

The effect of salts on the isoelectric point of casein.

by

T. Erdős.

(Chinoïn research fellow).

It has been described by MICHAELIS and SZENT-GYÖRGYI¹ that small concentrations of neutral salts shift the pH range, in which casein is insoluble, either to the acid or to the alkaline side. I have attempted a more detailed study of this phenomenon.

Hammarsten's casein (Merck) was used in the experiments. A stock solution was prepared by dissolving 0.1 g casein in 100 ml of a 0.004 M Na-acetate solution. The buffer solutions used were acetic acid-Na-acetate mixtures, covering the pH range of 3.0 — 6.0 the difference between each two members of the series being 0.05 pH units.

The experiments were made in the following way: 1 ml of the 0.1% casein solution was given to 8 ml of buffer and made up to 10 ml with distilled water. The contents of the tubes were mixed thoroughly, left to stand at room temperature (21—23°) for 24 hours, and the results noted. If sedimentation or visible flocculation was observed, the result was marked „precipitated“, if there was only opalescence but no flocculation, it was marked „not precipitated“. After reading the results, the pH of the solutions was controlled electrometrically.

It is seen from Table I that an increase in the concentration of the buffer will shift the precipitation zone to the acid side. There is no difference between the effect of 0.02 and 0.01 M buffers. In these buffer solutions casein is precipitated between pH 4.32—5.00. The middle of this range, pH 4.66, agrees well with the known values of the isoelectric point of casein:

Table I.

M concentration of the buffer	Range of precipitation
1.00	3.70 — 4.70
0.50	3.92 — 4.78
0.20	4.15 — 4.85
0.10	4.20 — 4.90
0.04	4.27 — 4.96
0.02	4.32 — 5.00
0.01	4.32 — 5.00

pH 4,7 according to RONA and MICHAELIS,² 4,6 according to MICHAELIS and PECHSTEIN.³

In the following experiments I have used a 0,02 M buffer to adjust the pH of the casein solutions. In certain cases, where a higher concentration of the buffer was needed, I have made allowance for the modifying action of the buffer concentration.

1 ml of a 1,0 M salt solution or the corresponding amount of salt in substance was added to 8 ml acetate buffer and 1 ml 0,1% casein. The volume was made up to 10 ml with dist. water. After standing at room temperature for 24 hours the precipitation was noted. In Fig. 1. the results of these experiments are summarized. The horizontal lines indicate the pH-range in which casein was precipitated. The salt added to the buffered casein is indicated by its formula over the precipitation zone. The final concentration of these added salts was 0,1 M except in the case of CH_3COOAg , whose final concentration was 0,05 M and AgNO_3 which was studied in both of these concentrations. Whereas in most cases the concentration of the buffer was 0,02 M, in some cases the concentration was 0,1 M and in others the buffer was a 0,5 M acetate buffer. In these cases allowance was made for the shift in the precipitation zone due to the buffer and this being subtracted the net effect of the salt is shown in Fig. 1. In the case of Na_2HPO_4 there is no acetate buffer, but phosphoric acid was added to give the desired pH. The horizontal line marked „0“ shows the precipitation pH-range of casein in 0,02 M acetate buffer.

Special attention was paid to the effect of the alkali haloids.

It is seen at first sight from Fig. 1. that most of the salts shift the precipitation zone towards the acid side. This shift is obviously due to the anion, its extent being the function of the atomic weight. Cations have a pronounced secondary

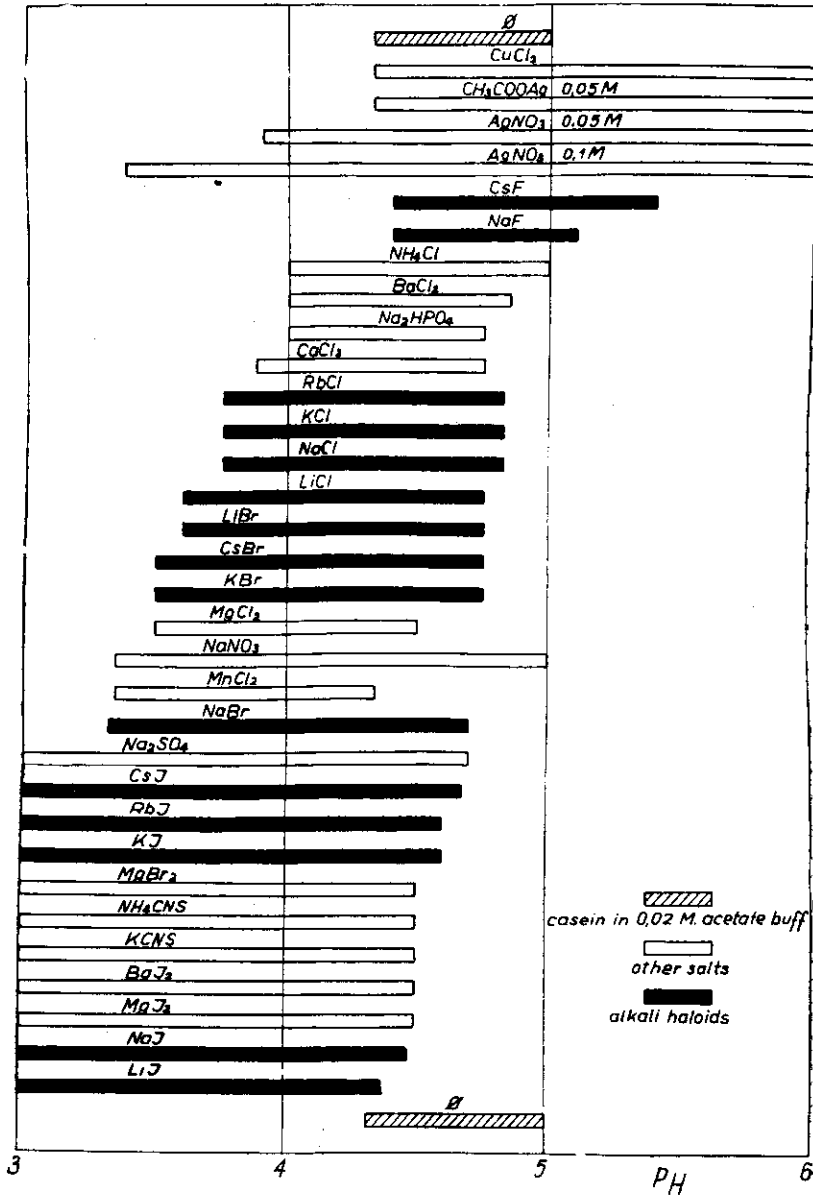


Fig. 1.

influence, shifting the precipitation zone towards the alkaline side. In this case too, the effect is proportional to the atomic weight. In two cases (the two fluorides) the precipitation zone is shifted altogether towards the alkaline side, showing that in these salts the cations take the upper hand. The iodides have a remarkable precipitating action, they would precipitate casein even at pH 1 (not shown in the figure). Similar results have been obtained with the Mg, Ca and Ba haloids. LiBr had exceptional influence, the effect of this salt would not fit into the system.

We may draw the conclusion that the effect of a salt results from the effect of its anion and cation. If the effect of its anion is stronger, the precipitation zone will be shifted to the acid side. If the effect of the cation is stronger the reverse will happen. On the whole the results confirm the data of MICHAELIS and SZENT-GYÖRGYI.

The precipitations obtained at these different pH values in presence of salts are all reversible and do not change the properties of casein. This is demonstrated by the following experiment. The precipitate obtained at pH 3,0 in presence of NaJ was centrifuged off and the small volume of precipitate dissolved in a 0,02 M acetate buffer: its precipitation zone was now identical with the original precipitation zone of casein. i. e. 4,32—5,00.

Next I have studied the influence of the salts on the acid-base binding capacity of casein. Two salts, NaCl and NaJ have been chosen for these experiments, the former giving a small effect, the latter a strong effect in the precipitation experiments. It was found that both salts have identical effects, both of them increase the acid-base binding capacity to the same extent and neither of them changes the isoionic point of casein.

First I made an electrometric titration curve by adding 0,1 N HCl to a 0,01 N NaOH solution and a similar curve by adding 0,1 N NaOH to a 0,01 N HCl solution. 1% casein solutions were made in 0,01 N NaOH and 0,01 N HCl, and again the electrometric titration curve was made by adding different quantities of NaOH or HCl. The titration curve was naturally different of the titration curve of the pure acid or base owing to the acid-base binding capacity of the casein. The difference of the two curves gives the acid-base binding capacity, the

minimum of which (i. e. the isoionic point) was in my experiments at pH 4,6 which agrees well with the accepted value in the literature. Then the experiments have been repeated in the presence of 0,1 and 1,0 M NaCl and NaJ respectively. The acid-base binding capacity was increased by the presence of either salt the more, the higher the concentration of the salt was in the solution. There was however no difference between the

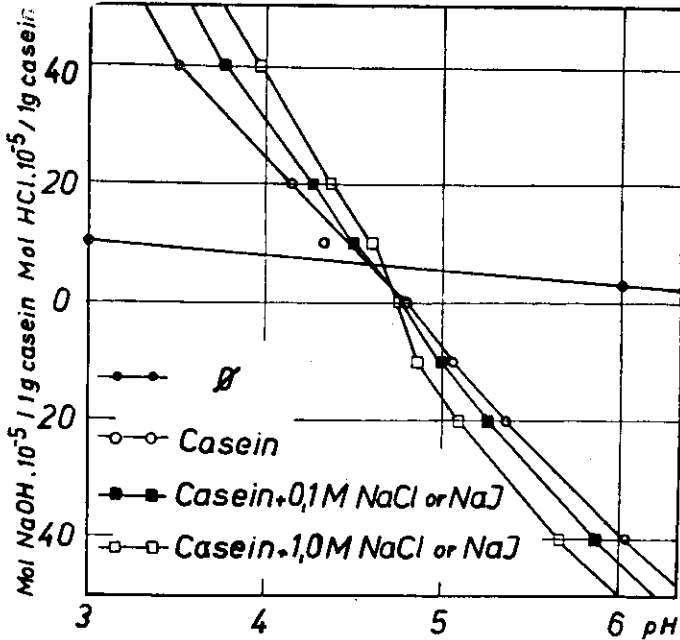


Fig. 2.

effect of NaCl and NaJ. The isoionic point was not changed by the presence of the salts. (Fig. 2.)

We may conclude therefore that the change in the iso-electric pH range of casein due to the presence of different salts in the solution cannot be explained by assuming an effect of the salts on the acid-base binding capacity of casein.

Literature.

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2. P. Rona u. L. Michaelis, *Biochem. Z.*, 28, 193 (1910).
3. L. Michaelis u. H. Pechstein, *Biochem. Z.*, 47, 260 (1912).