AGING OF SURFACES OF SOLUTIONS

Part VI. Surface Aging of Casein Solutions*

By G. N. Subba Rao, K. S. Gururaja Doss† and Basrur Sanjiva Rao
(*Department of Chemistry, Central College, Bangalore, S. India*)

Received January 31, 1946

Introduction

Aqueous solutions of casein are known to produce foams of great stability. The foaming is due to the surface accumulation of the protein and the formation of adsorption films. These adsorption films are insoluble and exhibit surface pressure in the Langmuir trough. It is thus possible to study accumulation by the method developed by Doss[1] and independently by McBain and co-workers.[9, 10] Surface accumulation studies on casein are of particular interest since the area occupied by a casein molecule at the surface can be determined by direct spreading measurements. The surface aging of these solutions has been investigated in the present work by studying (*a*) the rate of accumulation and (*b*) the rate of change of surface tension with time. In studying the latter, a direct comparison has been made of the trough and the ring methods. The variation of surface tension has been studied by Johlin[6] by the capillary rise method. This method, however, is subject to many sources of error.[15]

Experimental

Casein used in the present work was an isodisperse fraction prepared according to the method of Svedberg, Carpenter and Carpenter.[13] The aging was studied by employing the surface film balance described previously.[2] The zero position of the float was adjusted to be vertically below the torsion wire so as to eliminate completely the effect of any changes in the vertical component of the force due to surface tension or buoyancy.[14] This is very necessary in the study of the variation of surface tension with time.

Results

1. *Accumulation studies.*—Casein has been found to accumulate very rapidly. It is therefore very necessary to work with a highly dilute solution

---

* Part of Thesis submitted by G. N. S. in partial fulfilment of the requirements for the degree of Master of Science of the Mysore University.

† Physical Chemist, Imperial Institute of Sugar Technology, Cawnpore.

in order to obtain a measurable rate of accumulation. 0·00025% solution in 0·01 M hydrochloric acid is found suitable.

The accumulated casein film is of a highly condensed type and it is therefore possible to measure the rate of accumulation by determining the area of the film. The rate of accumulation was measured as follows. The trough was thoroughly cleaned using Nekal BX which gave an extremely clear trough. A blank was invariably tried with distilled water before each study. The extent of contamination of a surface of distilled water was measured and found to be small, being invariably within 5% of the rate of accumulation of the casein solution. The trough was filled with the casein solution. The surfaces on either side of the float were swept clean by a barrier and the time noted. A known area of the surface on the left-hand side of the float was allowed to age for a definite amount of time and the area of the accumulated casein was measured at 4 dynes/cm. pressure by moving the barrier towards the float. At this pressure the force-area curve for spread films of casein was steep and hence area measurements were accurate. Since the area occupied by a molecular of casein at 4 dynes/cm. pressure was known from direct spreading, the rate of accumulation could be computed. The casein solution employed being exceedingly dilute, the process of accumulation impoverished the solution appreciably. This difficulty did not arise with the studies on benzopurpurine

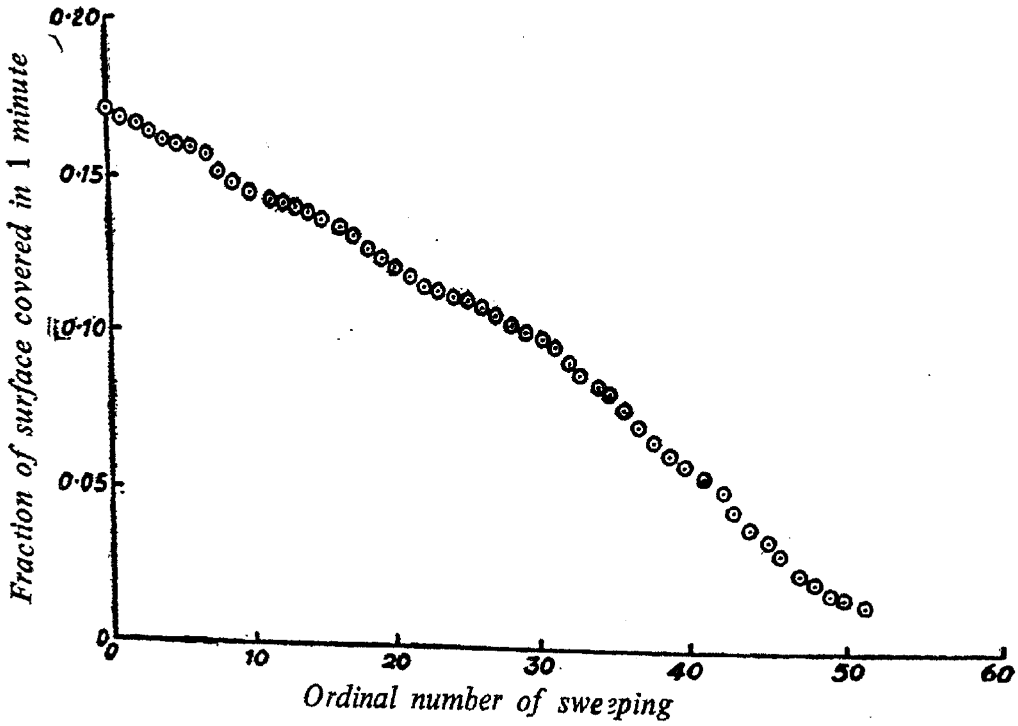

FIG. 1. Effect of repeated sweeping on the rate of accumulation of casein.

10 B since comparatively strong solutions (M/250, M/500) could be employed.[5] Fig. 1 illustrates this effect clearly. The successive one minute-values for the area covered with the same solution against the ordinal number of the sweepings have been plotted.

In order to get the time-area curve the following procedure was therefore adopted. For the value corresponding to each time-interval a fresh quantity of solution was started with. The surface was swept and accumulation measured thrice and the average of the three values taken. The results are given in Table I.

TABLE I

Time-area relationship to the accumulation of casein

0·00025 % casein in 0·01 M hydrochloric acid.

Area measured at 4 dynes/cm. Temperature 25° C.

Time in minutes	Fraction of the surface covered
1	0·17
2	0·26
4	0·38
8	0·59
16	0·83

The effect of salts on the rate of accumulation was studied and it was found that the rate of accumulation was not altered by the addition of potassium chloride or barium chloride.

The effect of temperature on the rate of accumulation was investigated. The trough was kept in a refrigerator at 13·5°C. It was found that the rate of accumulation was practically the same as at 25°C., the laboratory temperature. Attempts to measure accumulation at 40° C. were not successful owing to the serious disturbing effects caused by convection currents. Abnormally high accumulation was noticed—a 10° rise producing a twofold increases in the rate.

Accumulation of formolised casein.—Formolised casein was prepared by mixing solutions of Hammersten casein in aqueous sodium acetate with formaldehyde[8] and allowing to stand for three days. The rate of accumulation of 0·00025% solutions in 0·01 M-hydrochloric acid was studied. The results are given in Table II.

continued until a practically steady value was obtained. Then the surface on the right-hand side was cleared and allowed to age. The variation of surface tension of this surface was measured using the fully aged surface on the left as reference. The surface tension of the fully aged surface was then determined directly, by the ring method using the silica spring,[7] and the value employed in the calculation of the data given in Fig. 2.

When a fully aged surface was compressed (to 26 dynes/cm.), it exhibited a rise of surface tension on aging. The rise was about 5 dynes/cm. in ten minutes. The rise was steep initially and the behaviour was similar to that of benzopurpurine 6 B.[2]

A comparative study of the ring and trough methods was made, employing identical surfaces, and measuring the surface tension alternately by the two methods at various intervals of time. In the ring method a flat spiral of silica constructed in this Laboratory[7] was used for measuring the maximum pull. The results are illustrated in Fig. 3, and show that comparable results can be obtained by both the methods.

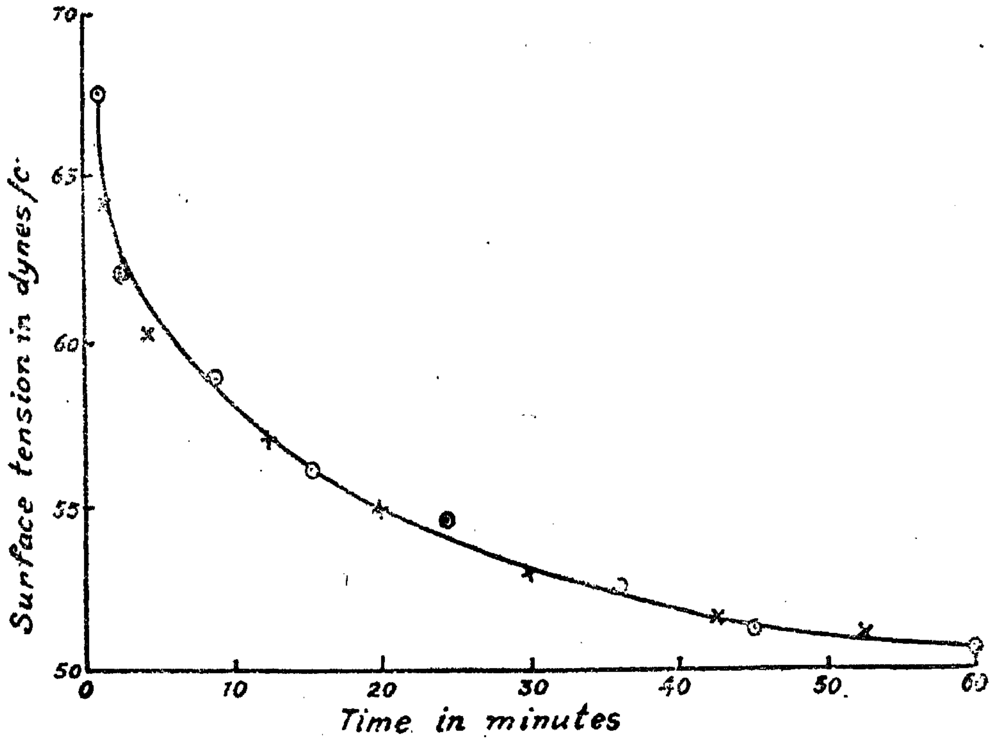

FIG. 3. Comparison of trough and ring method. ⊙Ring method. ×Trough method.

For 0·1% solutions, of casein, the trough method could not be used for studying the variation of surface tension since the solution wetted the paraffin and leakage past barriers occurred. The variation was therefore studied by the ring method. A two-minute old surface showed a surface tension of 52·2 dynes/cm. Further aging caused a very slow change, the surface tension dropping to 50·8 dynes/cm. after one hour.

Discussion

1. *Calculation of the theoretical rate of accumulation.*—The order of time needed for covering any definite fraction of the total surface by accumulation can be estimated roughly as follows. For covering a fraction of the surface, the depth of the solution (below the surface) which is to be denuded $= \frac{\chi \times 10^{-7}}{2 \cdot 5 \times 10^{-6}}$ or 0·04 cm. (10^{-7} = No. of g. of casein present per sq. cm. of the casein monolayer at a pressure of 4 dynes/cm. on 0·01 M hydrochloric acid and $2 \cdot 5 \times 10^{-6}$ = concentration of casein in g. per c.c. of solution).

Consider the Brownian displacement of the molecules parallel to the vertical direction. Assuming that half the molecules move towards the surface by Brownian displacements, the distance Δ that a molecule has to cover to appear at the surface would be twice the above depth, *i.e.*, 0·08χ cm.

According to Einstein's equation, we have,

$$\frac{\Delta^2}{t} = \frac{RT}{N} \cdot \frac{1}{3\pi\eta r}$$

where t is the time for the displacement Δ, R = gas constant, T = absolute temperature, η = viscosity, N = Avogadro number and r = radius of the casein molecule. r can be estimated by the equation $\frac{4}{3}\pi r^3 = \frac{M}{\rho N}$ where M and ρ are the molecular weight and density of casein respectively. Substituting the appropriate values in the Einstein's equation, we get

$$\chi = 0 \cdot 012\sqrt{t}, \text{ where } t \text{ is in seconds.}$$

2. *The high rate of acculumation.*—From the above equation, the fraction of the total surface covered in 60 seconds works out to be 0·091 whereas the experimentally observed value is 0·17, *i.e.*, nearly twice the theoretical value. A similar calculation for the later successive intervals would show that the experimental values of the accumulation are higher than the maximum possible theoretical values. This is a surprising feature. All the systems studied so far by the earlier investigators[1, 11] exhibit slow accumulation. The abnormally high rate of accumulation now noticed cannot be explained on the basis of the presence in casein of any fractions of low molecular weight. For, the casein preparation used is known to be practically isodisperse. Moreover, to account for the observed high rate,

an average molecular weight of about 9,000 has to be assumed, a value which is obviously inadmissible. The observed high rate may at first sight appear to be caused by the dragging effect of the gegenions associated with the casein molecules. But the experiments on the effect of neutral salts definitely show that the influence of gegenions is negligible. It is of interest to note that a high rate of accumulation is not peculiar to casein. It is also exhibited by egg albumin.[12] Our observations suggest that this phenomenon is caused by (*a*) undulations produced during the formation of the new surface and (*b*) existence of eddy currents near the surface which disturb the concentration gradient set up by the diffusion of protein molecules into the surface.

3. *Effect of temperature on the rate of accumulation.*—The temperature coefficient of accumulation is negligible. The accumulation is therefore not activated. This observation supports the other fact that the accumulation is very fast.

As already stated, convection currents interfered with measurements attempted at 40° C. and gave very high values. Since the accumulation of casein is not of the activated type, any molecule of the protein reaching the surface by diffusion, sticks to the surface. Therefore the rate of accumulation is high and one is obliged to work with very dilute solutions. In such solutions accumulation causes a concentration gradient. Convection currents would disturb this gradient and promote diffusion, thereby increasing the rate of accumulation. It may be pointed out that this complication does not arise with systems like benzopurpurine 10 B giving activated accumulation,[3] for, the accumulation being slow no appreciable concentration gradient can occur in such system.

4. *Effect of neutral salts.* Neutral salts have negligible influence on the rate of accumulation. This shows that there is no electrical potential barrier inhibiting accumulation.[15] It also confirms the view that the high rate of accumulation is not due to the influence of the gegenions, for, if they enhance the diffusion rate of casein molecules to the surface, neutral salts should reduce the rate of accumulation.

5. *The time-area curve.* Langmuir's theory of adsorption can be applied to this system. The rate of increase of total surface covered by casein molecules is given by the equation

$$\frac{d\chi}{dt} = K_1(1-\chi) - K_2\chi$$

where χ is the fraction of the total surface covered by casein molecules in a time-interval t, K_1, the specific velocity of adsorption and K_2 the velocity of desorption. As the adsorption is practically irreversible K_2 may be taken as zero. (Actually K_2 may have a negative value. For, χ becomes unity when the surface pressure due to accumulation becomes 4 dynes/cm., the pressure at which the area of accumulated film is measured; but even at this stage accumulation continues, increasing the surface pressure much further.) The equation can then be written as

$$\frac{d\chi}{dt} = K_1 C (1 - \chi) \text{ or } K_1 = \frac{1}{(t_1 - 1)} \ln \frac{1-\chi_1}{1-\chi},$$

where χ_1 is the area covered in one minute. In Table III the values of K_1 for the accumulation of casein are given.

TABLE III

Accumulation of casein from 0·00025% *solution in* 0·01 *M hydrochloric acid*

t	K_1
2	0·050
4	0·042
8	0·044
16	0·046

In this discussion, the first minute value has not been taken into account owing to the disturbing factors that operate during the formation of a new surface. The examination of the table shows that the system obeys fairly well the Langmuir's equation. This shows that (*a*) the molecules of casein at the surface hinder accumulation of other casein molecules only to the extent of the surface they occupy at the surface pressure of 4 dynes/cm. and (*b*) the eddy current effects are nearly the same in all the measurements. A totally different result has been obtained with egg albumin[12] which does not obey Langmuir's equation. Molecules of albumin inhibit accumulation over an area about three times their cross-section. This is probably due to the influence of long-range electrical forces.

6. *Effect of formaldehyde.*—Accumulation of casein treated with formaldehyde shows a large diminution in the rate, if the fraction of total surface covered within a definite interval of time is considered. But taking into consideration the fact that the formolised casein occupies nearly half the area occupied by untreated casein, one finds the amount (in mg. per sq. m. of the surface) that has accumulated is nearly the same for the ordinary as well as the treated casein. This result shows that treatment with formaldehyde does not alter the state of aggregation of the molecule.

7. *Variation of surface tension with time*.—The surface tension time curves for dilute solutions of casein can be interpreted, taking into account the condensed nature of the adsorption film. The accumulation starts, directly the new surface is formed. But accumulated molecules form a highly condensed film, having negligible surface vapour pressure. Consequently the surface tension remains constant though the accumulation proceeds. This goes on until the surface gets almost completely covered. At this stage further accumulation causes a large change in surface tension. At the next stage, when the surface gets highly packed up with molecules, the rate of penetration of casein molecules from the bulk solution into the surface decreases; so the slope of the surface tension-time curve decreases. In more concentrated solutions, accumulation goes on so rapidly that the initial horizontal portion of the curve is not noticeable. Similar behaviour has been noticed with solutions of lauric acid[16] and benzopurpurine 6 B.[4]

An examination of Fig. 2 shows that the values of surface tension are not so dependable when the fully aged surface is used as a reference surface. This is due to the fact that the surface tension of the fully aged surface is markedly upset by the irregular movements of the float during the determination.

When the adsorption film is compressed to a high pressure the surface tension of the compressed surface exhibits a rise in surface tension with time. This may be attributed either to a partial collapse of the film or to a change in the orientation of the molecules on the surface. With casein films, partial collapse has been noticed at a pressure of 26 dynes/cm.

Summary

1. The rate of accumulation of isodisperse casein has been investigated, employing a surface film balance. The rate is found to be much higher than the value expected from theory. This surprising feature, reported for the first time in the present work, has been explained.

2. Neutral salts have no effect on the rate of accumulation, thus showing the absence of influence of gegenions and of any electrical potential barrier.

3. Temperature coefficient of accumulation is negligible. Therefore the process is not activated.

4. Accumulation of formaldehyde-treated casein has been found to be essentially of the same order as of ordinary casein.

5. The time-area curve for casein obeys Langmuir's adsorption equation. There is no potential barrier inhibiting the accumulation.

6. Comparative study of the trough and ring methods for measuring surface tension has been made under identical conditions. The two methods are found to yield comparable results.

7. Variation of surface tension with time has been studied by the trough method.

References

1. Doss .. *Curr. Sci.*, 1935, **4**, 405.
2. ——— .. *Proc. Ind. Acad. Sci.*, A, 1936, **4**, 97.
3. ——— .. *Curr. Sci.*, 1937, **5**, 645.
4. ——— .. *Ibid.*, 1938, **7**, 182.
5. ——— .. *Kolloid-Z.*, 1938, **84**, 138.
6. Johlin .. *J. Phys. Chem.*, 1925, **29**, 271, 897.
7. Krishnappa, Doss and Rao .. Unpublished.
8. Lindstrom-Lang .. *Comp. rend. Lab. Carlsberg*, 1925, **16**, 48.
9. McBain and Wilson .. *J. Amer. Chem. Soc.*, 1936, **58**, 380.
10. ——— and Perry .. *Ind. Eng. Chem.*, 1939, **31**, 35.

11*a*. Nutting, Long and Harkins .. *J. Amer. Chem. Soc.*, 1940, **62**, 1500.
b. ——— and Long .. *Ibid.*, 1941, **63**, 87.
c. McBain, Ford and Mills .. *Ibid.*, 1940, **62**, 1324.
d. Ward and Tordai .. *Nature*, 1944, **154**, 146.

12. Rao, G. N. S., Doss and Rao, B. S. .. Unpublished.
13. Svedberg, Carpenter and Carpenter .. *J. Amer. Chem. Soc.*, 1930, **52**, 241.
14. Venkatachala, Doss and Rao .. Unpublished.
15. Doss .. *Kolloid-Z.*, 1939, **86**, 205.
16. Harkins and co-workers .. *Nature*, 1936, **138**, 406.