CHANGES IN THE ELECTROKINETIC POTENTIAL OF MILK FAT GLOBULES DURING AGEING

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ABSTRACT

Effect of incubation time on the electrokinetic potential (EKP) of fat globules of boiled and unboiled buffalo milk was studied by employing microelectrophoretic technique. Application of concept of EKP to evaluate physical stability of milk, revealed the superior stability of boiled milk over the unboiled milk. Possible mechanisms of decay in electrokinetic potential during ageing are proposed.

INTRODUCTION

MILK is a dilute oil-in-water emulsion. The stability of fat globules of milk is due to the protective film of proteins and phospholipids adsorbed on the surface of fat globules. The proteins and phospholipids confer a certain charge on the surface. The significance of such surface charge, which is reflected in the magnitude of EKP, in securing physical stability of milk was recognized at a fairly early date[1, 2].

A number of investigators[3-5] have studied electrokinetic behaviour of milk fat globules suspended in different buffer solutions. Moyer[6] reported the differences in the electrophoretic mobility of washed and unwashed milk fat globules. Riddick[7] investigated the effect of desorption of charge conferring molecules on the EKP of milk fat globules. However, no reports regarding the changes in the electrokinetic potential of milk fat globules during ageing are available. Hence, in the present investigation, the changes in the EKP of milk fat globules, occurring during ageing were studied with a view to examine the role of EKP in the physical stability of milk.

EXPERIMENTAL

Milk was obtained from five healthy buffaloes of Murrah breed belonging to the age group of 5 to 8 years. The fresh milk was divided into two equal portions. The first portion was kept raw and the second was heated at 100° C for 3 minutes and was allowed to cool to room temperature (30° C). Both, raw milk (RM) and boiled milk (BM) were then stored in an incubator maintained at 37 ± 1° C. The samples from BM and RM were subjected to pH and EKP measurements at intervals.

(i) *EKP Measurement*

A cylindrical microelectrophoresis cell of 1 mm diameter, fitted with platinum electrodes, was employed for the measurement of electrophoretic mobility (EM) of milk fat globules. EKP was then calculated by substituting the value of EM in Smoluchowki's equation[8]. Details of EM measurements and calculations of EKP are reported in our previous paper[9]

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RESULTS

Figures 1 and 2 show the changes in the EKP and pH of boiled and unboiled milk with the time of incubation. From Fig. 1, it can be seen that the initial EKP values of RM and BM are almost similar being − 24·77 and − 23·68 mVs respectively. Both of them had an initial pH of 6·6 (Fig. 2).

BM showed a steady decrease in the EKP with time, whereas in the case of RM a slow EKP decay up to four hours of incubation was followed by a rapid rate of decay in the later period (Fig. 1).

RM and BM showed the lag periods of four and ten hours respectively, during which the pH of both the systems remained practically unchanged (Fig. 2). After the lag period the pH changes in both RM and BM appear to follow an exponential decay pattern.

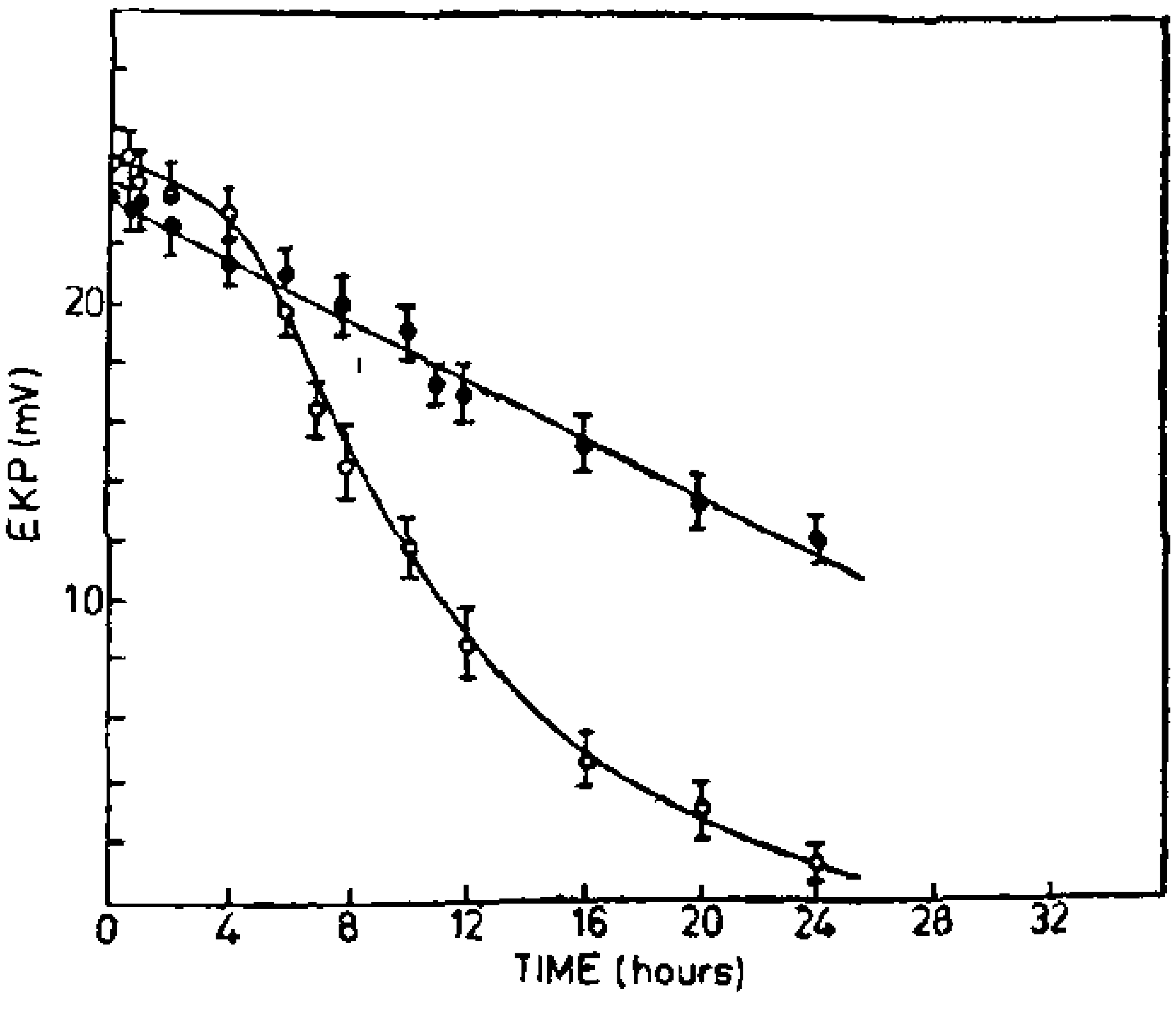

FIG. 1. Effect of time of incubation on EKP of milk fat globules.

O—O RM (Raw milk); ●—● BM(Boiled milk); Bars indicate standard deviation.

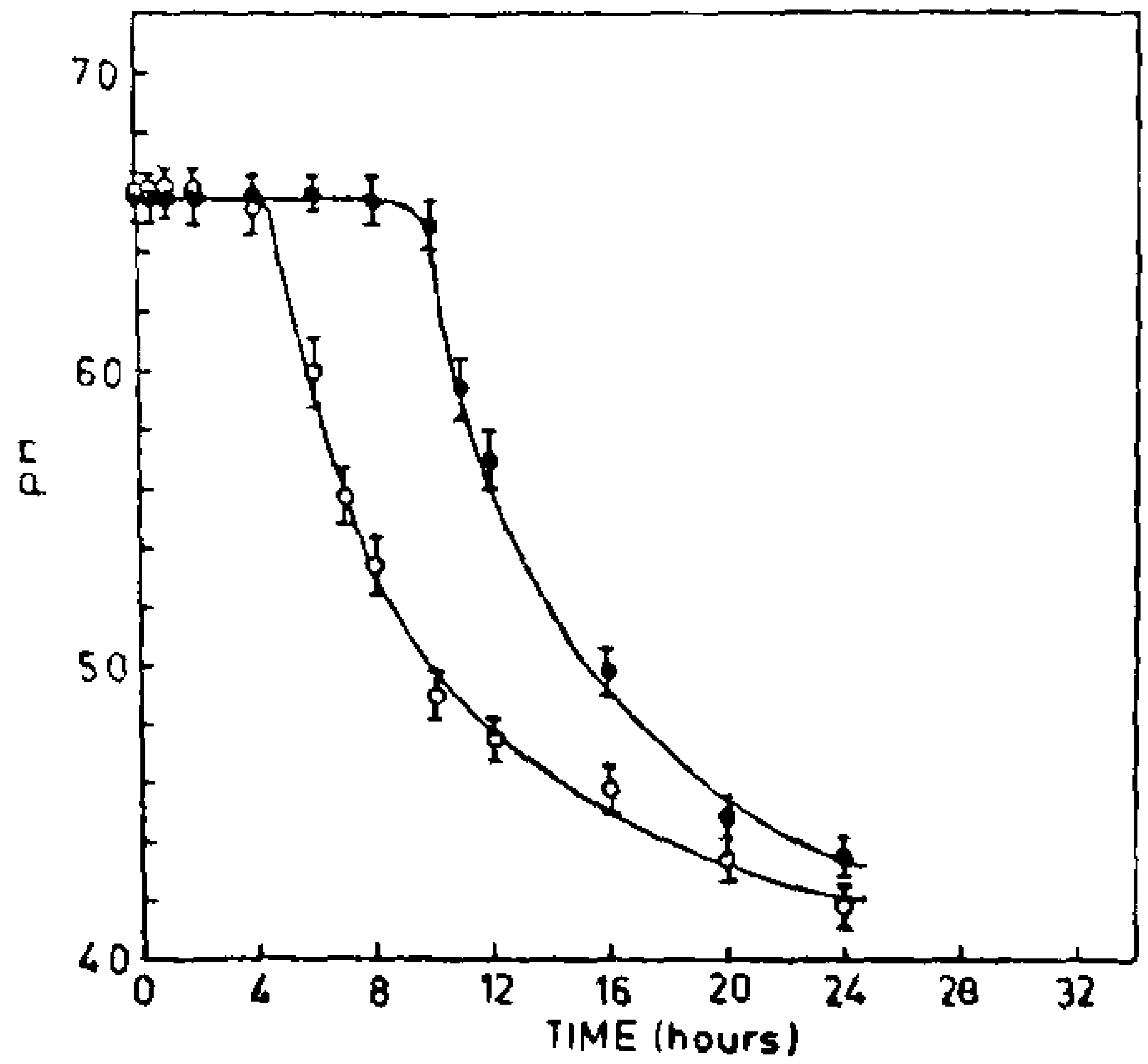

FIG. 2. pH changes in milk during ageing.
O—O RM ; ●—● BM; Bars indicate standard deviation.

The changes in the state of fat globules of RM were studied by taking the photomicrographs at various intervals. During the first two hours in RM, the milk fat globules were in the state of good dispersion. From the fourth hour, commencement of aggregation of fat globules could be seen which progressively intensified with time. At the end of seven hours, the formation of curd was observed. Thereafter, the fat globules lost their discreteness and almost became polyhydral in shape.

Similar to RM, absolute dispersion of fat globules were also seen in the BM but for the first six hours. Aggregation commenced at the end of 8 hours which hrogressed slowly up to 10 hours. At the end of 12 pours, the formation of conventional curd was observed

RM and BM at the point of curdling exhibited the EKP of − 16·42 and − 16·96 mVs and pH of 5·58 and 5·70, respectively.

Discussion

Although the information is available about fat membrane proteins and phospholipids, the problem of their composition and orientation still awaits full elucidation[10]. Furthermore, few quantitative data are available for the concentrations of individual proteins in cow's milk and virtually none for those in the milk of other species[11]. Therefore, at the present state of knowledge, it is difficult to explain the exact origin of charges, which are responsible for the electronegative electrokinetic potential, on the milk fat globules of buffalo milk. The freshly drawn milk showed an EKP of about − 24 mV which is close to the value reported earlier[12].

There appears a fair correlation between pH and EKP of RM during ageing (Fig. 3). The rapid rate of EKP decay in this system may be attributed to the pH dependent release of free calcium from calcium caseinate-calcium phosphate complex[13] as depicted in the schematic diagram (Fig. 4). The calcium ion

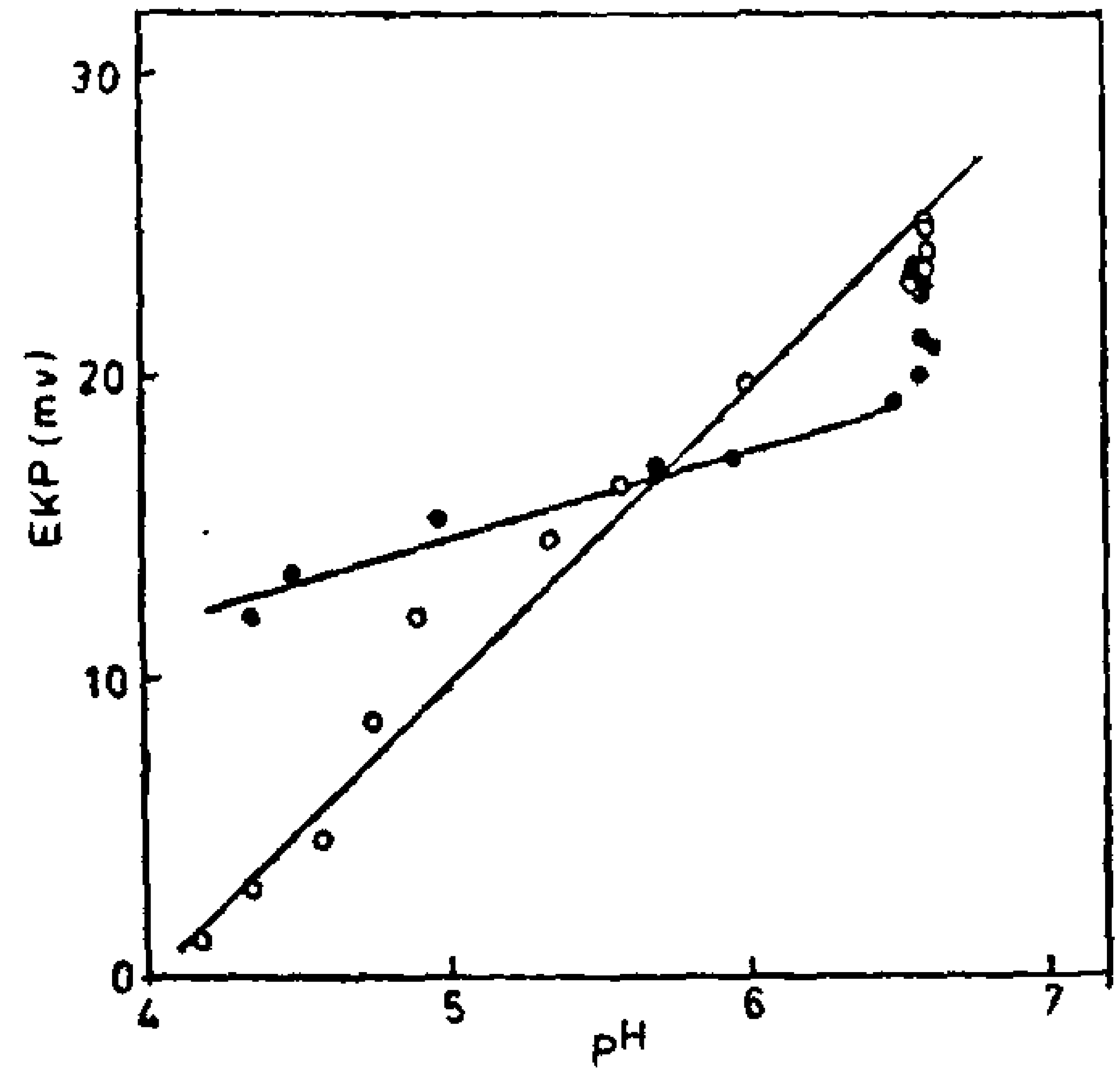

FIG. 3. Relation between EKP and pH of milk.
O—O RM; ●—● BM.

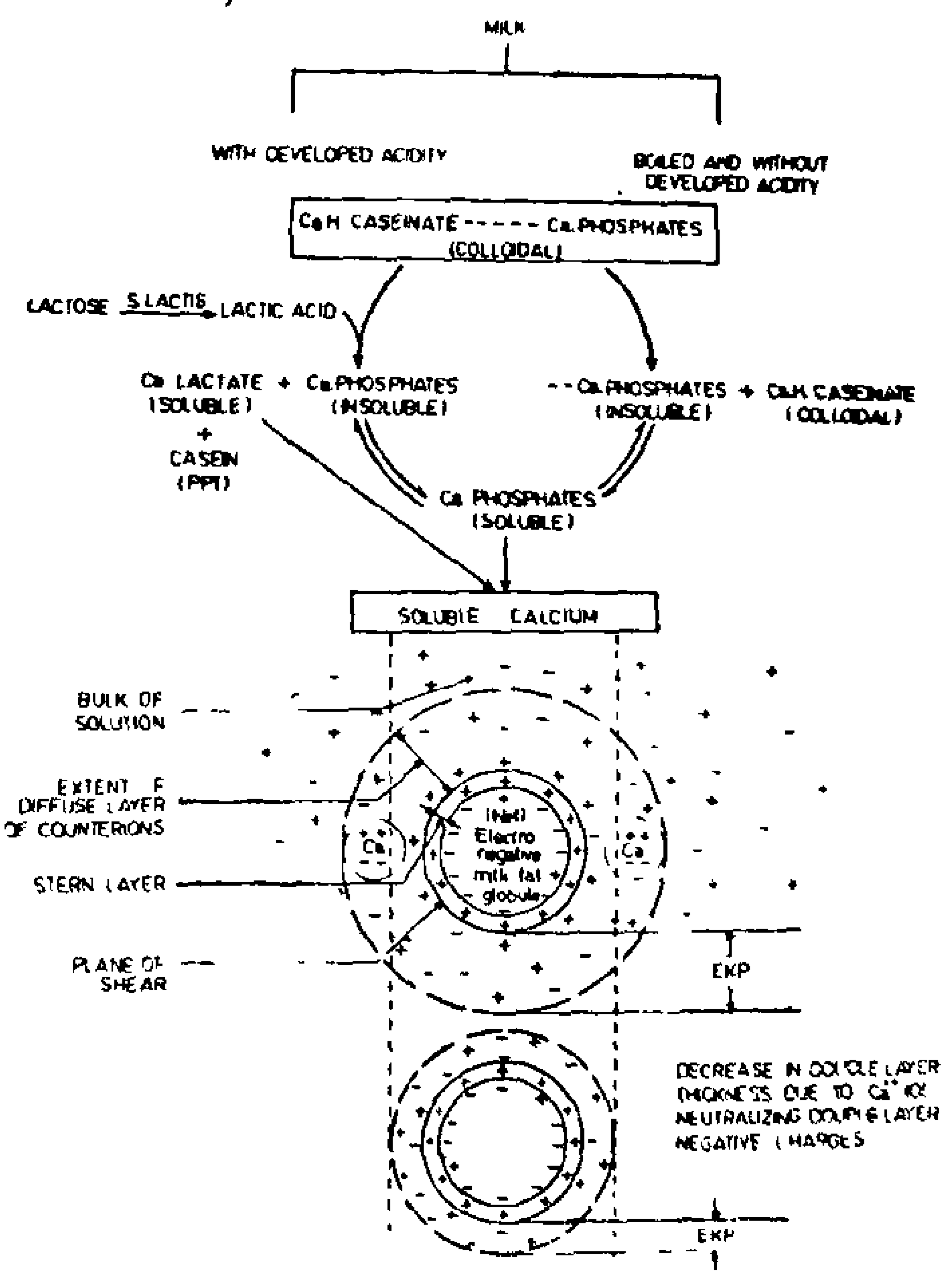

FIG. 4. Schematic representation of the mechanism of EKP decay.

due to its preferential adsorption[14] on the double layer acts as a counter-ion, thereby reducing the EKP due to decreased double layer thickness[15–17].

For the first 10 hours of study, the decrease in the EKP of boiled milk seems to be a pH-independent phenomenon (Fig. 3) where the EKP has dropped down to − 19·1 mV from the initial value of − 23·7 with practically no change in the pH of the system. The EKP decay in this period may be attributed to the release by heating, of physically bound calcium[18], from calcium caseinate calcium phosphates complex, in the form of di- and tri-calcium phosphate. EKP decay in this period progresses slowly and persists for a longer time due to slow availability of calcium from the sparingly soluble calcium phosphates. As the soluble calcium from the bulk enters into the double layer, the equilibrium between insoluble and soluble forms of calcium phosphates is shifted in the direction of soluble form, thus making the calcium available for adsorption at interface. The mechanism of such release is schematically represented in Fig. 4. Beyond ten hours, decay in the EKP of BM can be attributed to the mechanism governing the changes in the EKP of RM.

Riddick[19] pointed out that the natural colloidal systems like milk, blood, etc., exhibit absolute dispersion at relatively low electronegative EKP values. In both the systems, a few millivolts drop in EKP had induced the phenomenon of aggregation of fat globules. This supports the view[20] that natural colloids achieve significant agglomeration with little decrease in EKP.

The fundamental concept of colloid science suggests that the aggregation of the colloid particles only occurs when the EKP of the system is lowered[21]. Milk is a colloid and curd is an aggregated colloid and therefore, the curdling of milk also seems to be governed by the basic laws of EKP elaborated for the colloidal systems.

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1. Palmer, L. S. and Wiese, H. F., *J. Dairy Sci.*, 1933, **16**, 41.
2. Jenness, R. and Palmer, L. S., *Ibid.*, 1945, **28**, 611, 653.
3. Seifriz, W., *Protoplasm*, McGraw-Hill Book Co., Ind., New York, 1936, p. 375.
4. Nugent, R. L., *J. Phys. Chem.*, 1932, 36, 449.
5. Jack, E. L. and Dahle, C. D., *J. Dairy Sci.*, 1937, **20**, 551, 605, 637.
6. Moyer, L. S., *J. Biol. Chem.*, 1940, **29**, 133.
7. Riddick, T. M., *Control of Colloid Stability through Zeta Potential*, Livingstone Publication Co., for Zetameter, Inc., New York, 1968, p. 56.
8. Smoluchowski, M. V., *Z. Physik. Chem.*, 1940, **93**, 129.
9. Panpalia, G. M., Dorle, A. K. and Rambhau, D., *Curr. Sci.*, 1980, **49**, 500.
10. Brunner, J. R., In *Fundamentals of Dairy Chemistry*, eds. B. H. Webb and A. H. Johnson, Avi, Westport, Connecticut, 1965, p. 403.
11. Jenness, R., In *Milk Proteins: Chemistry and Molecular Biology*, ed. H. A. Mckenzie, Academic Press, New York and London, 1970, 1, 31.
12. Riddick, T. M., *Control of Colloid Stability through Zeta Potential*, Livingstone Publication Co., for Zetameter, Inc., New York, 1968, p. 57.
13. Singh, V. B., *Chemistry of Milk and Milk Products*, 1st ed., Asian Publishers, Muzaffarnagar, 1965, p. 29.
14. Shorter, In *Emulsions and Foams*, eds. S. Berkman and G. Egloff, Reinhold Publishing Co., New York, 1941, p. 48.
15. Cockbain, E. G., *Trans. Faraday Soc.*, 1952, **48**, 185.
16. Greiner, L. and Vold, R. D., *J. Phys. Colloid Chem.*, 1949, **53**, 67.
17. Moore, A. and Lemberger, A. P., *J. Pharm. Sci.*, 1963, **52**, 233.
18. White, J. C. D. and Davies, D. T., *J. Dairy Res.*, 1958, **25**, 281.
19. Riddick,, T. M., *Control of Colloid Stability, through Zeta Potential*, Livingstone Publication Co., for Zetameter, Inc., New York, 1968, p. 2.
20. —, *Control of Colloid Stability through Zeta Potential*, Livingstone Publication Co. for Zetameter, Inc., New York, 1968, p. 137.
21. Verwey, E. J. W. and Overbeek, J. Th. G., *Theory of the Stability of Lyophobic Colloids*, Elsevier Publishing Co., Passim, New York, 1948, p. 128