence of each other

Ca [mmol l ⁻¹]	Ba [mmol l ⁻¹]	Ca : Ba	± ΔCa [%]	± ΔBa [%]	φ
0.40	1.60	1 : 4	1.8	0.5	
1.00	1.00	1 : 1	0.9	0.9	<i>A</i> ₄₀₅ / <i>A</i> ₅₂₅
1.60	0.40	4 : 1	0.7	2.7	
0.50	50.0	1 : 100	9.1		
1.00	50.0	1 : 50	4.6		
1.50	50.0	1 : 33	3.0		<i>A</i> ₄₀₅ / <i>A</i> ₅₃₀
2.00	50.0	1 : 25	2.3		
10.0	40.0	1 : 4	0.8	0.2	
25.0	25.0	1 : 1	0.5	0.5	<i>A</i> ₄₉₀ / <i>A</i> ₅₂₅
40.0	10.0	4 : 1	0.5	2.1	

The errors of analyses for various concentrations of calcium and barium are given in Table 4. In contrast to magnesium, barium forms a complex with tetramethylmurexide. Calcium determination in the presence of a large excess of barium ($\text{Ba} : \text{Ca} > 30 : 1$) is, therefore, less accurate ($\Delta\text{Ca} > \pm 3\%$).

*Determination of calcium and strontium
in the presence of each other*

Absorption spectra of CaCl_2 and SrCl_2 solutions are situated rather close together (Figs. 1 and 2). For this reason, the calibration curves are less steep. The calibration curves are shown in Fig. 4 (curve 3) and in Fig. 5 (curve 3) for 0.05 M and 0.002 M solutions, respectively. The errors of analyses for various concentrations of calcium and strontium in solution are given in Table 5. Calcium can be determined with sufficient accuracy ($\Delta\text{Ca} = \pm 3\%$) even in the presence of excess of strontium (1 : 20).

Table 5

Determination of calcium and strontium in the presence of each other

Ca [mmol l ⁻¹]	Sr [mmol l ⁻¹]	Ca : Sr	$\pm \Delta\text{Ca}$ [%]	$\pm \Delta\text{Sr}$ [%]	φ
0.40	1.60	1 : 4	1.8	0.5	A_{485}/A_{530}
1.00	1.00	1 : 1	0.9	0.9	
1.60	0.40	4 : 1	0.6	2.4	
0.50	20.0	1 : 40	5.8		A_{480}/A_{530}
1.00	20.0	1 : 20	3.2		
1.50	20.0	1 : 13	2.3		
2.00	20.0	1 : 10	1.9		
10.0	40.0	1 : 4	1.4	0.4	A_{480}/A_{520}
25.0	25.0	1 : 1	0.9	0.9	
40.0	10.0	4 : 1	0.8	3.2	

*Determination of strontium and magnesium
in the presence of each other*

The calibration curves are given in Fig. 6 (curve 1) and in Fig. 7 (curve 1) for 0.05 M and 0.002 M solutions, respectively. According to results given in Table 6, strontium can be determined even in the presence of a large excess of magnesium (1 : 50) with an error of $\pm 3.6\%$. The magnesium determination is, however, less accurate when the strontium is present in a 4-fold excess ($\Delta\text{Mg} = \pm 4$ up to $\pm 5\%$).

*Determination of strontium and barium
in the presence of each other*

The absorption spectra of SrCl_2 and BaCl_2 solutions are rather close, especially for 0.002 M solutions (Fig. 2). The calibration curve is not too steep in this concentration region and a very accurate determination of the quotient φ is therefore needed. The calibration curves are shown in Fig. 6 (curve 3) and in Fig. 7 (curve 3)

Fig. 6. Calibration curves for the determination of cation pairs Sr—Mg, Ba—Mg and Sr—Ba (0.05 M solutions).

1. Sr—Mg, $\varphi = A_{500}/A_{540}$;
a) [Sr], b) [Mg].
2. Ba—Mg, $\varphi = A_{510}/A_{560}$;
a) [Ba], b) [Mg].
3. Sr—Ba, $\varphi = A_{490}/A_{540}$;
a) [Sr], b) [Ba].

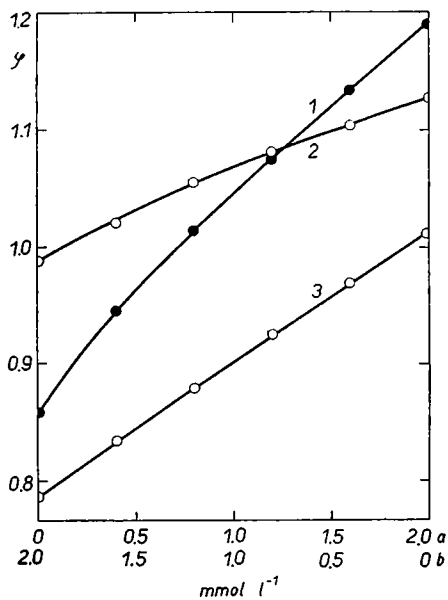
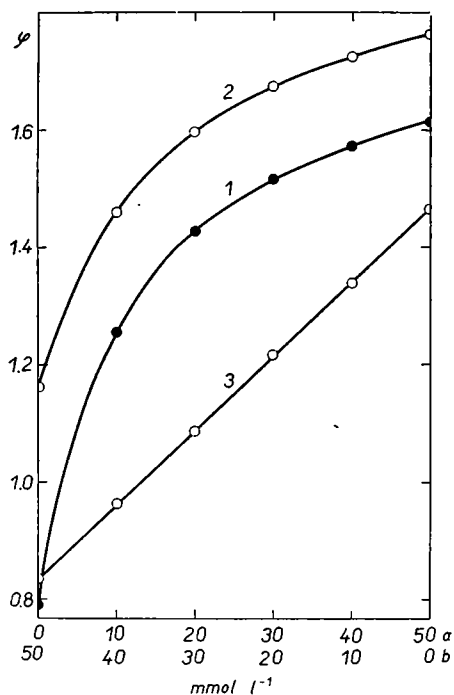


Fig. 7. Calibration curves for the determination of cation pairs Sr—Mg, Ba—Mg and Sr—Ba (0.002 M solutions).

1. Sr—Mg, $\varphi = A_{500}/A_{550}$;
a) [Sr], b) [Mg].
2. Ba—Mg, $\varphi = A_{500}/A_{560}$;
a) [Ba], b) [Mg].
3. Sr—Ba, $\varphi = A_{490}/A_{550}$;
a) [Sr], b) [Ba].

Table 6

Determination of strontium and magnesium in the presence of each other

Sr [mmol l ⁻¹]	Mg [mmol l ⁻¹]	Sr : Mg	$\pm \Delta Sr$ [%]	$\pm \Delta Mg$ [%]	φ
0.40	1.60	1 : 4	3.3	0.8	A_{500}/A_{550}
1.00	1.00	1 : 1	1.6	1.6	
1.60	0.40	4 : 1	1.1	4.4	
1.00	50.0	1 : 50	3.6		A_{500}/A_{540}
2.50	50.0	1 : 20	1.9		
5.00	50.0	1 : 10	1.2		
7.50	50.0	1 : 7	1.1		
10.00	50.0	1 : 5	1.0		
10.0	40.0	1 : 4	0.9	0.2	
25.0	25.0	1 : 1	1.1	1.1	A_{500}/A_{540}
40.0	10.0	4 : 1	1.3	5.3	

for 0.05 M and 0.002 M solutions, respectively. Since both cations form with tetramethylurexide complexes having rather similar stability constants, it is not possible, in contrast to other analyses, to determine one of these cations in the presence of a greater amount of the other. The errors of analysis are given in Table 7. In dilute solutions 0.002 M, the error in strontium determination for a ratio of strontium to barium of 1 : 4 is $\pm 5.5\%$. Likewise, the error in barium determination for a ratio of strontium to barium of 4 : 1 is $\pm 5.7\%$.

Table 7

Determination of strontium and barium in the presence of each other

Sr [mmol l ⁻¹]	Ba [mmol l ⁻¹]	Sr : Ba	$\pm \Delta Sr$ [%]	$\pm \Delta Ba$ [%]	φ
0.40	1.60	1 : 4	5.5	1.4	A_{490}/A_{550}
1.00	1.00	1 : 1	2.2	2.2	
1.60	0.40	4 : 1	1.4	5.7	
10.0	40.0	1 : 4	2.0	0.5	A_{490}/A_{540}
25.0	25.0	1 : 1	0.8	0.8	
40.0	10.0	4 : 1	0.5	2.0	

*Determination of barium and magnesium
in the presence of each other*

The calibration curves are presented in Fig. 6 (curve 2) and in Fig. 7 (curve 2) for 0.05 M and 0.002 M solutions, respectively. The calibration curve is rather flat for 0.002 M solutions of barium and magnesium chloride, due to a very small difference in their absorption spectra, especially in the wavelength region over 530 nm (Fig. 2). The errors of analysis are given in Table 8.

Table 8

Determination of barium and magnesium in the presence of each other

Ba [mmol l ⁻¹]	Mg [mmol l ⁻¹]	Ba : Mg	$\pm \Delta Ba$ [%]	$\pm \Delta Mg$ [%]	φ
0.40	1.60	1 : 4	7.4	1.9	
1.00	1.00	1 : 1	3.8	3.8	A_{500}/A_{560}
1.60	0.40	4 : 1	2.6	10.3	
1.00	50.0	1 : 50	6.2		
2.50	50.0	1 : 20	3.0		
5.00	50.0	1 : 10	2.0		A_{500}/A_{560}
7.50	50.0	1 : 7	1.6		
10.00	50.0	1 : 5	1.4		
10.0	40.0	1 : 4	1.3	0.3	
25.0	25.0	1 : 1	1.3	1.3	A_{510}/A_{560}
40.0	10.0	4 : 1	1.4	5.5	

A sufficient accuracy can still be achieved in 0.05 M solutions. Since magnesium does not form any complex with tetramethylmurexide, it is possible to determine barium in the presence of a rather large excess of magnesium (ratio 1 : 20) with an error $\Delta Ba = \pm 3.0\%$.

The determination of barium and magnesium in the presence of each other in 0.002 M solutions is the least accurate of the determinations of cation pairs taken from the series Ca^{2+} , Sr^{2+} , Ba^{2+} and Mg^{2+} . When the ratio of barium and magnesium is 1 : 4 or the reverse (*i.e.* 4 : 1), the determination of the cation with the smaller concentration is then impaired with a considerable error ($\Delta Ba = \pm 7.4\%$; $\Delta Mg = \pm 10.3\%$). The error of determination is $\pm 3.8\%$ for both cations if these are present in a ratio of 1 : 1.

*Determination of calcium, strontium and barium
in the presence of potassium*

Potassium does not form any complex with tetramethylmurexide. The absorption spectrum of tetramethylmurexide in 0.004–0.15 M solutions of potassium chloride is identical with that of tetramethylmurexide in magnesium chloride solutions. It does not depend on the potassium chloride concentration within the stated limits. The conclusions, valid for the determination of calcium, strontium and barium in the presence of magnesium, hold also for the determination of these cations in the presence of potassium. The Ca^{2+} , Sr^{2+} and Ba^{2+} cations can be determined accurately in the presence of a very large excess of K^+ ions.

It follows from the above results that by using tetramethylmurexide as an auxiliary ligand, it is possible to determine two arbitrary elements of the series magnesium (or potassium), calcium, strontium and barium coexisting in pairs in solutions the concentration range of which is rather broad. Although method has been developed for pure systems, it may also be extended to systems containing other adventitious salts not interfering with the tetramethylmurexide. In such a case, however,

the concentration of non-interfering salts must be the same in both calibration and investigated solutions, because the ionic strength of the investigated solutions must equal the ionic strength of calibration solutions. The described method has proved to be fully suitable for the study of the binding of calcium, strontium and barium to the carboxyl groups of pectin.

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