

*Citation:*

R. Brinkman & E. van Dam, A method for the determination of the ion concentration in ultra filtrated and other protein free solutions, in:

KNAW, Proceedings, 22 II, 1920, Amsterdam, 1920, pp. 762-771

**Physiology.** — “*A method for the determination of the ion concentration in ultra filtrates and other protein free solutions*”.  
 By Dr. R. BRINKMAN and Miss E. VAN DAM (Communicated by Prof. HAMBURGER).

(Communicated at the meeting of October 25, 1919).

*A. Determination of the concentration of free calcium ions.*

With regard to the biological actions of salts the actions of ions claim the first consideration. It is therefore desirable that we have at our disposal a method by which the ion concentrations are measured.

Up to this only the concentration of the free H<sup>+</sup>-ions have been measured directly; the concentrations of other, also physiologically important ions were not measured at all or determined only indirectly by calculation.

The concentration chain method can be applied only with great difficulty to the physiologically important metals owing to the disturbances brought about by the liberation of gas. DRUCKER<sup>1)</sup> has offered a method in which Ba-amalgam was used as an electrode. An analogous method can perhaps be worked out for the alkali metals. Such determinations have, however, not been made as yet

As an example of a case where it is necessary to know the ion concentration, we can point to the state in which the calcium occurs in the blood. It occurs there namely in three forms: as Ca<sup>++</sup> ion, as undissociated calcium salt (Ca (HCO<sub>3</sub>)<sub>2</sub>) and as colloidal calcium-protein compound. More or less 25 % of the total quantity of calcium occurs in the latter state. According to RONA and TAKAHASHI<sup>2)</sup> the ion concentration of the calcium in the serum is determined by the equation

$$\frac{[Ca^{++}].[HCO_3^-]}{[H^+]} = k. (k = 350 \text{ on an average}).$$

For the serum which has the physiological [H<sup>+</sup>] and carbonic acid tension, this means a [Ca<sup>++</sup>] of 20—25 mgr. per L. Of the more or less 100 mgr. per L. of calcium which occurs in the serum, therefore, only  $\frac{1}{4}$  part is present in the ion form. We learn from

<sup>1)</sup> Zeitschr. für Elektrochemie 19, 804 (1913).

<sup>2)</sup> Biochem. Zeitschr. 49 p. 390.

the equation that this concentration of Ca ions is not directly dependent upon the total quantity of calcium; the concentration of the physiologically most important part of the plasma calcium is thus not governed by the amount of calcium salts present, but by the concentration of the hydrogen and bicarbonate ions.

By means of the method offered by us it is now possible in a simple way to measure directly the Ca<sup>++</sup> ion concentration. In principle the method can equally well be applied to other ions.

We started with the determination of the concentration of Ca<sup>++</sup> ions, because the results of the determination can in this case very easily be controlled by calculation.

I. *General principle of the Method. A few technical remarks.*

If in a binary electrolyte the concentration of the anion =  $C_A$ , that of the cation =  $C_k$  and that of the undissociated salt =  $C_n$ , then, according to the law of mass action, the following relation exists

$$c_A \cdot c_k = k \cdot c_n, \text{ where } k \text{ is a constant.}$$

If the electrolyte is only slightly soluble the salt is practically completely dissociated and the concentration of the undissociated part may be neglected.

If now the solubility of the slightly soluble salt =  $A$ , then  $C_A = C_k = A$ , and the product  $C_A \cdot C_k = A^2$  has a constant value (solubility product).

If this product and the concentration of one of the ions is known, the concentration of the other can therefore be calculated.

Supposing that the solution has a concentration of Ca<sup>++</sup> ions =  $C_{Ca}$ , then the concentration of the  $C_2O_4$  ions which can exist free beside these Ca<sup>++</sup> ions, maximally =  $\frac{P}{C_{Ca}}$ , if  $P$  represents the solution product of  $CaC_2O_4$ . If now still more  $C_2O_4$  be added, then the  $CaC_2O_4$  will be precipitated or will remain in supersaturated solution.

If the formation of a supersaturated solution can be avoided, then it will be noticed, that, upon the gradual addition of  $C_2O_4$  ions to the solution containing Ca<sup>++</sup> ions, at a certain moment a slight turbidity due to  $CaC_2O_4$  results. At this stage the concentration of  $C_2O_4$  ions has become so strong that the solubility product is just exceeded. The  $C_2O_4$  ion concentration is then known, and also the solution product and the Ca<sup>++</sup> ion concentration can thus be calculated. Vice versa, if we start with a known [Ca] we are able to determine the value and constancy of the solubility product.

Where this method is used therefore it is necessary to observe

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how great the  $[C_2O_4^{2-}]$  is while only the merest sign of turbidity due to  $CaC_2O_4$  can be detected. In general it can be done in the following way:

A series of small tubes, each containing 1 c.c. of a known  $CaCl_2$  solution, was taken, and to the tubes in succession quantities of oxalate solution increasing gradually with each new tube were added with a capillary pipet divided into tentousandths of c.c. The tubes were then left to themselves for from  $\frac{1}{2}$  to 1 hour and consequently it was observed in which tube the first sign of turbidity due to  $CaC_2O_4$  appeared.

It is clear that the formation of supersaturated  $CaC_2O_4$  solutions has to be avoided.

In cases where the solutions held other salts besides (e.g. RINGER solution, ultra filtrate) we have never noticed supersaturation. As a matter of fact supersaturation occurred in the case of pure solutions of  $CaC_2O_4$ . This can be avoided by setting to work in the following way:

With a capillary pipet the desired quantities of a, say 0.05, N. strong oxalate solution is brought into the dry tubes. In a waterbath the tubes are evaporated down to dryness. After this the liquid containing the  $Ca^{2+}$  is introduced into the tubes. In this way is prevented the formation of already supersaturated solutions.

For the determination of the calcium ion concentration it is moreover necessary to use tubes that are well closed with ground glass stoppers. This is necessary to keep the water free of carbonic acid or to keep a fixed carbonic acid tension constant.

It is necessary for the judging of the appearance or non-appearance of the  $CaC_2O_4$  precipitate that the tubes should be cleaned as thoroughly as possible; this can be done in the usual way (chromic acid, ABEGG's steaming process etc.).

The best way for viewing the tubes is in a box with a slit in the bottom from which the light falls through the solution. Care should be taken that the light does not fall on the eye of the observer. The Tyndall phenomenon makes it possible to appreciate the slightest turbidity. Should the solution before the experiment already evince a slight opalescence (not due to  $CaC_2O_4$ ) as is sometimes the case with serum and ultra filtrate, it is advisable to view the solutions by red light. The wavelength of this light being too great to cause refraction the opalescence is not apparent. The temperature during the experiment must of course remain constant. It is therefore best to work in a waterbath of constant temperature.

The results obtained by the above method can be controlled in

another way still, viz. by measuring the electrical conductivity of the solutions.

If to a solution containing  $\text{Ca}^{++}$  and  $\text{Cl}'$  ions,  $\text{C}_2\text{O}_4''$  and  $\text{Na}'$  ions be added, then the product  $\text{Ca}^{++} \times \text{C}_2\text{O}_4''$  cannot exceed the square of the solubility of  $\text{CaC}_2\text{O}_4$ . If too many  $\text{C}_2\text{O}_4''$  ions have been added, then undissociated  $\text{CaC}_2\text{O}_4$  must be formed. How much  $\text{CaC}_2\text{O}_4$  will be formed, if the product is exceeded by a fixed quantity of  $\text{C}_2\text{O}_4''$ ?

To a binary electrolyte with a solubility  $A$ , a salt which has an anion in common with the first is added in a concentration  $x$ . Through this the solubility of the first salt is changed to  $A'$ . The total concentration of the anion then amounts to  $A' + x$ , that of the kation to  $A'$ . The solubility product is therefore:  $A'(A' + x)$ , and because this is constant we have:

$$A'(A' + x) = A^2$$

or

$$A' = \frac{-x \pm \sqrt{4A^2 + x^2}}{2}$$

The quantity of undissociated salt which results when  $x$  Mol salt that has 1 ion in common with the first is added, therefore is:

$$A - \frac{-x \pm \sqrt{4A^2 + x^2}}{2}, \dots \dots \dots (1)$$

if  $A$  represents the solubility of the first salt.

We have now e.g. 5 c.c. of an aqueous solution of  $\text{CaCl}_2 \cdot 6 \text{aq}$ , free of  $\text{CO}_2$ , containing per litre 0.56 millimol  $\text{Ca}^{++}$  and (2  $\text{Cl}'$ ). To this there is added several times successively 0.0050 c.c. of a 0.05 N solution of  $\text{Na}_2\text{C}_2\text{O}_4$ . After every addition the conductivity is measured. The  $\text{Na}_2\text{C}_2\text{O}_4$  may here be added in solution, for here the solution may be supersaturated.

By means of the first method the value now found for the solubility product is 0.055. From this it follows that a  $\text{C}_2\text{O}_4''$  concentration of a magnitude 1 millimol corresponds to the 0.56 millimol  $\text{Ca}^{++}$ . Upon every addition of 0.0050 c.c. 0.05 N.  $\text{Na}_2\text{C}_2\text{O}_4$  to 5 cc. of a solution of  $\text{CaCl}_2 \cdot 6 \text{aq}$ . the  $\text{C}_2\text{O}_4''$  concentration increases by 0.25 mm. After 4 additions therefore the solubility product is reached. What is the relation between the total concentrations of ions during these additions?

For the first addition the total ion concentration is

$$0,56 \text{ millimol } \text{Ca}^{++} + 0,56 \text{ m.m. } (2 \text{ Cl}') = 1,12 \text{ m.m.}$$

After the first addition of 0.025 mm.  $\text{Na}_2\text{C}_2\text{O}_4$

$$0,56 \text{ Ca}^{++} + 0,56 (2 \text{ Cl}') + 0,025 \text{ C}_2\text{O}_4'' + 0,025 (2 \text{ Na}') = 1,17 \text{ m.m.}$$

Thus the total ion concentration after the 2nd addition is 1.22 m.m., after the 3rd 1.27 mm. and after the 4th 1.32 m.m. Upon the 5th addition the solubility product is exceeded. According to the deduced formula (1) the amount of undissociated  $\text{CaC}_2\text{O}_4$  formed =

$$\sqrt{0,055} - \frac{-0,025 \pm \sqrt{4 \times 0,055 + 0,025^2}}{2} = 0,0115 \text{ mm.}$$

The total ion concentration becomes thus after the 5th addition

1.32 m.m. + 0.025  $\text{C}_2\text{O}_4''$  + 0.025 (2 Na'')—0.0115 Ca''—0.0115  $\text{C}_2\text{O}_4''$  = 1.347 m.m.

The total concentration of ions therefore does not increase by 0.05 m.m. but only by 0.027 m.m.

With the 6th addition we get a value for the undissociated  $\text{CaC}_2\text{O}_4$  of:

$$\sqrt{0,055} - \frac{0,050 + \sqrt{4 \times 0,55 + 0,05^2}}{2} = 0,024 \text{ m.m.}$$

The total concentration of ions after the 6th addition is 1.372 m.m., the increase is 0.025 m.m.

After the 7th addition it is found, calculated in the same way, that the total concentration of the ions is 1.402, the increase 0.03 m.m.,

It appears thus that the ion concentration with the first 4 additions increases regularly by 0.05 m.m. From the 5<sup>th</sup> addition onward, however, it increases only by 0.025 to 0.030 m.m. If now the electrical conductivity be examined after every addition it must appear to increase also in analogy with the increase of the total concentration of ions. Should it be found now that after the first 4 additions the conductivity increases only by half of the original value, then it is a proof that the true value has been found for the solubility product.

## II. *Determination of the concentration of Calcium ions in a solution of pure $\text{CaCl}_2 \cdot 6 \text{ aq.}$*

In 8 tubes with ground stoppers are brought respectively 0.0010, 0.0015, 0.0020, 0.0025, 0.0030, 0.0035, 0.0040, 0.0045 c.c. of a 0.05 N solution of  $\text{Na}_2\text{C}_2\text{O}_4$ . Consequently the tubes are placed in a waterbath for some time till the oxalate solutions are evaporated down to dryness. Hereupon into each tube there is introduced 1 c.c. of a  $\text{CaCl}_2 \cdot 6 \text{ aq.}$  solution which contains 125 mgr. per L. After an hour the result is observed.

The solution of  $\text{CaCl}_2 \cdot 6 \text{ aq.}$  was made from a chemically pure substance (The British Drug Houses); the strength of the solution was controlled by chlorine determination. The salt was dissolved in carefully boiled distilled water. All observations were made in small tubes of 2 cc. contents with ground stoppers.

The  $\text{Na}_2\text{C}_2\text{O}_4$  solution was made from pure  $\text{Na}_2\text{C}_2\text{O}_4$  after SÖRENSEN (KAHLBAUM). It contains no water of crystallisation, is not hygroscopic and is not affected by temperatures below 200°.

It appears that the first 6 tubes have remained perfectly clear but that the tubes with 0.040 and 0.045 c.c. oxalate solution show a faint turbidity.

The solubility product was reached thus, if, on an average, 0.0375 c.c.

$\frac{N}{20}$   $\text{Na}_2\text{C}_2\text{O}_4$  solution was added to 1 c.c. of a solution of  $\text{CaCl}_2 \cdot 6 \text{ aq.}$  ;

the  $\text{CaCl}_2$ , 6 aq. solution contained 125 mgr.  $\text{CaCl}_2$ , 6 aq. or 0.57 millimol  $\text{Ca}^{++}$  per L.

The concentration of oxalate, therefore, was 0.095 m.m., the solubility product is found to be  $0.095 \times 0.57 = 0.054$  mm. per L. The temperature during all the experiments was  $20^\circ$ . Table I gives the results of a series of such experiments.

TABLE I.

| Strength of $\text{Ca}^{++}$ concentration. | Strength of the $\text{C}_2\text{O}_4^{--}$ conc. that had to be added to show just a precipitate. | Solubility product. |
|---|--|---------------------|
| 0.57 millimol                               | 0.095 m.m.   | 0.054               |
| 0.55 "                                      | 0.095 "  | 0.052               |
| 0.38 "                                      | 0.145 "  | 0.055               |
| 0.37 "                                      | 0.15 "   | 0.054               |
| 0.28 "                                      | 0.20 "   | 0.056               |
| 0.28 "                                      | 0.20 "   | 0.056               |
| 0.10 "                                      | 0.54 "   | 0.054               |
| 1.00 "                                      | 0.056 "  | 0.056               |

From this table it appears thus that, with solutions of pure  $\text{CaCl}_2$ , 6 aq. of different strengths, a constant solubility product of  $\text{CaC}_2\text{O}_4$  is found, *namely* 0.055 mm. per L.

Let this value now be controlled by the measurement of the electrical conductivity as it is described above.

The conductivity was determined in a "resistance vessel" according to HAMBURGER. The method is found described in Osmot. Druck u. Ionenlehre Bd. 1, pag. 98. The temperature was constant at  $25^\circ$ .

To 5 c.c. of a solution of  $\text{CaCl}_2$ , 6 aq. which contained 125 mgr. per L., repeated additions of a 0.05 N solution of  $\text{Na}_2\text{C}_2\text{O}_4$  were made. Subsequent to every addition the conductivity was measured after it had become constant.

The resistance of the pure  $\text{CaCl}_2$ , 6 aq. solution was

$$8.709 \times 2000 \text{ C Ohm (C = capacity of the resistance vessel).}$$

Table II gives the decrease in the resistance after every addition of oxalate solution. (See Table following page).

It is observed that after the 4<sup>th</sup> addition of oxalate the decrease in resistance is diminished to less than the half. With the 4<sup>th</sup> addition, therefore, the solubility product was reached. The  $\text{C}_2\text{O}_4$  concentration then was 0.1 millimol the  $\text{Ca}^{++}$  concentration 0.56 millimol and the product thus 0.056 millimol.

TABLE II.

| Composition of solution.  | Resistance.                | Decrease in resistance.    |
|---|----------------------------|----------------------------|
| 5 cc. $\text{CaCl}_2$ 6 aq.   | $8.709 \times 2000$ c. Ohm | —                          |
| 5 cc. $\text{CaCl}_2$ 6 aq. + 0.005 cc. $\text{Na}_2\text{C}_2\text{O}_4$ | $8.452 \times 2000$ c. Ohm | $0.257 \times 2000$ c. Ohm |
| 5 cc. $\text{CaCl}_2$ 6 aq. + 0.010 cc. $\text{Na}_2\text{C}_2\text{O}_4$ | $8.200 \times 2000$ c. Ohm | $0.252 \times 2000$ c. Ohm |
| 5 cc. $\text{CaCl}_2$ 6 aq. + 0.015 cc. $\text{Na}_2\text{C}_2\text{O}_4$ | $7.929 \times 2000$ c. Ohm | $0.271 \times 2000$ c. Ohm |
| 5 cc. $\text{CaCl}_2$ 6 aq. + 0.020 cc. $\text{Na}_2\text{C}_2\text{O}_4$ | $7.696 \times 2000$ c. Ohm | $0.233 \times 2000$ c. Ohm |
| 5 cc. $\text{CaCl}_2$ 6 aq. + 0.025 cc. $\text{Na}_2\text{C}_2\text{O}_4$ | $7.600 \times 2000$ c. Ohm | $0.096 \times 2000$ c. Ohm |
| 5 cc. $\text{CaCl}_2$ 6 aq. + 0.030 cc. $\text{Na}_2\text{C}_2\text{O}_4$ | $7.500 \times 2000$ c. Ohm | $0.100 \times 2000$ c. Ohm |
| 5 cc. $\text{CaCl}_2$ 6 aq. + 0.035 cc. $\text{Na}_2\text{C}_2\text{O}_4$ | $7.410 \times 2000$ c. Ohm | $0.090 \times 2000$ c. Ohm |

Subsequently more determinations of a similar kind were made by us, which always gave a result of 0.053—0.58, — a mean of **0.055** — for the solubility product.

Here it must be remarked still that it cannot be expected that the solubility product will just have been reached at the end of an addition; the mean value, therefore, has to be taken.

There is still the possibility that the decrease in resistance came about because the oxalate added in such large quantities did practically not dissociate completely; the way in which the decrease would take place then would not be such a sudden one. To control this the same quantities of oxalate were added to 5 c.c. of distilled water; the conductivity kept increasing proportionally to the quantities added.

We have now found by two methods which are independent of each other the constant value of **0.055** m.m. for the solubility product, when  $\text{Ca}^{++}$  and  $\text{C}_2\text{O}_4^{--}$  ions are added together.

The solubility of  $\text{CaC}_2\text{O}_4$  has been found by KOHLRAUSCH to be  $4.35 \cdot 10^{-5}$  Mol per L. ( $18^\circ$ ); the solubility product calculated from this is 0.0019 m.m. per L. and this is much below the value found by us.

KOHLRAUSCH measured the conductivity of a saturated solution of  $\text{CaC}_2\text{O}_4$ ; he therefore did not start out from the individual ions.

HERZ u. MUHS<sup>1)</sup> found by adding together the ions a value of 0.034 gram per L. for the solubility of  $\text{CaC}_2\text{O}_4$  from which follows

<sup>1)</sup> Ber. 36. 4, p. 3717.



a solubility product of **0.054**. This product thus agrees perfectly with ours.

*The determination of HERZ u. MUHS and our own determinations, by two methods, show thus conclusively that we have to reckon with a solubility product of 0.055.*

### III. Determination of the concentration of $\text{Ca}^{++}$ ions in solutions which hold other salts besides.

#### 1. The concentration of $\text{Ca}^{++}$ ions of 0.02% $\text{CaCl}_2$ 6 aq. in 0.5% $\text{NaCl}$ .

For the system  $\text{CaCl}_2 \rightleftharpoons \text{Ca}^{++} + 2 \text{Cl}^-$  the following also holds:  $\text{CaCl}_2 \rightleftharpoons K \text{Ca}^{++} \text{Cl}^{-2}$ .  $K$  can be found if the degree of ionisation  $\alpha$  of a given  $\text{CaCl}_2$  solution is known. For  $\text{Ca}(\text{NO}_3)_2$  0,1% (= 6 m.M. per L.)  $\alpha$  is 0,67<sup>1)</sup>.

We have therefore

$$[\text{Ca}(\text{NO}_3)_2] - \alpha [\text{Ca}(\text{NO}_3)_2] = K \alpha \text{Ca}^{++} \alpha^2 (\text{NO}_3)^2$$

or because

$$[\text{CaNO}_3] = [\text{Ca}^{++}] = [\text{NO}_3] = 6 \text{ m.M. per L.}$$

$$1 - 0,67 = K \times 0,67^3 (\text{NO}_3)^2$$

$$1 - 0,67 = K \times 0,67^3 \times 0,036$$

$$K = 30.$$

This is therefore the dissociation constant for  $\text{Ca}(\text{NO}_3)_2$ ; that for  $\text{CaCl}_2$  will differ very slightly from it.

For 0,02%  $\text{CaCl}_2$  6 aq. or 0,91 millimol per L., in 0,5%  $\text{NaCl}$  also holds:

$$[\text{CaCl}_2] = K \text{Ca}^{++} \text{Cl}^{-2}.$$

The conc. of  $\text{Cl}^-$  is given by the dissociation of 0,5%  $\text{NaCl}$ . Here  $\alpha = 0,82$  (Osmot. Druck u. Ionenlehre, p. 53);  $[\text{Cl}^-]$  thus becomes 7 m.M. In addition to this there is still  $[\text{Cl}^-]$  of 0,91 m.M.  $\text{CaCl}_2$ ,  $\pm$  half of which we may consider to be dissociated without committing a large error. The total  $[\text{Cl}^-]$  then becomes  $\pm 8$  m.M.

Thus

$$\text{CaCl}_2 = K (0,91 - \text{CaCl}_2) 0,064. \quad K = 30. \quad \text{CaCl}_2 = 0,60 \text{ m.m.}$$

Of 0,91 m.m.  $\text{CaCl}_2$  thus 0,06 m.m. is not dissociated while 0,31 m.m. is dissociated. The solution therefore contains 12,4 mgr. free  $\text{Ca}^{++}$  per L.

Experimentally it appears that an oxalate concentration of 0.18 m.m. is necessary before turbidity results in a solution of  $\text{NaCl}$  0.5% +  $\text{CaCl}_2$  6 aq. 0.02%. From this follows a  $[\text{Ca}^{++}]$  of  $-0.055 : 0.18 = 0.30$  m.m., or 12 mgr. per L. This determination thus is perfectly in correspondence with the calculation.

#### 2. Determination of the concentration of Calcium ions in physiological salt solutions.

In a solution of the composition:  $\text{NaCl}$  0.7%,  $\text{NaHCO}_3 \pm 0.18\%$ ,  $\text{KCl}$  0.02%, and  $\text{CaCl}_2$  6 aq. 0.040%, with a certain carbonic acid

<sup>1)</sup> Osmot. Druck u. Ionenlehre. I, p. 53.

tension which was not exactly known, the concentration of hydrogen ions was  $0.3 \cdot 10^{-7}$  (determined with neutral red after SÖRENSEN) and the concentration of bicarbonate ions 0.02 N (determined by titration with 0.01 N. HCl and methyl-orange).

From this follows for the concentration of Calcium

$$[Ca^{++}] = 350 \frac{0,3 \cdot 10^{-7}}{0,02} = 20 \text{ mgr. per L.}$$

Experimentally a  $CaC_2O_4$  turbidity resulted with a concentration 0.1 millimol oxalate. From this follows a

$$[Ca^{++}] \text{ of } 0,055 : 0,1 = 0,55 \text{ m.M.} = 22 \text{ mgr. per L.}$$

In a similar solution in which the  $[H^+]$  however was  $0.45 \cdot 10^{-7}$  and the  $[HCO_3^-] = 0,02$  N., the  $CaC_2O_4$  milkiness was seen with  $[C_2O_4^{--}] = 0,07$  m.m. Thus:

$$[Ca^{++}] = 0.055 : 0.07 = 0,8 \text{ m.m.} = 32 \text{ mgr. per L.}$$

From the calculation  $[Ca^{++}] = 350 \frac{0,45 \cdot 10^{-7}}{0,02}$  m.m. = 30 mgr. per L.

### 3. Determination of the concentration of calcium in ultra filtrate.

Human serum was centrifuged for 2 hours in ultra filters after DE WAARD <sup>1)</sup>.  $CO_2$  was passed through the ultra filtrate until  $[H^+] = 0.3 \cdot 10^{-7}$ . (This was ensured by comparing the colour of neutral red in the ultra filtrate with neutral red in a phosphate mixture, according to SÖRENSEN, which had an  $[H^+] = 0.3 \cdot 10^{-7}$ ).

A precipitate of  $CaC_2O_4$  occurred with a  $[C_2O_4^{--}]$  of 0.1 m.m. per L. From this follows a  $[Ca^{++}]$  of 0.55 m.m. or 22 mgr.  $[Ca^{++}]$  ions per L., as has also been made probable by TAKAHASHI and RONA.

An attempt to apply these measurements directly in serum often fails because the turbidity due to  $CaC_2O_4$  is very much less evident, and the opalescence which normally occurs so often in serum is a drawback.

In the limited number of instances where we could notice a definite turning point the same concentrations of  $Ca^{++}$  ions as in ultra filtrate were found.

As a rule, however, it is necessary where serum determinations have to be made to make ultra filtrate, which, after DE WAARD, is very simple.

<sup>1)</sup> Arch. Néerl. de phys., 2 530 (1918).

*Summary.*

A simple method is described by which it is possible to measure the concentration of  $\text{Ca}^{++}$  ions in a solution of a mixture of salts e.g. ultra filtrate.

The method is based upon the following principle:

To a solution containing  $\text{Ca}^{++}$  there are added so many  $\text{C}_2\text{O}_4^{--}$  ions till the solubility product of  $\text{Ca}_2\text{C}_2\text{O}_4$  is just reached. The juncture at which so many  $\text{C}_2\text{O}_4^{--}$  ions are added that this product is just exceeded, is ascertained by the appearance of a slight milkiess due to  $\text{CaC}_2\text{O}_4$ . It does not matter whether in the mixture of salts there are present other ions still that can give a precipitate with oxalate. It is only necessary that  $\text{CaC}_2\text{O}_4$  should be the most insoluble substance which can result in the solution.

The method is correct to 2—3 mgr  $\text{Ca}^{++}$  per L. The value of the solubility product was tested by the measurement of the electrical conductivity of the solution.

The principle of the method can likewise be applied for the determination of other ions. The only condition is the disposal of a reagent that gives a salt which is very slightly soluble with the ion whose concentration has to be measured.

*Physiological laboratory of the  
University of Groningen.*

September 1919.