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A NEW AND EASY METHOD FOR THE POTENTIOMETRIC DETERMINATION OF CALCIUM CONCENTRATIONS IN SOLUTIONS

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It has been shown in a previous paper (1) that minerals can be used as materials for electrodes. I shall now describe more in detail researches on fluorite as a calcium electrode.

Fluorite, CaF_2, is a mineral which can be obtained in beautiful crystals. From a crystal a thin plate is split off, which is sealed on a glass tube by means of sealing-wax or some other substance not reacting with water. After being sealed the plate is ground and polished to make it as thin as possible, care being taken that the plate does not become porous. The tube is then placed for 24 hours with the open end in a beaker of water; if the water rises in the tube, the electrode is porous.

Inside the tube is placed a solution of calcium chloride, *e.g.* 0.05 M; in this solution a wire of silver, covered with silver chloride, is placed. The silver-silver chloride electrode was prepared as follows:

A platinum wire is covered by a silver layer by electrolysis (4 volts, 1 milliampere) of a solution obtained by mixing equal volumes of 13 per cent KCN solution and 18 per cent $AgNO_3$ solution. After washing, the silver-plated platinum wire is electrolytically covered with a thin layer of AgCl, a 1 N solution of HCl and a current of 3.5 milliamperes being used for 20 minutes. Then the electrode is thoroughly washed with hot distilled water.

The calcium electrode is then checked in solutions of $Ca(NO_3)_2$. The standard solution of calcium nitrate was analyzed by oxidimetric titration with $KMnO_4$ of the calcium oxalate precipitated in the ordinary way.

From the standard solution dilutions were made and the E.M.F. of the cell

$$Ag, AgCl \mid CaCl_2 \mid CaF_2 \mid Ca(NO_3)_2 \mid KCl \text{ (saturated)} \mid KCl \text{ (0.1 M)}, Hg_2Cl_2 \mid Hg$$

was measured at room temperature.

The E.M.F. was measured with a Leeds and Northrup Students' potentiometer. Since the resistance is very great, it is necessary to use vacuum tubes. We used two tubes and a scheme given by Janssen (2). The first tube was a Philips No. 4060; the anode current of the second tube (Philips No. B-405) was counteracted by a current so as to make the galvanometer a null point indicator.

When measurements are to be made, it is necessary first of all

TABLE I

Relation between Electromotive Force and Calcium Concentration

Concentration of Ca nitrate	pCa = − log c	E.M.F. of cell
m.-eq. per l.		*mv.*
1000	0	68.5
100	1	54.5
10	2	41.0
1	3	28.5
0.1	4	13.5
0.01	5	12.0
0.001	6	12.0

to check the CaF_2 electrode with known calcium nitrate solutions. The equilibrium is attained in a few minutes. By way of example I give the results with Electrode 35 in Table I.

Fig. 1 shows the curve of the E.M.F. of the cells plotted against the values of pCa. There is a linear relation between pCa and the E.M.F. but deviations occur at pCa 5 and higher. This is probably due to the solubility of CaF_2 itself.

The solubility product is

$$[Ca^{++}]\,[F^-]^2 = 3.4 \times 10^{-11} \text{ (at } 18°)$$
$$[Ca^{++}] = 3.2 \times 10^{-4}$$

Thus in a saturated solution of CaF_2 the calcium concentration is 0.64 milli-equivalent per liter. This means that the CaF_2 elec-

trode itself, placed in solutions of calcium nitrate weaker than 0.64 milli-equivalent per liter, gives rise to a higher concentration which remains practically constant, thus making all measurements misleading. Therefore, it is impossible to use concentrations weaker than about 1 milli-equivalent per liter.

As it is of great importance to know the distribution of calcium in protein sols, we tried the method in the study of gelatin sols and milk.[1] The adsorption of calcium by gelatin has been studied by Eversole, Ford, and Thomas (3).

Isoelectric gelatin, prepared from the purest gelatin of the Lym-en Gelatinefabriek, Delft, was dissolved and by adding hydrochloric acid or sodium hydroxide was brought to different pH

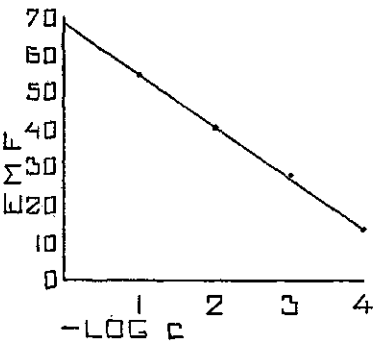

FIG. 1. Relationship between E.M.F. and calcium concentration

values. The pH was measured with a glass electrode; the glass was from the Corning Glass Works, made according to the specifications of MacInnes.

The measurements were made with 1 per cent gelatin sols of pH 4.32, 4.55, and 5.40. First of all, it was found that the CaF_2 electrode showed exactly the same potential in each of these three gelatin sols. This means that the potential of this electrode is not influenced by hydrogen ions.

The mixtures of gelatin and calcium nitrate were made by adding 25 cc. of calcium nitrate solution to 25 cc. of gelatin sol. In a few minutes a constant E.M.F. is obtained; after each measurement the electrode is thoroughly rinsed with distilled water.

[1] These experiments on gelatin and milk were carried out in collaboration with Mr. B. M. Krol.

The measured E.M.F. of the cell gives the pCa = $-\log c$, which can be read from the curve of Fig. 1. It is necessary to standardize every time before a series of measurements; sometimes small

TABLE II

Calcium-Binding Power of Gelatin

$Ca(NO_3)_2$ added	Gelatin, pH 5.40			Gelatin, pH 4.55			Gelatin, pH 4.32		
	pCa	Ca concentration	Ca bound by 10 gm. gelatin	pCa	Ca concentration	Ca bound by 10 gm. gelatin	pCa	Ca concentration	Ca bound by 10 gm. gelatin
m.-eq. per l.		*m.-eq. per l.*	*m.-eq.*		*m.-eq. per l.*	*m.-eq.*		*m.-eq. per l.*	*m.-eq.*
364	1.10	89.1	274.9	0.85	158.5	205.5	0.60	251.2	112.8
	0.97			0.70			0.57		
182	1.30	56.2	125.8	0.95	125.9	56.1	0.85	141.2	40.8
	1.20			0.83			0.80		
91	1.50	31.6	59.4	1.10	79.4	11.6	1.15	79.4	11.6
	1.45			1.05			1.05		
36.4	1.87	12.6	23.8	1.47	31.6	4.8	1.57	31.6	4.8
	1.93			1.55			1.45		
18.2	2.33	5.0	13.2	1.93	12.6	5.6	1.87	14.1	4.1
	2.25			1.87			1.85		
9.1	2.50	3.2	5.9	2.25	5.6	3.5	2.13	7.7	2.0
	2.45			2.20			2.17		
7.3	2.80	1.6	5.7	2.45	3.6	3.7	2.33	4.5	2.8
	2.80			2.40			2.40		

TABLE III

Influence of Gelatin Concentration on Calcium Adsorption

Concentration of gelatin	pCa	Ca concentration	Ca bound	Ca bound per gm. gelatin
per cent		*m.-eq. per l.*	*m.-eq. per l.*	*m.-eq.*
2½	1.35	44.7	455.3	18.2
2	1.20	63.1	436.9	21.8
1½	0.90	125.9	374.1	24.9
1	0.80	158.5	341.5	34.1
½	0.50	316.2	183.8	37.1

differences may occur, depending on the room temperature. All determinations have been made twice. From the figures for pCa one sees that the reproducibility is very good. The results are presented in Table II.

From these results it follows that calcium is bound or adsorbed by gelatin. As it is well known that at pH >4.7 the gelatin is negative, the binding of calcium will also be greater than at pH <4.7, where gelatin is positive. From the figures it is clear that the binding of calcium becomes less on lowering the pH. The amount of calcium which is adsorbed by gelatin is fairly great.

Also the influence of the concentration of gelatin, the concentration of calcium nitrate being kept constant at 500 milli-equivalents per liter, has been studied. The results are given in Table III.

From these results it follows that the adsorption of calcium per gm. of gelatin is greater the smaller the gelatin concentration. This fact could be expected *a priori*.

Attention was paid next to the adsorption of calcium in milk. The amount of soluble calcium varies, according to Van Slyke and Bosworth (4) between 0.0343 and 0.0734 per cent. The amount of soluble calcium is that part of the total calcium content present which can be measured with the CaF_2 electrode. This amount must be practically equal to the amount of calcium in the milk serum. First of all I determined the pH and pCa of a sample of fresh milk; afterwards the milk was filtered through a collodion membrane, and the pH and pCa of the ultrafiltrate were again determined. The following results were obtained.

	pH	pCa	Calcium	
			m.-eq. per l.	*per cent*
Whole milk	6.63	1.47	33.9	0.068
Milk serum	6.73	1.45	35.5	0.071

Though the calcium concentrations are not exactly the same, it is justifiable to regard them as equal. This control proves that the method is reliable.

The pH of milk serum is higher than the pH of whole milk. This must be ascribed to the well known "suspension effect" of Wiegner. It might be possible that on further study of the calcium concentrations under different conditions a suspension effect for this ion will be found.

Next, different amounts of calcium nitrate were added to milk. The mixtures were made by mixing 10 cc. of milk and 10 cc. of a

calcium nitrate solution; also a mixture of 10 cc. of milk and 10 cc. of water was made. Similar measurements were made with the same sample of milk which had been previously acidified with

TABLE IV

Calcium-Binding Power of Milk

A is the concentration of calcium in milk diluted with 1 volume of water, as measured with the CaF_2 electrode; *B*, the amount of calcium, added as nitrate; *C*, the concentration of calcium in the mixtures with calcium nitrate, as measured with the electrode (*A*, *B*, and *C* are expressed in milli-equivalents per liter). Thus $C - A$ is the increase of the calcium concentration in the intermicellar liquid, and $B - (C - A)$ is the amount of calcium adsorbed.

$Ca(NO_3)_2$ added (*B*)	Fresh milk					Acidified milk				
	pH	pCa	Ca concentration found (*A*)	$C - A$	$B - (C - A)$	pH	pCa	Ca concentration found (*A*)	$C - A$	$B - (C - A)$
m.-eq. per l.			*m.-eq. per l.*					*m.-eq. per l.*		
0	7.14	1.80	15.8			5.98	1.63	25.1		
	7.20	1.80				5.93	1.60			
			(*C*)					(*C*)		
364	5.78	0.83	147.9	132.1	231.9	4.80	0.60	251.2	226.1	137.9
	5.78	0.83				4.78	0.58			
182	5.93	1.10	79.4	63.6	118.4	5.00	0.83	141.2	116.1	65.9
	6.00	1.08				5.00	0.88			
91	6.23	1.28	52.5	36.7	54.3	5.15	1.08	89.1	64.0	27.0
	6.15	1.25				5.18	1.00			
36.4	6.55	1.65	25.1	9.3	26.7	5.45	1.40	39.8	14.7	21.7
	6.63	1.58				5.38	1.35			
18.2	6.85	1.80	15.8	0	18.2	5.55	1.60	25.1	0	18.2
	6.85	1.78				5.63	1.58			
9.1	6.98	1.80	15.8	0	9.1	5.85	1.60	25.1	0	9.1
	7.05	1.80				5.70	1.60			
7.3	6.98	1.80	15.8	0	7.3	5.80	1.60	25.1	0	7.3
	7.05	1.80				5.80	1.60			

lactic acid. All mixtures were made twice. In Table IV the results are presented. From these data it follows that the reproducibility of the measurements is very good. The mean values of pCa are used in the calculations.

The adsorption of calcium is less in the acidified milk. Small amounts of calcium added are completely adsorbed. From Table I it can be seen that between 100 and 10 milli-equivalents every 10 milli-equivalents correspond with 1.35 millivolts, and between 10 and 1 milli-equivalents every milli-equivalent corresponds with 1.25 millivolts. Therefore, a difference in adsorption by adding 18.2 or 7.3 milli-equivalents of calcium nitrate per liter respectively would have been detectable if the adsorption had not been complete. The fact that no difference occurs proves that the added calcium is completely adsorbed.

It is open to discussion whether the calcium is adsorbed, or bound, by casein or the phosphates. In every case we may conclude that milk behaves as a calcium buffer.

SUMMARY

A method has been developed to determine potentiometrically concentrations of calcium in solutions and in protein sols. The CaF_2 electrode can be used at calcium concentrations of 1 milli-equivalent per liter and higher.

It has been shown that calcium is adsorbed by proteins and that the adsorption decreases with increasing acidity of the protein sols.

The use of CaF_2 as an electrode provides the great advantage that no foreign substances are introduced into the systems to be studied, as is the case when electrodes of the second or third order are used.

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