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CHEMICAL AND PHYSICAL BEHAVIOR OF CASEIN SOLUTIONS.

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1. L. L. Van Slyke and J. C. Baker described in 1918¹ a method for preparing "pure casein" from skimmed milk, which consisted in "the gradual addition of acid and its immediate distribution through the mass of milk without causing coagulation of casein at the point where the acid first comes into contact with a portion of the milk. This result can be accomplished by introducing the acid below the surface of the milk with simultaneous high-speed mechanical stirring. . . . After standing under gentle stirring for 3 hours with acidity just below the point of casein coagulation, addition of acid is continued slowly, accompanied as before by rapid stirring in order to obtain the particles of casein coagulum in the finest possible state of division." The coagulated casein is then centrifuged and after repeated washings is found free from Ca and P. As Van Slyke and Baker point out, the pH of this casein coagulum is about 4.5 to 4.6; *i.e.*, it is slightly below the isoelectric point. The essential feature of Van Slyke and Baker's method, therefore, consists in slowly bringing the milk or casein solution approximately to the pH of the isoelectric point of casein. The writer has shown that gelatin gives off all ionogenic impurities at the isoelectric point² and Van Slyke and Baker's experiments show that the same method works also with casein. The casein prepared after Van Slyke and Baker's method is also free from albumin since this latter protein is soluble at pH 4.5 or 4.7, and is hence removed from the insoluble isoelectric casein by washing.

¹ Van Slyke, L. L., and Baker, J. C., *J. Biol. Chem.*, 1918, xxxv, 127.

² Loeb, J., *J. Gen. Physiol.*, 1918-19, i, 237.

In our experiments we used casein prepared after Van Slyke and Baker's method from skimmed milk and in addition from a commercial "pure casein." Both preparations gave practically the same result. In order to remove traces of fat from the casein the latter was washed in acetone.

2. In previous publications the writer had shown that weak dibasic and tribasic acids combine in molecular proportions with crystalline egg albumin, prepared after Sørensen and with gelatin.³ It can be shown that the same is true for casein. 1 gm. of isoelectric casein, prepared after Van Slyke and Baker, was put into 100 cc. of watery solution containing 1, 2, 3, etc. cc. of 0.1 N HCl or 0.1 N H₃PO₄. The pH of the casein solution was ascertained potentiometrically and the number of cc. of 0.1 N acid required to bring the 1 per cent casein solution to the same pH was plotted as ordinates over the final pH of the casein solution as abscissæ. The casein chloride or casein phosphate is not completely soluble in a 1 per cent solution at room temperature until the pH is about 3.0 or a trifle below. When too much acid is added, *i.e.* when the pH is 1.6 or possibly a little above, casein precipitates out again from a 1 per cent solution.

Fig. 1 gives the curves for HCl and H₃PO₄, drawn out within those limits of pH within which the casein salts are soluble in a 1 per cent solution at room temperature. The curves show that about three times as many cc. of 0.1 N H₃PO₄ as of 0.1 N HCl are required to bring 1 gm. of originally isoelectric casein in a 1 per cent solution to the same pH; or in other words, H₃PO₄ combines with casein in molecular proportions, as we should expect if casein phosphate is a true chemical compound.

It was not possible to plot the corresponding curves for casein sulfate and casein oxalate since these salts are too sparingly soluble. This is true also for casein salts with other acids; *e.g.*, triacetic acid.

3. The writer had shown that the influence of different acids on the physical properties of gelatin or crystalline egg albumin depends only upon the valency and not upon the nature of the ion in combination with the protein.³ Thus the values of osmotic pressure or viscosity of gelatin chloride are identical with those of gelatin phosphate for

³ Loeb, J., *J. Gen. Physiol.*, 1918-19, i, 559; 1920-21, iii, 85, 247; *Science*, 1920, lii, 449.

the same pH and the same concentration of originally isoelectric gelatin; and the same is true for crystalline egg albumin. The reason is that in the case of gelatin or albumin phosphate the anion is the

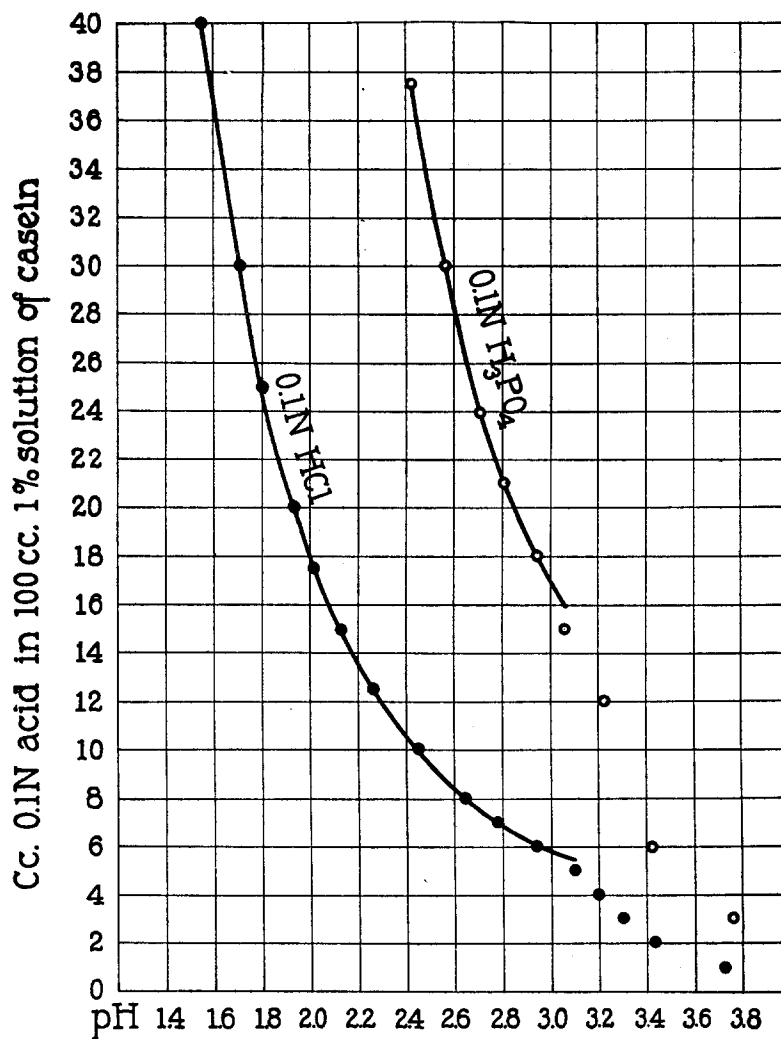


FIG. 1. Ordinates represent the cc. of 0.1 N HCl or H₃PO₄ in 100 cc. of 1 per cent casein solution. The abscissæ are the pH of the solution. Approximately three times as many cc. of 0.1 N H₃PO₄ as of 0.1 N HCl are required to bring 1 gm. of casein to the same pH.

monovalent anion H_2PO_4 and not the trivalent anion PO_4 . If the same rule holds for casein, the osmotic pressure and viscosity of casein phosphate should be practically identical with that of casein chloride when plotted over the same pH and when the concentration of (originally isoelectric) casein is the same in both cases (1 gm. in 100 cc. of solution). Fig. 2 shows that the osmotic pressure curves for casein chloride and casein phosphate (in 1 per cent solutions) are

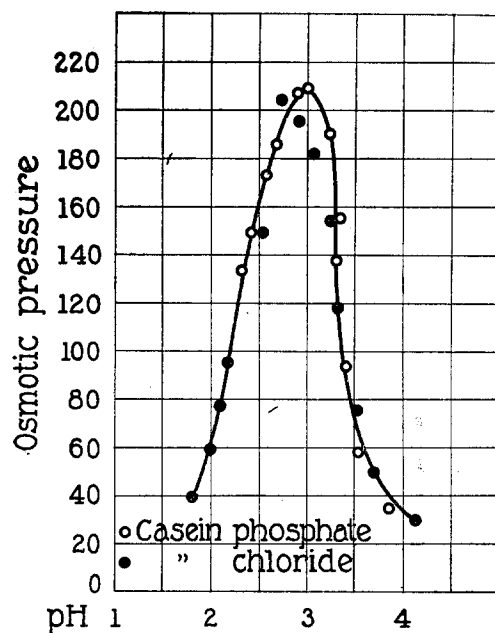


FIG. 2. Osmotic pressure of 1 per cent solutions of casein chloride and casein phosphate as function of pH. The two curves are almost identical.

almost identical. The curve includes also the osmotic pressure at a pH between 4.0 and 3.0 where the two casein salts are not completely soluble in 1 per cent solutions, but since the relative solubilities of casein chloride and casein phosphate are also practically identical, the osmotic pressure curves for the pH where the solubility of the two salts is not complete remain approximately the same.

Fig. 3 gives the viscosity curve for 1 per cent solutions of casein chloride and casein phosphate over pH as abscissæ. The ordinates

are the quotients of the time of outflow of the casein solutions over the time of outflow of distilled water through the same viscometer. The time of outflow for distilled water was 60 seconds. We will, for the sake of brevity, call this ratio specific viscosity. We see that this specific viscosity does not rise above 1 as long as the pH is above

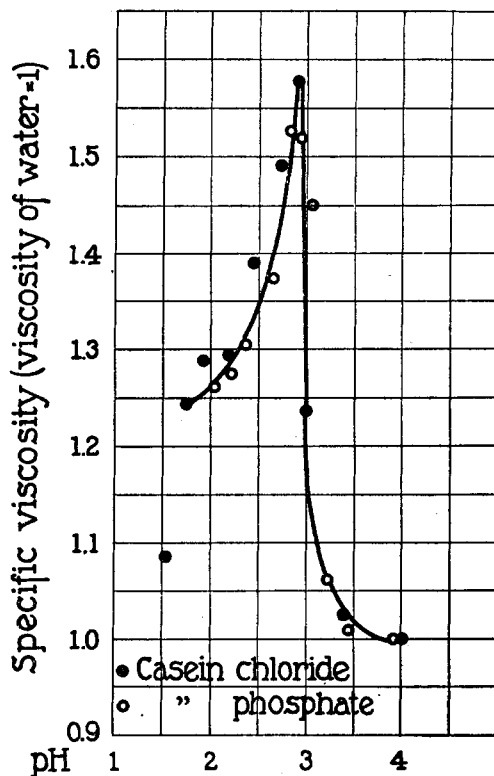


FIG. 3. Viscosity of 1 per cent solutions of casein chloride and casein phosphate. The curves are approximately identical.

3.5. At a pH of 3.0 a sharp rise occurs, because the solubility of the casein increases at this point considerably. With a further fall of pH the viscosity diminishes again. Fig. 3 shows that the viscosity curves for casein chloride and casein phosphate are almost identical, which was to be expected if the rules found for gelatin and crystalline egg albumin are also true for casein.

We had shown that when we add $\text{Ca}(\text{OH})_2$ or $\text{Ba}(\text{OH})_2$ to isoelectric gelatin or isoelectric crystalline egg albumin the two alkalies combine with the protein in equivalent proportion.³ Hence the same number

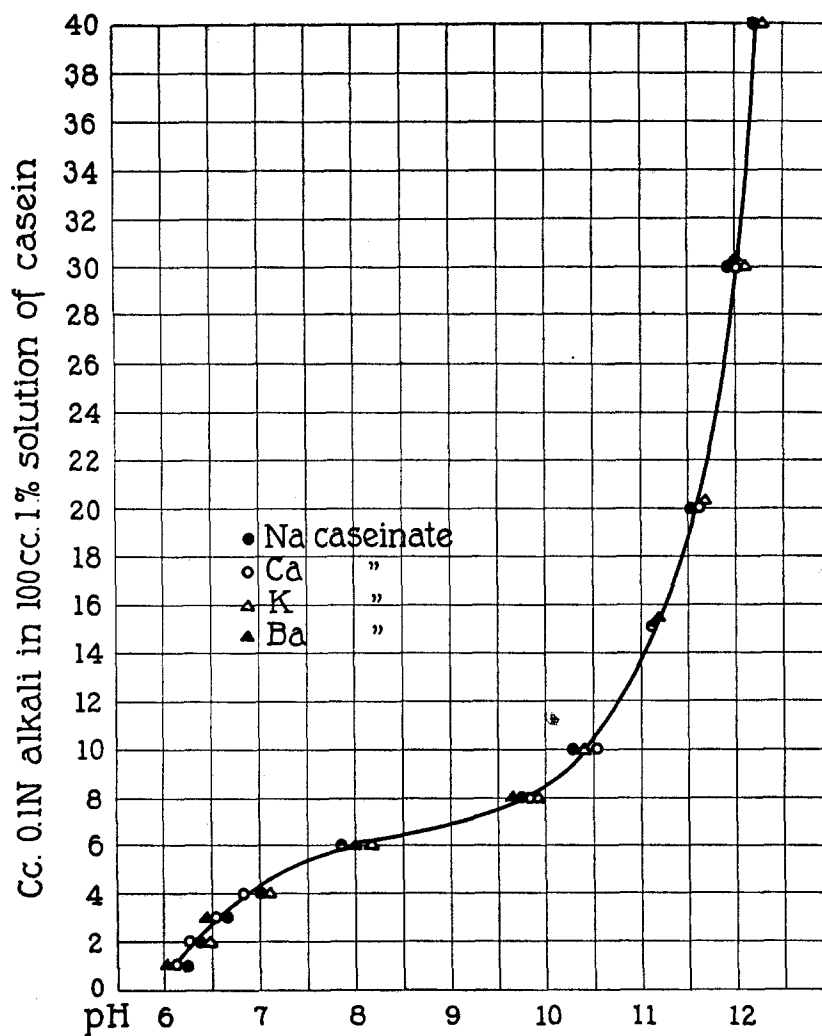


FIG. 4. Ordinates are the cc. of 0.1 N NaOH, KOH, $\text{Ca}(\text{OH})_2$, and $\text{Ba}(\text{OH})_2$ in 100 cc. of 1 per cent solution of casein. Abscissæ are the pH of the solution. The curves for the four alkalies are identical, proving that Ba and Ca combine with casein in equivalent proportion.

of cc. of 0.1 N Ba(OH)₂ or Ca(OH)₂ was required to bring a 1 per cent solution of isoelectric gelatin or crystalline egg albumin to a given pH as was required in the case of NaOH or KOH. It can be shown

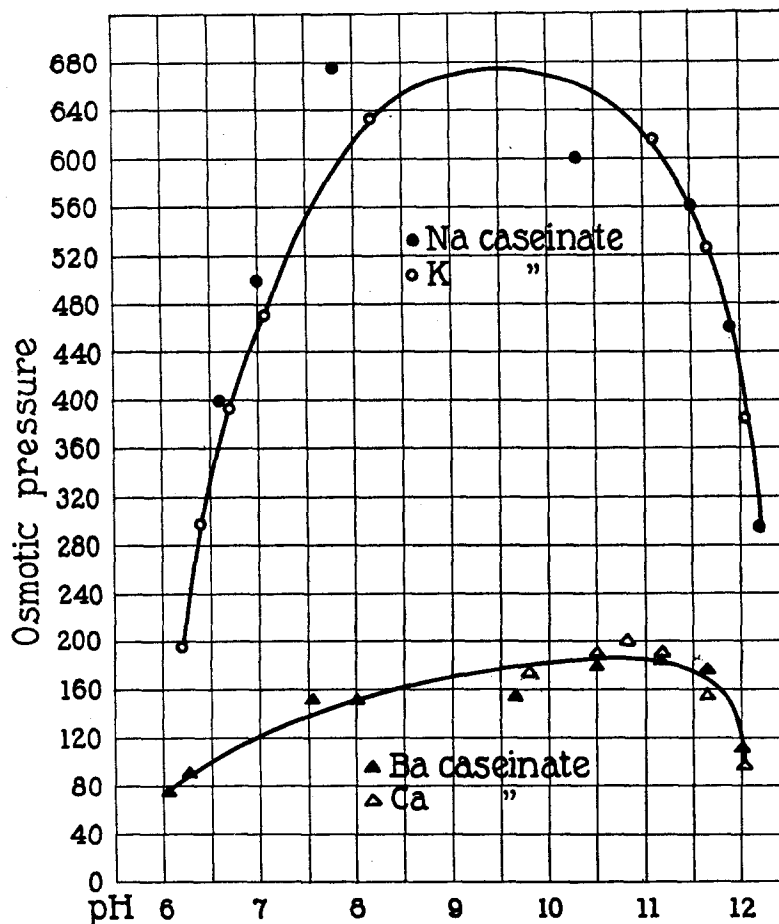


FIG. 5. Osmotic pressure of Na caseinate, K caseinate, Ba caseinate, and Ca caseinate. The curves for the former two caseinates are identical and considerably higher than those for the latter two caseinates.

that the same is true for casein. In Fig. 4 the abscissæ are the pH, the ordinates the number of cc. of 0.1 N NaOH, KOH, Ca(OH)₂, and Ba(OH)₂ that must be contained in 100 cc. of a 1 per cent solution

of isoelectric casein to bring it to the same pH. The curves for the four alkalies are identical (Fig. 4), thus proving that $\text{Ca}(\text{OH})_2$ and $\text{Ba}(\text{OH})_2$ combine with casein in equivalent proportion. Table I gives the limits of pH between which the four metal caseinates are completely soluble in a 1 per cent solution at room temperature.

TABLE I.

1	per	cent	Na	caseinate	completely	soluble	between	pH	7.02	and	>	12.20
1	"	"	K	"	"	"	"	"	7.09	"	>	12.28
1	"	"	Ca	"	"	"	"	"	10.53	"	>	12.00
1	"	"	Ba	"	"	"	"	"	10.50	"	>	12.26

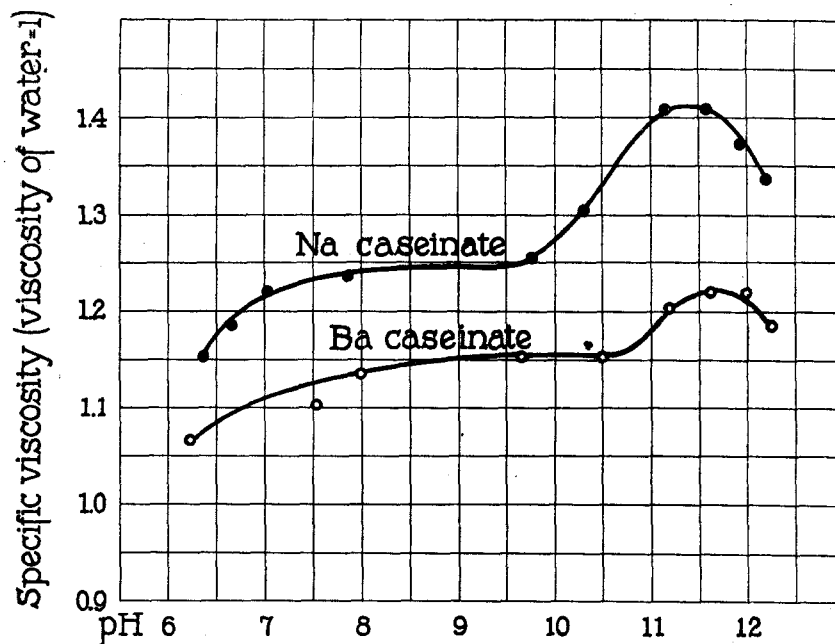


FIG. 6. Viscosity curves for Na caseinate and Ba caseinate.

On account of the incomplete solubility of Ba caseinate and Ca caseinate at a pH between 4.7 and 10.5 it is not possible to draw any conclusion from the relative osmotic pressure or relative viscosity of metal caseinates with monovalent and divalent cation between a pH of 4.7 and 10.5. This should be kept in mind in judging the curves in Figs. 5 and 6.

When we plot the osmotic pressure of these solutions as ordinates over the pH as abscissæ (Fig. 5), we notice that the curves for the osmotic pressure of Na and K caseinate are alike; the curves for the casein salts are, however, over three times as high when the cation is monovalent (Na or K) than when the cation is bivalent (Ca or Ba). This is, however, chiefly the result of the fact that Ca and Ba caseinate are incompletely soluble up to a pH of 10.5.

Fig. 6 gives the viscosity curves for Na caseinate and Ba caseinate. The difference in height between the two casein salts is between pH 11 and 12 of a similar order as in the case of Na and Ba gelatinate.

SUMMARY AND CONCLUSION.

The experiments on casein solutions therefore confirm the conclusion at which we arrived from the behavior of gelatin and crystalline egg albumin that the forces determining the combination between proteins and acids or alkalies are the same forces of primary valency which also determine the reaction between acids and alkalies with crystalloids, and that the valency and not the nature of the ion in combination with a protein determines the effect on the physical properties of the protein.

The measurements mentioned in this paper were made by Dr. E. Brakeley, Mr. M. Kunitz, and Mr. N. Wuest of this Laboratory, to whom I wish to express my indebtedness.