A critical analysis of the various results obtained during the present investigation, detailed in the preceding chapter, has been attempted. Comparison of several data corresponding to the two systems, namely, buffalo milk and cow milk, has also been undertaken. A discussion on such analysis and comparison follows in the present chapter, which has been divided into two sections. The first one, Section 'A', deals with discussion on findings with model milk systems, while the second, Section 'B', is related to study of evaporated milk.
The comparison of the data presented in Table 2 discloses that buffalo skim milk (SKM) contains higher proportions of all the major milk constituents with the exception of two ions, citrate and chloride. Total proteins and individually caseins and whey proteins are present more in buffalo milk, as has been reported by Ghosh and Anantakrishnan (1965) also. Of the minerals, calcium (Anantakrishnan et al., 1943; Singh Verma and Anantakrishnan, 1946; Schneider et al., 1948; and Basu et al., 1962), magnesium (Acharya and Devadatta, 1939) and phosphorus (Basu and Mukerjee, 1943; Anantakrishnan et al., 1943; Schneider et al., 1948; Basu et al., 1962) contents in buffalo milk were higher than in cow milk. As in the present study citrate was reported to be lower in buffalo milk by Anantakrishnan et al., (1943) and Basu, et al (1962). Presence of lower chloride content in buffalo milk has also been corroborated by several workers (Sen and Dastur, 1947; Schneider et al., 1948; Praphulla and Anantakrishnan, 1958, Singh et al., 1961; Basu et al., 1962). Higher lactose content of buffalo milk is well established (Schneider et al., 1948; Basu et al., 1962; Ghosh and Anantakrishnan, 1965).

The milk serum (MS) prepared by dialysis showed a higher content of lactose than the corresponding skim milk, 5.67 and 5.55 g per 100 g for buffalo milk serum and skim milk, respectively (Table 2). Since milk serum did not contain the protein and has relatively less of minerals, the excess
of lactose is essential to maintain iso-osmotic nature of the two fluids. A comparison of the mineral contents of milk serums from buffalo and cow milks discloses that calcium, phosphate, citrate and chloride were greater in cow milk serum, while only magnesium was greater in buffalo milk serum. These mineral contents in milk serum represent the proportion of soluble, ionic forms of the minerals in the milks from the two species. Deficiency of soluble calcium in buffalo milk has been reported by earlier workers (Verma and Anantakrishnan, 1946). Reference to partitioning of other minerals in buffalo milk is, however, not available.

The composition of model milk system (MMS) differed from that of corresponding skim milk. The total milk solids (TMS) of BMS was about 14.24% while that of buffalo SKM was 10.37%. The casein concentration was higher in MMS but lactose, calcium, citrate and phosphate were lower in MMS. Similar observations were recorded in CMS also (Table 2). In MMS as in the case of milk serum osmotic equilibrium has been attained mostly through the exchange of lactose and two proteins. The chloride contents of SKM, MS and MMS were almost similar in both cases of buffalo and cow milks.

pH of different systems

It was observed from preliminary study that the pH of MMS had considerable influence in its viscosity and hence the pH of all the systems were studied regularly. Data from Tables 3, 4 and 5 (and Figures 1-4 in Plate 1) reveal that both for SKM and MMS, the buffalo systems recorded higher pH than the cow systems. Such observations are corroborated
by earlier findings of Bhimsena Rao and Dastur (1955 and 1956 b). From the frequency distribution for pH, it is clear, however, that variation of pH in both buffalo MMS and SKM was greater than that in cow MMS and SKM. In cow SKM five out of eleven samples had their pH between 6.5 and 6.60 and 3 others had between 6.61 and 6.70. But in buffalo SKM 7 out of 16 samples had their pH in the range 6.61 - 6.70 and 6 such samples in the range of 6.51 to 6.60. In case of MMS, pH of 8 CMS samples out of 11 were in the range of 6.61 - 6.70 while only 4 BMS samples out of 16 had their pH in this range. pH of 5 BMS samples were in the higher range of 6.71 - 6.80 and pH of remaining BMS samples varied between 6.35 to 6.60 and 6.81 to 7.00.

**Viscosity of MMS with variable casein**

The viscosity of MMS with varying concentrations of casein alone showed a logarithmic relation between viscosity and casein concentration (Figures 9 and 10, Plate 3). Although comparable data for such variation in MMS is not available, in artificial skim milk similar increase in viscosity with increasing caseinate concentration has been reported (Eilers et al. 1947). It is clear from the figures in Plate 3 that the rise in viscosity with increasing casein concentration is more pronounced in CMS than in BMS. Further, viscosity of CMS was always greater than that of BMS having corresponding casein concentration (Table 6). Difference in the state of hydration and shape of the dispersed casein micelles in BMS and CMS may be one of the causes for such disparity in their viscosities. The axial
ratio of cow milk casein has been reported to be larger than that of buffalo milk casein under identical temperature and pH (Roy and Bhalerao, 1968). The casein from two milks present in colloidal suspension differed in their densities, cow milk casein having higher density in comparison to that of buffalo milk casein (Roy, 1968). Thus CMS containing denser and more elongated ellipsoidal dispersed phase is likely to give higher viscosity than the BMS having same casein concentration. Of several other factors which may be responsible for such difference in viscosities of CMS and BMS, a difference in the chemical make-up may be one.

Southward and Dolby (1968) reported that presence of $\alpha_s^1$-A genetic variant in casein rendered solutions of casein less viscous. The influence of varying properties of $\alpha$, $\beta$, and $\gamma$-fractions of casein on viscosity of its solution is not, however, clear. Casein from buffalo milk contains more $\alpha$-fraction (55.5%) and less $\beta$-fraction (39.1%) as compared to cow milk casein, 44.5% $\alpha$- and 52.4% $\beta$-fractions (Ganguli and Bhalerao, 1964). Aschaffenburg et al. (1968) reported predominance of $\alpha_s^1$-C, $\beta$-A and $\kappa$-A genetic variants in caseins from Indian cow's milk; such information about buffalo milk casein is not, however, available. Consequently, paucity of data prevent further elaboration in this aspect.

Exponential relationship exists between viscosity and casein of BMS (Figures 9 and 10, Plate 3). In BMS two exponential equations exist; one each for casein concentration higher and lower than 6.30 g/100g. In CMS, however,
3 such equations could be derived, one corresponding to casein concentration below 7.80, second between 7.80 and 11.70 and third above 11.70 g/100 g (Table 7). All these equations have general form of the classical Arrhenius equation, namely,

$$\log \eta_v = K_1C + K_2$$

(where $K_1$ and $K_2$ are two constants, $C$ is concentration of dispersed phase and $\eta_v$ is relative viscosity of the dispersion).

Different values of $K_1$ and $K_2$ in the 5 equations derived indicate deviation from a single Arrhenius equation applicable to either 8MB or CMS. It is probable that such deviations at definite casein concentrations indicate occurrence of interaction to various extents between the constituents present as dispersed phase (Muck and Tobias, 1962).

A relatively sharper increase in viscosity of either 8MB or CMS with higher concentrations of casein in the system may be explained due to interaction of casein with water. It is likely that with the increased concentration of casein in the dispersion phase a greater extent of hydration will take place thereby rendering the free water, that is, the dispersion medium less in quantity. Consequently, a greater increase in viscosity will result.

During the next phase of study concentration of lactose in the MMS was varied from its original, normal level to log and 20 g per 100 g to assess the influence of lactose on the viscosity and pH of the MMS. Increasing concentrations of lactose changed the viscosity of MMS in logarithmic manner.
The magnitude of such change, however, depended on the concentration of casein in the MM3. When the casein concentration in the MM3 was lower than a critical value, the influence of lactose was to increase the viscosity of the MM3. But lactose decreased the viscosity of MM3 containing casein above this critical concentration (Tables 8 and 9 and Figures 11 and 12 of Plate 4). Such critical concentration of casein capable to reverse the influence of lactose on viscosity of MM3 was different for the two systems, namely, 8.64 g/100 g for the MM3 and 8.20 g/100 g for the CMS (Figure 13, of Plate 5). It is difficult to ascribe any particular cause for such a difference. However, differences in the composition of casein constituents (Ganguli and Bhalaria, 1964) in the shape (Roy and Bhalaria, 1966) and in density (Roy, 1966) of the two casein from buffalo and cow milk may have their influence on such critical concentration. The increasing concentrations of lactose lowered the pH of the MM3 in some samples to a slight extent, not more than 0.1 unit, while in others no such influence of lactose was observed. The foregoing results indicated the presence of different types of interaction between casein and lactose, depending upon the casein concentration of the MM3. At casein concentrations below the critical value increase in concentration of lactose probably lead only to modification and enhancement of hydration of both casein and lactose particles, thereby leading to increased voluminosity of the dissolved units reflected by the increase in viscosity of such systems (Eilers et al., 1947). In case of MM3 with casein concentration
higher than the critical value, interaction between casein and lactose might have set in, probably accompanied with release of water bound to the casein and lactose units. Such interaction and consequent contraction in voluminosity of the dissolved units and simultaneous increase in free water content are likely to decrease viscosity of the system, as has been observed. Increasing lactose concentration might have intensified such casein-lactose interaction.

Results for viscosity of either BMS or CMS (Tables 8 and 9, respectively) indicate that although in general there was greater viscosity for a MMS with higher casein concentration, in few cases such order was reversed. A critical analysis of the data from these tables reveals that the pH of the MMS had an influence on its viscosity, besides casein concentration. System with higher pH but slightly lower casein concentration showed greater viscosity than a system having more casein and lower pH. Such anomalous viscosity has been reported earlier also (Southward and Dolby, 1968), but the role of pH of the solutions has not been taken into account. From the present study the influence of pH of the MMS on its viscosity has been established. There can be, however, other reasons for such anomalous viscosity, e.g., nature of the casein prepared from milk of early or late lactation period, the former variety giving more viscous solution than the latter one (Southward and Dolby, 1968).

A regular decrease in both viscosity and pH of the MMS was observed during storage (Tables 10-13). Such lowering in viscosity might have been due to enhancement of casein-lactose interaction with continued storing. Lowering of
viscosity was not caused by development of acidity, as manifested by decrease in pH of systems, and the same has been concluded from the results presented in Tables 14 and 15 and Figures 14, 15 (Plate 6) and 16 (Plate 7). Addition of toluene as a preservative for the MMS although prevented considerable lowering in pH of the MMS during storage could not arrest the fall in their viscosity. Considerable lowering in the pH of MMS, to a range of 5.2 to 5.8, on prolonged storing, however, had an influence on viscosity. In this pH range, the system clotted and prior to such clotting a sharp increase in viscosity was invariably obtained. The BMS clotted at relatively higher pH than the CMS. However, either at comparatively higher pH maintained at constant level with addition of toluene or at gradually falling pH due to absence of preservative a decrease in viscosity of the MMS was observed, indicating that other factors besides acidity were responsible for such viscosity lowering. On the other hand, the concentration of casein in the MMS had shown to influence considerably viscosity of MMS, more so when lactose was also in larger proportion. For a casein concentration of 6.19 g/100 g of BMS without addition of lactose the viscosity decreased very little on storing even for 7 days; a decrease of only 2.27 per cent with lowering of 1.62% in pH was recorded in the sample, data for which is being reported. When lactose was added to the above system, decrease in viscosity was 3.92% and that in pH 1.80%. One BMS containing 9.88 g/100g casein showed a considerable decrease in viscosity even on storing for 24 hours, irrespective of any lowering in pH. Viscosity lowering in this
case was found to be 19.44% accompanied by only 1.36% drop in pH, which continued steadily to 44.3% drop when no preservative was added. The decrease in viscosity in the corresponding toluene preserved system was 9.48% without any change in pH, and 39.1% with decrease in pH by 5.1%. The decrease in viscosity for a CMS containing 14.15 g/100g casein to which lactose has been added was found to be 53.9% for increase in pH by 0.18%, on similar storage with toluene. Such viscosity lowering proceeded further to 55.2% and 61.7% for systems with and without preservative, respectively, during storage for 5 days. Likewise, viscosity of similar CMS decreased continuously with storage. On one particular sample with 11.99 g casein/100g decreases recorded, were 55.4% and 61.8%, respectively, for a system with and without preservative during storage of 3 days.

From foregoing results one is prone to infer that mutual interaction between casein and lactose takes place in concentrated milk system even at ordinary temperature, particularly when the proportion of casein present is high, and such interaction gets augmented on storage. There are a number of reports indicating protein-lactose interaction in heated milk systems (Grimbley, 1954; Nielsen et al., 1963; Henry et al., 1948; and Leviton and Pallansch, 1962). It has also been reported (Tarassuk and Tamsma, 1956) that milk of high solid contents (26.34%) resists gelation, i.e., increase in viscosity, better than milk of normal concentration when subjected to heat, although specific reason for the same has not been ascribed to. It is likely that the casein lactose interaction taking place at ordinary
temperature in highly concentrated casein-lactose system will be intensified on heating, thereby preventing rise in viscosity, as has been reported in the present study.

**MMS with micellar casein**

The behaviour of MMS prepared with micellar casein, i.e., casein separated from skim milk by high speed ultracentrifugation, could not be studied effectively, particularly with higher casein concentrations, since such systems were relatively unstable and clotted readily. However, a critical analysis of the results in Tables 18, 19 and 20 reveals some interesting observations. Viscosity of such systems during storage for 5 days although showing considerable decrease, did not change appreciably when more lactose was added, unlike the same observed in MMS prepared with acid precipitated casein. Calcium, present in micellar form of the casein, but almost absent in acid casein, might have some inhibitory effect on the casein-lactose interaction taking place in the MMS, particularly during storage. A difference between the behaviour of BMS and CMS prepared from micellar casein due to addition of 20% lactose and subsequent storing (Tables 19 and 20, and Figures 20 of Plate 8) lends further support to such an explanation. Buffalo micellar casein with higher colloidal calcium (Verma and Anantakrishnan, 1946) effected relatively less lowering in viscosity of the MMS on storing than cow micellar casein with lower calcium content.

**Influence of total whey proteins on viscosity of MMS**

A study of the role of addition of whey proteins
separated in the total form from milk (WP) on two physico-chemical properties of MMS, namely, viscosity and pH, revealed that there was hardly any change in the pH of MMS due to addition of WP. The viscosity of the MMS, however, changed considerably (Tables 21 and 22; Figures 24 of Plate 10). The increase in viscosity was fairly similar for both BMS and CMS when 2% and greater concentrations of WP was incorporated into the MMS. At lower WP concentration, 1.5%, the rise in viscosity in BMS was slightly higher, by 20.44% as compared to that in CMS by 13.72%. Forewarming by heating to 96°C ± 1°C for 5 minutes augmented this increase in viscosity of MMS due to WP (Tables 23 and 24 and Figures 24 of Plate 10). In heated samples, however, the magnitude of increase in viscosity was considerably greater in BMS for all concentrations of WP. Further, a contrasting behaviour was observed in the two systems, BMS and CMS, containing WP due to heating. On calculating the effect of heat alone on the rise in viscosity of MMS containing identical quantities of WP it appears that for BMS the effect of heat decreases with increasing WP concentration. For example, at 1.5% WP level, there was an increase in viscosity by 9.15%, which changed to 6.08% for 2.5% WP in the system. But in case of CMS heating caused greater change in viscosity with higher concentration of WP, namely from 3.45 to 5.12% with increasing WP from 1.50 to 2.50 g/100 g. It is possible that incorporation of WP in the MMS led to some interaction between this milk constituent with others. Presence of such interaction has been reported earlier from electrophoretic studies (Hartman and Swanson, 1965 and Beeby, 1966). More numerous
studies, however, have been reported to demonstrate interaction between two individual components of WP, namely $\alpha$-lactalbumin and $\beta$-lactoglobulin with other protein components like casein (Tzamtman and Swanson, 1950; Ntalianas and Grimbleby, 1962) with $\alpha$-casein (Kresheck, 1962), with $k$-casein (Kresheck, 1962; Long et al., 1963) and also between these components themselves (Yamauchi, 1961; Hunziker and Tarassuk, 1965 and Nakanishi et al., 1968).

Evaluation of the role of these individual WP components on viscosity of MMS has been attempted during the present study and the findings will be discussed elsewhere. The reason for a difference in the extent of interaction between WP and other milk constituents in BMS and CMS is difficult to explain. Presence of more casein and also of minerals in the BMS may be one of the factors for such discrepancy.

Incorporation of additional lactose in two MMS containing WP to make its concentration to 15 g/100 g increased the viscosity of the systems by about 8% in BMS and about 6% in CMS for all the concentrations of WP. Additional lactose to MMS without WP did not change appreciably the viscosity of the MMS. Such results indicate that lactose has some influence on WP or on other major milk constituents in presence of WP. The effect of lactose, however, is about one fourth of that of WP on the viscosity of either BMS or CMS (Tables 21 and 22). Forewarning of BMS after incorporation of lactose, effected the viscosity of BMS to slightly counteract the influence of WP (columns 3 and 5 of Table 23). In CMS, however, such effect of lactose
was less prominent than in BMS. Under identical conditions
the viscosity of BMS and CMS without whey proteins increased
slightly (11.47\% BMS and 8.02\% for CMS) which indicates
interaction between lactose and casein or minerals. A
possible interaction between WP and casein or minerals has
been pointed out earlier. Thus additional lactose above its
normal concentration is shown to counteract both types of
interactions, described in preceding lines. Ntaiilianas and
Grimbleby (1982) in their synthetic milk system study
observed that lactose minimized heat induced casein -
\(\beta\)-lactoglobulin interaction. Similar inhibitory influence
of lactose on interaction between different casein and whey
protein components has been confirmed by Yamauchi (1961)
and Yamauchi and Tsugo (1961). From the present study such
inhibitory action of lactose has been demonstrated to be
more in the case of BMS with buffalo WP than in CMS with
cow WP. Since the role of whey proteins in general and
\(\beta\)-lactoglobulin in particular on viscosity and gelation of
evaporated milk has been stressed upon by several workers
(Rose, 1981 and 1982; Davies, 1959; Pyne, 1958; White and
Davies, 1958; Pyne 1962; Rose, 1965; Tilley, 1960; and Morr
\textit{et al}, 1962), this aspect of the study was extended by
interchanging the WP separated from milk of one species
with casein from the other species present in the MMS.
Results from such study are discussed afterwards. Addition
of WP and also WP with lactose to the MMS did not change
appreciably the pH of the systems either at normal temperature
or after forewarming (Tables 21-24)

Change in viscosity and pH of either BMS or CMS
incorporated with WP and further with lactose due to storage showed fairly similar pattern (Tables 25 to 28; Figures 25 to 28 Plate 11). In the initial stage of storage there was slight increase in viscosity of the system, upto 3 days for BMS and upto 5 days for CMS. On further storing viscosity of both the systems decreased in a more or less similar manner. The control sample without addition of WP in BMS showed regular and almost similar lowering in viscosity during the entire period of storage. In CMS, however, decreasing viscosity of the control system was more than that in systems with WP. Likewise, in both BMS and CMS with addition of lactose a greater lowering in viscosity during storage was observed in the control systems. Such results indicated a viscosity stabilizing influence of WP from cow milk and also of WP from both milks in conjunction with lactose. Similar stabilizing influence of WP of buffalo milk although not established from the results cannot be ruled out, since the differences in the extent of viscosity lowering are not very great. During storage there was lowering in the pH of the systems and such lowering was decidedly greater with either increased concentration of WP or extended period of storage, for both BMS and CMS. Presence of lactose in both the systems prevented such lowering in pH to a considerable extent.

**Interchanged systems**

Interchanging of the constituents, both dialysable materials and the total whey proteins, from milk of one species with those from milk of the other species and study
of viscosity and pH of the resulting MMS has been undertaken. Analysis of the results described through the Tables 29 to 44 shows that it has not been possible to establish a definite and differential influence of whey proteins from milk of one species on the viscous property or on the pH of a model milk system containing either casein or the dialysable constituents from milk of the other species. For example, the viscosity of BMS prepared by dialysing buffalo milk casein (BMC) in buffalo skim milk (BSKM) showed an increase in viscosity by 37.37% at room temperature and by 67.92% after forewarming, due to addition of 2.5g/100g cow whey protein (CWP). In comparison viscosity of such a system due to addition of 2.5g/100g buffalo whey protein (BWP) increased by 36.1% at room temperature and by 70.96% after forewarming (calculated values from Tables 29 and 30). Changes in viscosity of such BMS incorporated with lactose, 15g/100g, due to addition of either CWP or BWP followed a pattern similar to the one described above. When a BMS was prepared by dialysing BMC in cow skim milk (CSKM) and 2.5g/100g CWP was added to such BMS its viscosity increased by 30.02% at room temperature and by 61.39% after forewarming. Viscosity of such a BMS incorporated with 2.5g/100 g BWP instead of CWP showed rise in viscosity by 33.75% at room temperature and by 61.69% after forewarming, as derived from results presented in Tables 37 and 38. Addition of lactose only increased the viscosity slightly more without altering the trend of change. Although the effect of BWP and CWP on viscosity of either BMS or CMS
was not distinctive, an influence of dialysing medium on the viscosity of the MM3 was observed. Comparison of the results from Tables 29, 33, 37 and 41 clearly indicates that when the dialysing medium is BSKM the MM3, whether BMS or CMS, is produced with relatively lower basal viscosity than the MM3 prepared by dialysing against CSKM has. It has been pointed out earlier while discussing the results (in Table 6) that CMC yielded CMS with greater viscosity than BMS could lead to in BMS having identical casein concentrations. It is now further shown that dialysable constituents from cow skim milk render the MM3 prepared with those more viscous. Since only difference in relative proportions of dialysable constituents like lactose (Ghosh and Anantakrishnan, 1965; and Table 2) or the minerals (Verma and Anantakrishnan, 1946; and Table 2) are known and no qualitative difference between the two has been reported, an explanation for the above mentioned influence of CSKM dialysable constituents may be sought in such quantitative differences. The influence of additional lactose, upto 15g/100g, can be seen to be independent of the type of the skim milk (as can be deduced from results in Tables 29, 33, 37 and 41). CSKM contains less calcium and more citrate than BSKM, correspondingly CMS from CSKM contains less calcium and more citrate in comparison to BMS from BSKM (Table 2). It can be seen from the results of Tables 70, 71 and 88, 89 and also the histograms in Figures 43 of Plate 17 that increased concentration of calcium ions decreased viscosities of both BMS and CMS while such increase in
citrate concentration raised their viscosities. It may be possible when BBS is prepared dialysing against B3KM comparatively more of calcium but less of citrate enters into the BBS and reverse action takes place in BMS prepared against CSKM, thereby giving a lower basal viscosity to the former class of BBS as compared to that of the latter class.

The influence of forewarming was mostly to augment the viscosity rise of either BMS and CMS prepared by normal and interchanged dialysis, without imparting any more characteristic difference to the effect of the WP from buffalo milk or cow milk. A similar non-characteristic change in the viscosity of either BMS or CMS due to WP from buffalo or cow milks is seen from results of Tables 31, 35, 39 and 43. If at all any difference can be concluded from such results, it is in the slightly better viscosity stabilising effect of the buffalo milk WP on both BMS and CMS.

Effect of **α**-lactalbumin

Analysis of the results in Tables 45 to 48 brings out informations regarding the role of **α**-lactalbumin (LA), one of the major components of the total whey proteins, on viscosity and pH of the MMS under different conditions. The pH of either BMS or CMS were not influenced by the addition of LA, alone or in conjunction with lactose, or by subsequent forewarming treatments at 96°C ± 1°C for 5 minutes. A similar inference was drawn in the case of WP also. Addition of LA caused linear increase in viscosity of both BMS and CMS with increasing concentration (Figures 29 Plate 12).

For the BMS viscosity increase ranged between 6.00 to 14.07
per cent corresponding to the LA concentration of 0.30 to 0.90 g/100 g. Such rise in viscosity in CMS due to LA was relatively less than in BMS, in the range of 3.53 to 9.00 per cent, due to addition of 0.30 to 0.90 g/100 g of the protein. A comparison of these results to those derived from Tables 21 and 22 detailing the influence of WP on viscosity of MMS reveals that LA caused slightly lower increase in viscosity of a MMS than did the WP, measured for successive, identical increments of the protein concentration. The influence of forewarming of MMS with added LA on their viscosity is seen to be controlled by the concentration of LA present. Increasing concentration of LA could prevent the decrease in viscosity of the control MMS due to heating. In fact, for higher LA concentration, 0.90 per cent for BMS and 0.60 per cent and above for CMS, there was viscosity rise due to heating. Addition of lactose along with LA appears to have a small influence to increase MMS viscosity. Such influence appears to be independent of the increasing whey protein concentration in both heated and unheated BMS samples (Table 45 and 47 and Figures 29 Plate 12). In CMS, however, the combined effect of heat and lactose was seen to be dependent on LA concentration. For higher LA concentration lactose and heat lead to interaction and subsequent decrease in viscosity. As in the case of WP with LA also the effect of heat was more prominent than that of lactose addition on the MMS viscosity.

Storage study on CMS incorporated with LA did not reveal any difference in the trend of such results as obtained with either BMS or CMS incorporated with respective WP.
In case of BMS however incorporation of LA stabilized viscosity of the system and as a consequence there was hardly any change in its viscosity during storage up to five days (Figures 25 to 28, Plate 11 and Figures 31 and 32 Plate 13). Thus, it has been observed that the LA from buffalo milk behaved differently from the same from cow milk, in so far as changes in viscosity with heating and with storing was concerned.

**Effect of \( \beta \)-lactoglobulin**

The role of \( \beta \)-lactoglobulin (LG), the second major constituent of milk whey protein, on viscosity and pH of BMS can be ascertained from the results through Tables 53 to 60 and also Figures 30 of Plate 12 and 32 of Plate 13. The LG, like the WP and also LA, was found to have very little influence on the pH of both BMS and CMS. During the present study relatively smaller amounts of LG from buffalo milk has been incorporated into BMS, 0.45 g/100g, since this fraction of the whey protein is present in buffalo milk at a much lower concentration than in cow milk (Ghosh and Anantakrishnan 1965, Ismail et al. 1970). Addition of LG, like that LA, increased the viscosity of both BMS and CMS linearly with increasing concentration of the protein (Figures 30 Plate 12). For the BMS viscosity increase ranged between 5.49 and 15.78 per cent for concentration of LG 0.15g to 0.45 g/100g of the system. Such rise in the viscosity of cow milk system were from 4.67 per cent to 10.36 per cent, which is comparatively lower than those in BMS. In this respect effect of LG was similar to that LA.
The influence of LG in raising the viscosity of BMS compared in terms of identical concentrations of the proteins, was more pronounced than that of LA, but less than that of the WP (results in column 3 of Tables 21, 45 and 53). In CMS, however, the roles of both the fractions, LG and LA, were similar, but the same was less pronounced than that of the WP. The effect of heat on viscosity of MMS incorporated with LG varied with the species. In BMS addition of LG from buffalo milk and subsequent forewarning caused gradually less pronounced lowering with increasing concentration of the protein. In the case of CMS, however, such lowering in viscosity was observed in samples with lower LG concentrations, and increased concentrations of the protein caused increase in viscosity, (Tables 53 to 56) from the results of the same tables it can be inferred that the effect of lactose on the viscosity of BMS is to moderately increase the same for all concentrations of LG. But in the case of CMS lactose caused an equal lowering in viscosity. When the samples containing both LG and lactose were heated the differential behaviour of the BMS and CMS was observed to a lesser extent. In BMS under these conditions there was a greater increase in viscosity, particularly at lower concentrations of LG indicating that lactose at ordinary temperature does not interfere with the effect of LG to any appreciable extent, but on heating it exerts definite influence, may be through inhibition of interaction between LG and other milk constituents. In case of CMS heating leads to augmented interference, as it is apparent from the
considerable lowering in viscosity of the systems particularly at greater concentration of LG, 0.45 and 0.60 g/100 g. Evidence of such interfering role of lactose has been cited by several workers (Yamauchi, 1961; Yamauchi and Tsugo, 1961; Ntalianas and Grimeley, 1962). From the foregoing discussion and also from relevant points discussed under the effects of WP and LA it can be pointed out that the influence of WP on viscosity of MMS was more prominent than that of either LA or LG; of these two fractions the LG one was observed to have a greater role on viscosity. Further, in general, the effects of the cow milk whey proteins, both total and fractional, on viscosity of CMS was more pronounced than those of their counterparts on BMS viscosity.

The effect of storage on viscosity of BMS incorporated with LG was appreciably contrasting to that of viscosity of CMS under identical conditions (Figures 33 and 34 Plate 13). In the former case LG not only prevented in the lowering of viscosity during storage upto 5 days, but also slightly increased the same; such increase in viscosity was more prominent with higher concentrations of the protein and also with addition of lactose. In case of CMS, however, there was a gradual, but slight fall in viscosity with progressive storing. Such increase or decrease in viscosity in BMS or CMS, respectively, were unaffected by pH, as is evident from continued lowering in pH with storing in both the systems (Tables 57 to 60).

Role of milk lipids on the viscosity of MMS

A knowledge of the role of milk lipids on the viscosity
of MMS is revealed from an examination of the results presented in Tables 62 to 65 which describe the viscosity changes in both MMS and CMS incorporated with milk lipids in concentrations ranging from 5 to 15 g/100g, and also with additional lactose to 15 g, at room temperature and after forewarming. Addition of lipids progressively increased the viscosity of both MMS (Table 62) and CMS (Table 63). The rise in viscosity in both the systems increased with increasing concentrations of milk lipids, as can be seen from the relative slopes of the straight lines depicting such changes in Figures 35 and 36 in Plate 14. Further, the viscosity rise was greater in CMS than in MMS. Calculation from data in Tables 62 and 63 discloses that in the MMS viscosity increased by 19.04, 59.92 and 171.5 per cent due to addition of 5, 10 and 15 g of lipids, respectively. But in CMS the corresponding increments were 33.15, 101.5 and 194.4 per cents.

Rutz and Whitnah (1957) in their study on viscosity of cow milk as affected by further fat and protein contents have also observed a high positive correlation between viscosity and butter fat content of the milk.

Forewarming of the MMS incorporated with lipids at different levels decreased their viscosity, but change was less appreciable in MMS with higher lipid concentrations and maximum in the control system. The percentage increase in viscosity due to successive addition of 5 per cent lipids was more in the heated samples than in those at room temperature. For example, in MMS at room temperature the viscosity increased by 19.40 per cent but when heated the same increased
by 28.12 per cent for addition of 8% lipids (Tables 62 and 64). Likewise, increments corresponding to 10 and 15 per cent lipids were 59.92 and 171.5 for BMS at room temperature and 83.44 and 202.3 for BMS after heating. From the foregoing discussion it appears that lipids interact with other milk constituents to add to viscosity of the system. Further, the influence of heat was to augment such interaction at least at the greater lipid concentrations from 10 and 15 per cent. Similar evidence of interaction between fat and other milk constituents and acceleration of the same due to heat has been reported by other workers (Mick and Tobias, 1962).

The effect of incorporation of lactose above its normal concentration to BMS was found to be clearly dependent on the lipid concentration and also on the specific nature of the system. In BMS (Table 62) at 5% lipid level lactose addition increased its viscosity by 7.07 per cent while such increase was less prominent, 5.14 per cent, at 10% lipid level. But at the maximum lipid level of 15% addition of lactose lowered the viscosity by 5.88 per cent. In the CMS (Table 63), on the contrary, addition of lactose lowered the viscosity at all levels of lipids, by 0.10 per cent at 5%, by 3.99 per cent at 10%, and by 12.23 per cent at 15 per cent level of lipids. Such a differential behaviour in BMS and CMS may be explained from difference in physical state of the lipids from these two milks. The cow milk fat globules are smaller in size as compared to those from buffalo milk (Kothavalla and Sunawalla 1937; Puri et al, 1952). Consequently, for identical mass of lipids the fat globules from
cow milk will have greater surface area and thereby will be likely to interact with other milk constituents, like the added lactose, to a greater extent as compared to buffalo milk fat globules. Hence, the lowering of viscosity in BMS observed only at 15 per cent lipids level could be encountered in CMS even at 5 per cent level of lipids. Prior to addition of lactose to 15% viscosity rise was decidedly greater in CMS, as pointed out earlier. In such cases cow milk fat globules might have been involved in interaction relatively more than buffalo milk fat globules with the protein constituents of the milk systems thereby leading to greater rise in viscosity. Addition of lactose appears to have disturbed such lipid-protein interaction leading to lowering in viscosity. Heating of the MMS subsequent to addition of lactose did not alter much the viscosity of either BMS of CMS at 5% lipid level. With increasing concentration of lipids, however, heating caused rise in MMS viscosity, slightly more in BMS than CMS. Heating in absence of excess lactose on the other hand caused relatively little less change in MMS viscosity. Although the data collected from the present study are not adequate to explain such changes, the effect of heat in melting the fat globules and thereby causing some dissociation from lipid-lactose interaction or augmentation of lipid-protein interaction can be a possible factor for such difference. From the study on the effect of storing on viscosity of the MMS it could be seen that incorporation of lipid did not alter the trend of moderate lowering in viscosity very much in BMS or CMS up to 5 days of storing (Tables 56 to 67 and Figures 37, Plate 15).
Similar inference could be drawn for MMS with 15 per cent lactose in addition to varying concentrations of lipids. During this period the lowering in pH of the MMS was also very little. On continued storing, however, the decrease in viscosity was more prominent and also was greater with higher lipid concentrations. Further, when compared between these two systems of BMS and CMS it was observed that addition of lactose to CMS led to relatively greater lowering in viscosity due to storage beyond 5th day. Incorporation of lactose led to change in viscosity of MMS which was fairly similar to those described in the preceding lines (Tables 68 and 69; Figures 38, Plate 15). Continued storage beyond 5th day led to considerable fall in pH in all the samples till those clotted. Addition of lipids although could prevent to a certain extent the decrease in pH but not the clotting of the samples (Tables 66 to 69). On the contrary, lipid-free samples clotted at much lower pH during same interval of storage, e.g., 15 days.

Role of the major milk minerals on viscosity and pH of MMS.

A comparative and analytical study of the results presented through Tables 70 to 103, and also through graphs and histograms in Plate 16 and 17, discloses a number of informations regarding the role of five major milk minerals on viscosity and pH of model milk system. These minerals are calcium, magnesium, chloride, citrate and phosphate. The first two, i.e., calcium and magnesium, are cationic, and the remaining three are anionic. A distinct difference between the influence of cations from the same of the anions
on the viscosity of MMS has been observed. Further, the role of each of these minerals on BMS and CMS has been found to be characteristic. Details of such observations are discussed in the following paragraphs.

Comparison of the graphs in Figures 39 to 42 (Plate 16) reveals the opposite nature of the effects of the milk cations and anions on viscosity of MMS. Calcium and magnesium decreased the viscosity of either BMS or CMS progressively with increased concentrations of the ions, whereas chloride, citrate and phosphate increased the viscosity. Moreover, quantitative differences were observed in the roles of calcium or magnesium and similarly in those of chloride, citrate and phosphate. The extents of such change due to addition of these minerals can be assessed by suitable calculations from the data presented in Tables 70, 71, 76, 77, 84, 88, 89, 96 and 97. Comparing the effects of calcium and magnesium ions on BMS viscosity (Tables 70 and 76) it can be seen that for an increase of calcium ions in BMS by about 160% viscosity decreased by 35.46 per cent while for a similar increase in magnesium ions the lowering in viscosity was about 11 per cent in the lower range of magnesium concentration. The effect of magnesium ions on viscosity of CMS was not much different from the same on BMS viscosity just described. The effect of calcium ions on CMS viscosity, however, was relatively less when compared to that on BMS viscosity, namely, decrease by 15.5 per cent from 160 per cent increasing calcium content at the initial state, followed by 26 per cent lowering in viscosity due to about 160 per cent rise in calcium concentration at the second step (Table 71).
Assessment of the roles of calcium and magnesium ions attempted during the present study, can not be compared with similar data from other workers since such data are not readily available. Influence of calcium ions in lowering the viscosity of skim milk has, however, been established by several other workers (Anon, 1942-43; Thompson, et al, 1957; Beeby and Kumetat, 1959). D'Yachenko and Ngo-Loi (1967) reported similar observation from their study with whole milk. One of the effects of addition of calcium or magnesium ions to the MMS may be formation of divalent ionic bonding with free negatively charged groups of casein moiety and subsequent replacement of hydrogen bonding leading to dehydration of casein micelles. The surface area of the dispersed casein micelles is decreased either through dehydration or through increased aggregation due to bonding with divalent cations, and thereby a lowering in viscosity of the dispersion, that is the MMS, follows. Progressively greater lowering in viscosity of CMS due to addition of increasing amounts of calcium ions (Table 71), and of magnesium ions (Table 77) and also of BMS due to addition of magnesium ions (Table 76) lends support to such an explanation. A similar explanation for the viscosity lowering capacity of cations through increased aggregation of casein has been offered by Zittle et al (1956) in their study with calcium caseinate sols. A greater ability of calcium ions to lower viscosity of MMS compared to that of magnesium ions may be due to larger ionic radius of the former than that of the latter (Glasstone, 1960). In case of BMS viscosity due to addition
of calcium ions other factors, such as a high initial concentration of colloidal calcium, that is calcium associated with buffalo milk casein (Verma and Anantakrishnan, 1948), might have interfered with further formation of double ionic bonding with calcium ions above a concentration of 175 mg per 100 g.

In discussing the relative effects of the three anions in increasing viscosity of MMS it is apparent that the influence from chloride ions is minimum but uniform throughout an increased concentration of over 450 per cent. The main objective for studying the effect of chloride ions added as NaCl on viscosity of BMS was to assess the influence of this anion in the role for calcium ions added as calcium chloride solution. Since a slight increase in viscosity of BMS, 4.35 per cent for about 300 per cent additional chloride, was observed (Table 84) while addition of calcium chloride caused several-fold decrease in viscosity (Figure 39, Plate 16) presence of any major role of the chloride ions in this respect was ruled out.

The effects of both citrate and phosphate ions on MMS viscosity were found to have considerable dependence on the species of MMS. Increase of citrate ions in BMS in the lower range of about 360 per cent caused viscosity rise by 6.75 per cent. But citrate ions in higher range of about 500 per cent increased concentration effected viscosity rise by 24.45 per cent (Table 88). In CMS, however, at the initial stage of increased citrate concentration by about 200 per cent, 15.37 per cent rise in viscosity occurred.
Further, increase in citrate concentration did not effect much augmentation of viscosity (Table 89). Such a differential behaviour of citrate ion on BMS and CMS viscosity is prominently disclosed by the slopes of the lines in Figures 42 of Plate 16. These figures additionally disclose that the influence of citrate ions on CMS viscosity is almost independent of ion concentration above 300 mg per 100 g, while in BMS a progressive viscosity increase takes place with greater citrate concentrations. It may be pointed out that in CMS the initial concentration of citrate ions is relatively greater than in BMS (Table 2). Such a limitation on the role of citrate ions in CMS is comparable to that of calcium ion in BMS discussed earlier.

The influence of phosphate ions on the viscosity of BMS and CMS was found to be reverse to that of citrate ions. The BMS viscosity increased considerably by 15.22 per cent due to rise by 200 per cent phosphate ions measured as P. Additional increase of phosphate by 150 per cent changed the viscosity further to 17.38 per cent, by only 2.66 per cent. In case of CMS, however, 220 per cent increase of phosphate ions effected a rise in viscosity by 6.20 per cent and addition of 150 per cent phosphate ion augmented the same by 9.36 per cent. The above results indicate an early saturation of the effect of phosphate ions on BMS but not on CMS. It may be pointed out that CMS contained relatively less phosphate than BMS (Table 2). The influence of citrate ions in increasing viscosity of either skim or whole milk has been reported by several workers (Anon 1942-43; D'Yachenko and Ngo-Loi, 1967; Vujicic, et al, 1968). Likewise, other
workers have recorded that addition of orthophosphate increased viscosity of milk (Anon, 1942-43; D'Yachenko and Ngo-Loi, 1967; Vujicie, et al., 1968). Many such workers have explained such increase in viscosity due to addition of citrate or phosphate ions through increased hydration of casein particles. Besides this explanation it may be pointed out that incorporation of either citrate or phosphate may lead to their preferential combination with divalent cations like calcium and magnesium and thereby removing those from casein complexes. Elimination of calcium and magnesium is likely to give place to hydrogen bond formation and consequent hydration. According to Pyne and McGann (1960) the colloidal calcium phosphate of milk is more like a colloidal calcium phosphate-citrate. Thus, addition of one of these two anions which is present in relatively lower concentration in the milk of a particular species is likely to have a comparatively greater influence on its viscosity change than the addition of the other anion, and the same have been observed during the present investigation. Incorporation of the minerals was done without causing considerable change in the pH of the MMS, particularly so in case of the anions, consequently, the slight changes in pH occured with addition of the minerals were not of significance.

The effect of heating MMS after incorporation of either calcium or magnesium appeared to be very little (Histograms in Figures 43, Plate 17) in either BMS or CMS. In case of anions, however, heating of MMS with citrate caused considerable increase in their viscosity, more
prominently in BMS. On the contrary phosphate added samples showed considerable lowering in viscosity due to heating and in this case CMS was affected much more than BMS. (Tables and Figures cited above). Differences exist in the nature of interactions between citrate and phosphate ions with casein and in the solubilities of calcium citrate and calcium phosphate, and such differences may be responsible for the differential behaviour in the influence of citrate and phosphate ions followed by heating. It has been reported by several workers (Hilgeman and Jenness, 1951; Morr, 1967; Vujicic, et al, 1968) that addition of citrate ions increases the soluble calcium content of skim milk while that of orthophosphate ions decreases the soluble calcium and increases the colloidal calcium content. Further, such effects are aggravated by heating. Similar observations for lowering the soluble calcium due to heating in the presence of phosphate ions in milk salt solution has been reported by Verma and Sommer (1958). It is quite possible that removal of calcium from the colloidal state, and particularly from the casein-calcium-complex aggregates, would be replaced by greater hydration of the casein complex leading to increased viscosity of the system. On the contrary, increase in calcium in the casein-calcium complex would hinder such hydration and as a result lower the viscosity. Thus, addition of phosphate ions and subsequent heating have lead to lowering of viscosity in both BMS and CMS, while heating subsequent to addition of citrate has increased viscosity. McGann and Pyne (1960) have also indicated considerable increase in viscosity of milk due to
removal of colloidal phosphate.

Addition of lactose in conjunction with the several minerals lead to slight modification in viscosity of the MMS. In all the cases of both cations and anions lactose incorporation has tended to slightly increase viscosity of the systems. The overall effect, however, has been different for the cations and anions. In the cases of the MMS containing added calcium and magnesium ions, addition of lactose has resulted in curtailing to some extent the ionic influence in decreasing the MMS viscosity. The effects of the anions, citrate and phosphate to increase MMS viscosity have been accentuated by the addition of lactose. Herrington (1934) has reported interaction between lactose and milk minerals, mainly calcium chloride. Smeets (1955), on the contrary, could not detect any soluble complex between lactose and calcium. The results from the present study, however, are indicative of some interaction between lactose and the cations, probably more with magnesium than with calcium ions. Removal of the divalent cations by such interactions might have helped to counteract the viscosity depressing action of such cations or to facilitate the viscosity increasing functions of the anions. In any case, this kind of lactose-cation interaction must have been weak and heat-labile in nature, since when the MMS incorporated with any of these minerals and lactose were heated a reversion of the influence of the ions added was recorded.

Analysis of the results presented through Tables 74, 75, 82, 83, 87, 94, 95, 102 and 103 revealing the effects of storing on viscosity and pH of model milk systems incorporated
with different minerals and lactose and forewarned, indicates that changes in viscosity due to storage followed a common pattern excepting those in the system containing additional calcium ions. Model milk system with added calcium ions could not be stored for more than 48 hours (Tables 70 and 71). With added magnesium ions although BMS showed clotting after 48 hours, CMS could be stored for a longer period (Tables 78 and 79). Such systems when stored subsequent to forewarming treatment showed better stability. MMS with added anions could be stored without clotting for a relatively longer period. When mineral incorporated systems were stored before heating there was considerable lowering in both viscosity and pH till viscosity started to rise with the appearance of early stage for clotting. In cases of MMS stored after heat treatment there was relatively less lowering in either viscosity or pH of the systems, excepting in the case of BMS containing added calcium and also calcium plus lactose. In the later cases a slight increase in the viscosity but very small decrease in pH were recorded during storage upto 5th day subsequent to which the system started to coagulate (Table 74). In some earlier paragraphs attention has been drawn to the characteristically different behaviour of calcium ions in BMS viscosity. Likewise, such difference is observed in the effect of storage on viscosity of BMS containing calcium ions. The influence of heat on MMS containing mineral ions, excepting in BMS with calcium ions, in minimising the viscosity lowering due to progressive storage may be ascribed to the attainment of some sort of equilibrated interaction between the ions and other
constituents of the MMS. In the case of unheated samples due to lack of such interaction viscosity of MMS might have undergone considerable lowering, as has been observed with all the control MMS samples without the addition of the minerals.

**Change in viscosity of milk serum**

Analysis of the influence of different major milk constituents presented so far in this chapter has been made taking into account the effect of these constituents on the viscosity of milk serum, whenever, possible. Results for variation in viscosity of serum due to such additional constituents have been presented through Tables 104, 106, 107, 112, 113, 118 and 119 and such results have been graphically described in Figures 21 of Plate 9, Figures 24 of Plate 10, and Figures 29 and 30 of Plate 12.

It can be seen on comparison that viscosity of either buffalo or cow milk serums are close to one another, averages of viscosities of 4 such samples being 1.13 and 1.10 for buffalo and cow milk serums, respectively. The viscosity of milk serums are, however, much less than those of model milk systems containing either 8.64 per cent buffalo milk casein or 8.20 per cent cow milk casein. In case of buffalo milk serum viscosity is about one-fifth of that of BMS; but in case of cow milk this proportion is one-seventh.

Acid precipitated casein or separated milk lipids could not be incorporated into milk serum. Addition of calcium or magnesium chloride and sodium citrate or phosphate
was found to cause hardly any change in the viscosity of milk serum (details of results not presented). Incorporation of lactose to make final concentration of the same 15g and 20g per 100g caused considerable increase in viscosity of milk sediments (Table 104 and Figures 21 of Plate 9). Such increase was found to be more marked up to 15 per cent lactose concentration. Further, increasing viscosity due to addition of lactose was relatively more in cow milk serum than in buffalo milk serum. Presence of interaction between lactose and some of the milk minerals, particularly the cations, might have been responsible for the observed viscosity rise. Preponderance of calcium in cow milk serum (Table 2) and consequent greater rise in viscosity due to addition of lactose in this serum supports such an explanation. Interaction between lactose and calcium ions in aqueous solution (Herrington, 1934) and also in methanol solution (Domovs and Freund, 1960) has been reported earlier.

Addition of whey proteins, either in total form or as separated α-lactalbumin or β-lactoglobulin, has shown to effect slight, linear rise in the viscosity of both buffalo and cow milk serums. Incorporation of lactose along with whey proteins caused rise in milk serum viscosity to almost the same extent as that caused by lactose alone. This observation and linearity as well as low magnitude of the influence of whey proteins on milk serum viscosity suggest that, in all probability, there is hardly any interaction between the whey proteins and milk minerals, particularly in absence of casein or lipids. The consistency of viscosity
of milk serums with continued storage (Figures 22, Plate 9) in spite of considerable decrease in their pH (Figures 23, Plate 9) also supports such a conclusion.
SECTION B

A discussion on the results presented in Section B of the Chapter III on the physico-chemical properties of evaporated milk, prepared in the laboratory scale from both buffalo and cow milks and the same suitably modified, is incorporated in this Section. Further, changes in the physico-chemical properties of evaporated milk due to storage at two different temperatures are being examined in this Section.

Preliminary studies on the effects of heating, forewarming as well as sterilization, on the buffalo milk constituents, reconstituted to give a model milk system which simulated evaporated milk revealed that such systems containing less than 8.64 per cent casein coagulated on sterilization. However, increasing the casein concentration to 8.64 per cent or more gave a product which could be sterilized at 121.5°C subsequent to forewarming at 96°C without coagulation. Although such buffalo milk system withstood sterilization it showed about 350 per cent rise in viscosity in comparison to about 70 per cent rise in viscosity of similar cow milk system. Subsequently, the forewarming temperature-time combination was changed to 120°C with no holding and such forewarmed MMS gave a sterilized product which was found to show less rise in viscosity, more so in buffalo milk system. Consequent to such preliminary observations, buffalo evaporated milk (BEM) and also cow evaporated milk (CEM) were prepared using such high temperature forewarming followed by condensation and sterilization, details of which have been given in the Section B of Chapter II. Such
evaporated milk samples were prepared from the original whole milk, the same standardised for fat, such milk incorporated with different amounts of major milk minerals, and milk modified by addition of acid precipitated casein and also of milk minerals. Changes in viscosity and pH during the various stages of preparation has been recorded in Tables 125, 132 and 138 for BEM and Tables 144 and 150 for CEM. The effects of storage on these two properties and also on some other properties, commonly known as defects of evaporated milk, namely, browning, sediment formation and fat separation, were also studied and their results reported through relevant tables.

Composition of milk and evaporated milk

Changes in composition of the total milk solids (TS) and individual components in buffalo milk due to concentration in the ratio of 2:1 were fairly proportional excepting in cases of lactose and also the protein fractions (Tables 124 and 131). Loss of lactose due to heating of milk, mainly through lactose-casein interaction and subsequent degradation of lactose to produce acidic components, has been established by several workers (Gould, 1945; Patton & Flipse 1953; Grimbleby, 1954; Ismael and Grimbleby, 1959; Nielson et al., 1963). Although the total protein content in BEM increased proportionately with concentration of the original milk, the ratio between the contents of casein to serum protein changed markedly. Whether such a change is real or is apparent, due to absence of any specific method for analysis of casein and the whey proteins partially denatured due to
heat, is an open question. Incorporation of casein into milk altered composition of the milk with respect to not only the total proteins and casein but also to the phosphate ions. In the case of composition of CBM samples similar changes, as discussed above, were observed (Tables 143 and 149) with the exception that the original milk and its constituents underwent concentration by about 2.3 times.

Viscosity change in buffalo milk on conversion to BSM

The results depicting changes in the viscosity and pH of buffalo milk during all the different stages in preparation of evaporated milk are reproduced for three batches of samples only (Tables 125, 132). Similar changes for a fourth batch are described in part only for milk and its evaporated product (Table 133). Such results for two more preliminarily batches are, however, not reproduced. From all these results a progressive but unequal increase in viscosity due to each stage of preparation is evident. On the contrary, a decrease in a like manner in pH has been recorded. Forewarning of the milk caused a slight increase in viscosity (13.2 - 16.1 per cent). The next stage of condensing the forewarmed milk resulted in large increase in viscosity (161.3 - 220.7 per cent). The following homogenization process again had very little influence in viscosity elevation (16.7 - 27.4 per cent). The final stage of sterilization rendered the maximum rise in viscosity, by 200.5 to 279.2 per cent, for the three different batches. A visual projection of such changes for the BSM samples of
batch 2 is available in Figures 45 of Plate 18. These data delineating a systematic change in viscosity of buffalo milk during the several standard processes leading to formation of evaporated milk were found to be consistent for all the batches studied during the present investigation. A comparison of these data to similar results in buffalo milk is not possible due to absence of any such published results. A comparison, however, can be drawn to similar data pertaining to cow milk processed to evaporated milk during the present study and also by a few Western investigators.

Before coming to such comparison a brief discussion on change in pH of buffalo milk during processing to IBM follows; for such finding also no comparison with data from earlier workers is possible.

**Change in pH of milk on conversion to evaporated milk**

The initial pH of the buffalo milk samples ranged between 6.88 and 6.70 and the same changed very little due to forewarming. Condensation of the milk brought down the pH to 6.45, uniformly for the 3 samples studied, thereby causing a fall of about 3.5 per cent in pH from initial milk. The next step of homogenization had again hardly any influence on the pH of the samples. The last stage in the process, that is, sterilization, resulted in a sharp lowering in the pH of the samples to 6.25 - 6.28. Such lowering amounts to about 6.40 per cent in the average from the pH of the initial milk. Scrutiny of the results presented in Tables 144 and 150 shows that the pH of cow milk samples,
6.64 and 6.66 changed considerably due to condensation and sterilization stages in the processing to evaporated milk as in the case of buffalo milk samples, while forewarming and homogenization had very little effects. The overall change in pH of the cow milk due to conversion to the sterilized concentrate was by 6.17 per cent resulting in final pH of 6.23 and 6.25 for the two batches of CEM. The lowering in pH due to condensation stage was about half of this value, 3.61 per cent in one sample and 3.15 per cent in the other. It can be seen that initial buffalo milk samples had slightly higher pH, than such samples of cow milk, by 0.02 to 0.06 units in the samples studied. Likewise, corresponding BEM showed a little higher pH than CEM, by 0.01 to 0.05 units. According to Bhimsena Rao and Dastur (1955 and 1956b) the pH of buffalo milk was significantly higher than that of milk from cows of Indian breeds. Hofi and associates (1966), however, could not find the difference in pH of buffalo and cow milks of Egyptian origin to be significant, although buffalo milk recorded relatively higher pH. These workers further reported a significant decrease in the pH of both cow and buffalo milks on sterilization at 120°C for 20 minutes, such decrease being relatively less in cow milk. Increase in acidity or lowering in pH of milk either due to concentration (Rogers et al., 1921; Howat and Wright, 1934) due to heating and sterilization (Whittier and Benton, 1927; Gould, 1945; and Sommer and Hart, 1919) or due to both concentration and sterilization (Keeney and Josephson, 1947) are already well known.
Numerous explanations have also been forwarded for such, of which formation of acid through destruction of lactose is one. Since buffalo milk contains more lactose, decomposition of the same might have caused production of acid in relatively larger proportion and thereby changed the initially higher pH of buffalo milk to finally slightly lower pH of the BSM in comparison to that of CRM.

Viscosity change in cow milk on conversion to CEM

Results from Tables 144 and 150 indicate changes in viscosity undergone by cow milk during various processing stages to follow those of buffalo milk processed under identical conditions, although the magnitude of changes are not identical. Similar conclusion is apparent from the Figures 45 and 46 of Plate 18. Both forewarming and homogenization caused slight increases in viscosity of cow milk, 11.3 per cent and 17.1 per cent in the average for the two batches, due to former and later processes, respectively. Such increases are comparable to those observed in buffalo milk. Condensation, however, caused a greater increase in viscosity in cow milk, by 27.4 per cent as compared to that in buffalo milk by 19.8 per cent, considering the average values. A greater variation was observed in the effect of the stage of sterilization on the milks from two species. While an average increase in viscosity of about 24.8 per cent was observed in the buffalo milk, corresponding increase in cow milk was as high as 71.5 per cent. Analysis of other results for changes in viscosity of the two milks modified by addition of casein (Tables 125, 133, 144 and 151) also
reveals such a greater viscosity rise in cow milk than in buffalo milk due to both condensation and sterilization. Viscosity rise in cow milk concentrate (31% TS) due to sterilization at 121.5°C for 7.5 minutes holding, observed during the present investigation (715 per cent) is fairly comparable (Mojonnier and Troy, 1922) to such rise of 742.5 per cent due to sterilization at 117.2°C (243°F) for 15 minutes with cow milk of 26% total solids concentration. According to Leviton and Pallansch (1961a) viscosity rise due to sterilization at 137.4°C for 15 seconds for cow milk, concentration 26 per cent TS, was 441.6%. In the context of the fact that high temperature, short-time sterilization yields a milk concentrate of low viscosity (Bell et al., 1944), the above results from Leviton and Pallansch is also comparable to the present findings. The author has not been able to come across any published data detailing the viscosity changes in cow milk during various processes leading to evaporated cow milk having 31% total solids, and therefore, a more specific comparison of his data in this regard could not be made. Thus it can be seen, that although buffalo milk is believed to be more susceptible to coagulation due to heat of sterilization (Srinivasan et al., 1967) the actual viscosity rise due to sterilization in buffalo milk is less than that in cow milk. Further discussion on this point will be taken up later.

Viscosity and pH of evaporated milk with additional minerals

From the details of results in Table 132 for BBM and Table 160 for CEM it is evident that incorporation of citrate
and phosphate led to reduce viscosity rise in both buffalo and cow milk systems due to the processes of forewarning, homogenization as well as condensation. In BEM prepared from raw buffalo milk the effect of forewarning was an increase in viscosity by 13.3 per cent as compared to increases by 4.5 per cent in BEM from milk with citrate, 4.0 per cent from milk with phosphate and 4.4 per cent from milk with citrate plus phosphate. Viscosity increases due to condensation following forewarning of the corresponding buffalo milk samples were 220.7, 204.5, 214.0 and 209.7 per cent. Similar increases in viscosity in cow milk with added minerals in the order just described above due to the stage of condensation were 279.0, 210.7, 244.6 and 238.3 per cent. In both these systems of cow and buffalo milks the influence of citrate ions was more prominent than that of phosphate ions. Referring to the increase in viscosity due to sterilisation alone it is seen that while normal buffalo milk showed a viscosity rise by 263.2 per cent, buffalo milk with phosphate or phosphate plus citrate showed rise by 253.9 and 273.9 per cent, respectively. But the buffalo milk with added citrate failed to withstand sterilization and clotted. Efforts to prepare BEM with different concentrations of citrate ions, ranging from 10 to 50 mg per 100 g milk, met with same fate. In the case of cow milk sample viscosity rise due to sterilization alone was 668.2 per cent for initial milk, 295.5 per cent for milk with citrate, 333.3 per cent for milk with phosphate and 336.9 for milk with phosphate - plus citrate. From all these results a few points may be brought out. Firstly, the influence of citrate
ions in retarding viscosity rise in cow milk system during all stages of processing to evaporated milk was more prominent than that of phosphate ions. Secondly, in buffalo milk system the effects of these two ions were fairly comparable excepting in the process of sterilization, where, citrate ions augmented increase in viscosity as a preliminary stage of coagulation. Thirdly, incorporation of both citrate and phosphate together was more effective than addition of phosphate alone but less so than that due to citrate, only at the stage of condensation. A differential behaviour of citrate ions on the viscosity of milks from buffalo and cow has been concluded from the present study with IMS, as discussed earlier, and as can be seen from the results of Tables 88, 89 and also Figures 42 of Plate 10. Referring to changes in pH due to addition of these minerals (results from the same tables) disclose that presence of citrate in milk, either from buffalo or cow, yielded evaporated milk which had distinctly, though slightly higher pH than the one prepared from milk alone. On the other hand, addition of phosphate to milk caused slightly more fall in pH than that from milk alone. Similar influence of citrate has been recorded in terms of checking increase in acidity of evaporated cow milk (Chekulaeva, 1956), which in other words amounts to rise in pH of the product.

The addition of phosphate ions to cow milk concentrate before sterilization, has been known to cause lowering in viscosity of sterilized product (Deysher et al., 1944; Ponomarev, 1946; Leviton et al., 1962; Leviton and Pallansch, 1962). Incorporation of citrate ions, although not so
extensively studied as that of phosphate ions, has been reported by Ponomarev (1946) to lower the viscosity of cow whole milk concentrate after sterilization. However, results from the present study provide a comparison of the influence of the phosphate and citrate ions on viscosity of whole milk concentrate after each stage of processing leading to sterilized evaporated whole milk. From the results on storage of the evaporated milk samples, to be discussed subsequently, differential behaviour of these two ions will be disclosed further.

Viscosity and pH of evaporated milk with additional casein and minerals

Addition of casein to whole milk by thorough blending caused in general increase in viscosity of the mixture, by 18.3 per cent for buffalo milk (average of 3 batches) and by 13.4 per cent for cow milk (average of 2 batches), as can be deduced from the results of Tables 125, 133, 144 and 161. When subjected to forewarming treatment the casein incorporated milk samples showed comparatively lower rise in viscosity than milk. The effect of all other treatments like condensation, homogenization and sterilization was, however, more pronounced in casein incorporated milk than in milk, both in buffalo and cow samples. Condensation led to an increase by 216.6 per cent in the average in viscosity of buffalo milk containing casein as compared to such rise by 198.2 per cent for buffalo milk alone. Likewise, in cow milk containing casein viscosity rise due to condensation was 350.0 per cent as compared to 274.0 per cent for cow milk alone.
Sterilization caused viscosity rise in HEM containing casein by 357.1 per cent as compared to that of 248.0 per cent for HEM from milk alone, corresponding viscosity rises for CEM with casein and without casein were 391.4 and 715.0 per cent, respectively. Comparable data showing viscosity change due to various stages in processing to evaporated milk from milk to which casein has been added, are not available from published results. However, Beeby and Loftus Hills (1966) from their study on gelation in evaporated milk (Details of processing not reported) have shown a correlation between the higher protein content of the milk and greater viscosity of concentrated skim milk.

Results from present study, however, indicate that despite such increased viscosity due to addition of casein to milk the keeping quality of the products was improved (Figures 47 and 48, Plate 18). Incorporation of citrate and phosphate in addition to casein also improved the viscous property of the product better, as will be evident from later discussion. It has been observed from study with model milk system that presence of casein at an optimum concentration of about 8.64 per cent in buffalo milk system or 8.20 per cent in cow milk system caused negligible change in viscosity of the model milk system due to addition of increasing amount of lactose and also due to subsequent forewarming. However, addition of either the whey proteins of the milk lipids caused slight increase in viscosity of the system, either in cold or after forewarming. Since the evaporated milk prepared from casein incorporated milk contained both whey proteins and lipids in addition to higher concentrations of lactose...
and casein it was expected that forewarming would cause a rise in viscosity. It has been seen that such a rise was decidedly less than that observed in milk without added casein, which is in agreement with the results obtained from study with model milk system. Analysis of the results presented in Tables 133 and 151 discloses the influence of citrate and phosphate ions on changes in viscosity and pH of casein incorporated milk samples due to different stages of processing to evaporated milk. It is clear that in both buffalo and cow milk systems addition of citrate leads to less pronounced lowering in pH due to sterilization while phosphate ions have little additional pH lowering ability. Changes in viscosity follow somewhat different pattern in buffalo and cow milk systems. In buffalo milk system rise in viscosity due to forewarming is minimum (5.6 per cent) when both citrate and phosphate were added together. Phosphate ions caused increase in viscosity due to forewarming by 7.6 per cent, while citrate ions did so by 9.6 per cent, as against by 13.5 per cent in milk without added anions. Effect on viscosity rise due to condensation alone was fairly similar for citrate and phosphate ions, severally and jointly, about 193 per cent as compared to 210 per cent for only casein incorporated milk. Presence of phosphate and citrate together caused the least rise in viscosity due to sterilization of the condensed and homogenized product, by about 297 per cent. Such rise due to phosphate alone was about 312 per cent, due to citrate alone 338 per cent and in sample without added minerals about 381 per cent. A similar trend in overall change of viscosity as well as pH
due to all the processes involved in preparation of evaporated milk as a result of addition of citrate and phosphate ions along with casein to buffalo milk is disclosed by the data of the Table 138 also, for a second batch sample.

In case of the cow milk system (Table 151) incorporation of citrate had the best beneficial effect in controlling viscosity rise due to all the processes involved, namely, by 753 per cent. Such increase in viscosity in milk without added anions was 1305 per cent, with added phosphate ions 849 per cent and with phosphate and citrate ions together 318 per cent. Changes in pH also was somewhat less in evaporated cow milk prepared after incorporation of citrate. A comparison of the results from the above cited tables, and also from the graphical presentation of such results in Figures 51 and 52 of Plate 19 and Figures 55 and 56 of Plate 20, clearly indicate that while in cow milk system addition of casein to milk alters the influence of citrate of phosphate ions only in magnitude but not in any qualitative manner.

In buffalo milk system it brings about changes of both kinds. When citrate ions were added to buffalo milk and such mixture was processed to evaporated milk clotting set in during sterilization, although the original buffalo milk did not clot during such process. But addition of citrate ions to buffalo milk subsequent to incorporation of casein provided milk which not only did not coagulate on sterilization but also gave an evaporated milk with less viscosity than that of the one without added citrate. A differential behaviour of citrate ions on the viscosity of buffalo or cow milks during processing to evaporated milk is consistent
with the presence of such behaviour on the viscosity of model milk systems from milk constituents of the two species, as has been indicated earlier also. A possible explanation for such behaviour on the basis of higher citrate in cow milk than in buffalo milk and higher colloidal calcium concentration in buffalo milk than in cow milk has also been attempted earlier. The impact of simultaneous addition of casein and citrate or phosphate has been observed to be more pronounced in viscosity change of evaporated milk during storage of the products and discussion on the same follows.

Changes in viscosity and pH of evaporated milk during storage

Details of changes in viscosity and pH of buffalo evaporated milk prepared from different samples with varying initial concentrations have been reported in Tables 126, 127, 134, 135, 139, 140. Changes in viscosity due to storage for one set of BSM samples, stored both at 37°C and 40-6°C, are described graphically in Figures 47 of Plate 18 and these results correspond to those for Batch No. 2 in Table 126. Likewise, viscosity changes for another batch of BSM, presented in Tables 134 and 135, are described graphically in Figures 51 and 52 of Plate 19. Similarly, the results for viscosity changes in CBM are depicted through Figures 48, Plate 18, corresponding to results of Table 145 and through Figures 55 and 56 of Plate 20 corresponding to results of Tables 152 and 153, respectively. Analysis of these graphical depictions reveals the trend shown in viscosity change of all evaporated milk samples studied.
Viscosity It is evident from Figures 47 (Plate 18) that BEM prepared from buffalo milk alone showed a progressive and constant increase in viscosity till it thickened to a gel in course of storage for 90 days. BEM prepared from milk incorporated with casein, however, showed a fall in viscosity followed by rise after one month storage (Figures 47, Plate 18; Figures 52, Plate 19). Such an initial lowering in viscosity has been observed in CBM prepared from cow milk alone or from cow milk incorporated with casein (Figures 48, Plate 18 and Figures 55 and 56, Plate 20). The initial lowering of viscosity of CBM at early stage of storage, known as thinning, is a well known phenomenon (Mojonnier and Troy, 1922; Deysher et al. 1944; and Webb et al., 1946).

Absence of such thinning at early storage period in BEM prepared from raw buffalo milk is noteworthy. The viscosity stabilizing ability of total whey proteins, or whey protein fractions, from buffalo milk on both buffalo and cow model milk during storage has been pointed out earlier. Buffalo milk contains larger amount of whey proteins as compared to cow milk (Ghosh and Anantakrishnan, 1965). Thus both the magnitude and the quality of whey proteins in buffalo evaporated milk might have rendered this unusual property to BEM, namely, absence of thinning in early stages of storing. Results from the study with model milk systems (Tables 18, 19 and 20) also indicated that micellar casein from buffalo milk in presence of higher lactose concentration showed a viscosity stabilization which was not
so marked in the system with cow micellar casein.

**Role of casein** Incorporation of casein to milk and subsequent processing to BEM has obviously lowered the proportion of whey proteins in such BEM and thereby removed the viscosity stabilizing influence of the buffalo whey proteins. The BEM prepared from milk with added casein showed a distinctly better keeping quality, since the product did not show gelation. A similar improvement in keeping quality of CBM prepared from milk with added casein was noted also. It is a standard practice with evaporated milk manufacture to maintain a definite fat-to-solids-not-fat (fat: SNF) ratio in the milk to be processed to evaporated milk (Hunziker, 1949; Hall and Hedrick, 1966). According to the U.S. Standard such ratio is 1:2.2785, but according to the British Standard the same is 1:2.4444 (B.S., 1951). Indian Standard specification also prescribes fat to SNF ratio same as that of British specification (I.S., 1957).

It can be seen from the review presented in Chapter-I that milk from cow of Indian breeds (*Bos indicus*) contains more fat and consequent lower fat: SNF ratio as compared to milk from cows of Western breeds (*Bos Taurus*). Furthermore, from the same review it is evident that early attempts to prepare evaporated milk in India, either from cow milk or from buffalo milk, have not met with success. It has been, however, gathered mostly from personal communications and also from Anantakrishnan and Kothavalla (1947) that such attempts were made to meet the British Standard specification, and without giving any consideration to the basic composition of milk.
from Indian buffalo or cow. It was observed from the present study with model milk systems, as discussed earlier, that variation in the viscosity of the system containing 10% fat and high concentration of lactose is less than in a system with 5% fat and same concentration of lactose, at least in the buffalo milk system. It was, therefore, thought that presence of about 10% fat in the evaporated milk may be more beneficial towards its viscosity, as well as keeping quality, as compared to 9% fat. Such high proportion of fat in the milk gave a comparatively low fat: SNF ratio, 1:2.05 and 1:1.86 for buffalo milk and cow milk, respectively, as representative values from the samples studied.

Incorporation of casein to milk increased the SNF in the fat: SNF ratio in the standardized starting material, 1:2.22 and 1:2.05 for the above referred buffalo milk and cow milk samples, respectively. It is apparent from the results of present study that increasing the fat: SNF ratio in milk led to a better product of evaporated milk, mainly through a predominant interaction between casein and some other major milk constituents. The other milk constituents involved in such interaction may be lactose, lipids and minerals, particularly, calcium ions. A greater concentration of casein in milk was shown to give better heat stability to the milk and such effect is explained due to diminished calcium ion activity through casein - calcium ion interaction (Evenhuis, 1958). The presence of casein in greater concentration might have its influence through minimising the interaction involving the whey proteins.
which present in concentration exceeding 0.9 per cent caused heat unstability of the milk (Rose, 1963). The improvement was not so much so in the initial viscosity of the product as was in rendering the milk concentrate, particularly from buffalo milk, more stable to heat to sterilization and in its keeping quality, measured from both viscosity and pH characteristics (Tables 126, 127, 134, 135, 139 and 140 for BSM; and Tables 145, 152 and 153 for CBM). Such improvement was also evident in colour, flavour and a few other properties of the BSM discussed subsequently.

Role of minerals

Incorporation of orthophosphate ions (50 mg/100g) or a mixture of orthophosphate and citrate ions (50 mg of each per 100 g) into the buffalo milk and processing to evaporated milk, yielded a product which showed distinctly less increase in viscosity during storage after 120 days. In this regard a mixture of orthophosphate and citrate ions had a slightly better effect than orthophosphate ions alone (Figures 51, Plate 19). Citrate ions as such showed definitely deleterious effect since milk concentrate with additional citrate ions clotted on sterilization. On the contrary CEM prepared from cow milk incorporated with citrate ions alone showed the best keeping quality on storage. But CEM from milk with added phosphate showed the maximum increase in viscosity during storage upto 3 months although it showed least change in pH. A similar "stabilizing effect" of citrate ions on the viscosity of sweetened condensed separated cow milk due to storage
for more than 2 weeks but a destabilizing effect of the orthophosphate ion during storage even for a shorter time has been reported by Samel and Meurs (1962). The influence of orthophosphate ions in decreasing the storage life of concentrated cow milk with 36% total solid has also been reported by Edmondson (1959), Leviton and associates (1962), and many other workers.

The beneficial effect of incorporation of casein to either buffalo milk or cow milk in preventing rise of viscosity during storage up to 120 days was observed to be further increased due to addition of the two anions studied (Figures 52, Plate 19; and Figures 56, Plate 20). In the case of BEM from milk incorporated with citrate along with casein overall increase in viscosity due to storage for this period was minimum, about 12 per cent of the original viscosity. In fact viscosity of such BEM was almost constant during storage for 60 days, subsequent to which a slight increase set in. The influence of orthophosphate ions or a mixture of orthophosphate and citrate ions was very similar to that of citrate ions during storage for 60 days. Due to prolonged storing, however, the former two samples showed greater increase in viscosity than the last one. Such superior capacity of the citrate ions in conjunction with casein in preventing gelation of evaporated milk was more prominent in CEM than in BEM. While the overall increase in viscosity in BEM from buffalo milk with added casein and citrate ions during storage for 90 days was about 6 per cent of the original viscosity (Table 135) the corresponding
increase in CEM of stellar composition during the same period of storage was only 3.5 per cent (Table 153). In CEM addition of orthophosphate ions along with casein led to a distinct sharp increase in viscosity after storage for 60 days (Figures 56 of Plate 20) in contrast to addition of either citrate ion or a mixture of citrate and orthophosphate ions. Such a greater detrimental effect of orthophosphate ions on viscosity rise in CEM is consistent with observations discussed earlier on the influence of phosphate ions in cow model milk system, and explanation offered for the latter observation may be valid in the case of CEM also.

pH Changes in pH of both BEM and CEM during storage at 37°C for varying periods up to 180 days was very moderate for all the samples studied. In samples of evaporated milk prepared from original milk standardized for fat alone maximum decrease in pH by about 7.5 per cent, from 6.28 to 5.81 for BEM (Table 126), and that by about 6 per cent, from 6.23 to 5.85 for CEM (Table 145), during storage for 180 days were recorded. In one BEM sample, the decrease in pH after storing for 120 days was only about 1.5 per cent (Table 134), and in a CEM sample only 0.5 per cent fall in pH during 90 days storage was observed (Table 152). Incorporation of casein to milk gave evaporated milk which showed still less change in pH. Addition of either phosphate or citrate ions along with casein or without casein to the original milk processed to evaporated milk gave products which in general showed negligible loss in pH, ranging
from 0 to 1.1 per cent in BEM (Tables 134 and 135) and from 0.3 to 1.0 per cent in CEM (Table 153) due to storage for 90 days.

Storage at 4°C-6°C

Better preservation of evaporated milk by lowering the temperature of storage below 15.5°C (60°F), and particularly at 4°C (40°F), as judged from minimum change in viscosity and pH and also in some other properties like colour, fat separation, etc., has been well established for CEM samples by Western workers (Mojonnier and Troy, 1922; Deysher et al., 1944; and Webb et al., 1951). Similar observations have been recorded during the present study for all the evaporated milk samples, both BEM and CEM. BEM prepared from buffalo milk alone when stored at low temperature for 180 days showed viscosity rise by about 129 per cent of the original value, while the same stored at 37°C gelled after storing for 90 days (Tables 126 and 127; Figures 47 of Plate 18). Lowering in pH in BEM stored at low temperature was about 1 per cent but the same due to storage at 37°C was about 7.5 per cent, during 180 days. BEM prepared from milk with added casein when stored at low temperature for 180 days increased in viscosity by about 35 per cent and decreased in pH by about 0.5 per cent of the original values, in comparison to about 238 per cent rise in viscosity and 6.7 per cent fall in pH when stored at 37°C, for an identical period. A similar trend, with slight difference in actual changes, was observed for CEM from milk alone as well as from milk with
added casein due to storage for 180 days at low temperature and at 37°C (Table 145, Figures 48 of Plate 13).

Without going into details of changes, namely, increase in viscosity and decrease in pH of both BEM and CEM prepared from milk modified with addition of both casein and the anions, observed due to storage at low temperature, it can be inferred that incorporation of citrate along with casein caused a least change during storage for 120 days of BEM and for 90 days of CEM. The effect of the phosphate ions to increase viscosity of both BEM and CEM was more than that due to citrate ions. Further, as in the case of storage at 37°C, phosphate addition was more detrimental towards CEM viscosity than to BEM viscosity during storage at low temperature (Figures 57-60, Plate 21). The effect of both these ions on decreasing the pH of either BEM or CEM during storage at low temperature was almost negligible, by not more than 0.02 units in any case (Tables 134, 135, 139, 140, 152 and 153).

Changes in other properties of evaporated milk during storage

**Browning**

The colour of the evaporated milk prepared was not much different before and after sterilization, since both forewarming and sterilization was done at high temperature with short time exposure to heat such observations were expected (Webb and Holm, 1930; Webb, 1935; Bell et al., 1944; Hunziker, 1949; Tarassuk and Simonson, 1950; and Simonson and Tarassuk, 1952).
The CEM, however, showed slightly deeper creamy yellow colour than BEM, as inferred from the absorbance values of 0.090 and 0.065 for CEM and BEM, respectively (Tables 146 and 128). These tables give representative results for one set of BEM and CEM each. The colouration produced for other samples of evaporated milk studied during the present investigation were also recorded and showed similar trend as described through these two tables. Incorporation of casein and also citrate and phosphate ions to milk produced evaporated milk which showed a slightly deeper colour than EM produced from milk alone, for example, showing absorbance of 0.073 and 0.065 for the former and latter classes of EM, respectively (Table 128). Deepening of colouration due to storage was, however, comparatively less in EM prepared from casein incorporated samples. It has been observed further (Tables 128 and 146) that deepening of colouration was relatively more in BEM than in CEM due to storage at both 37°C and 4°-6°C. Storing at 37°C showed somewhat greater discolouration, about 2.5 times in all the samples of both BEM and CEM, than due to storing at 4°-6°C. It can be noted, however, that during the present study for all the EM samples prepared the colour developed even after storing for 180 days at 37°C was well within acceptable standards.

Sediment formation

One of the characteristic defects of evaporated milk developed particularly during storage at higher temperature
is sediment formation at the bottom of the container (Hunziker, 1949). The present study also revealed formation of such sediments in both BEM and CEM due to storage for varying periods from 30 to 180 days. A reference to the Chapter III for the results describing the extent and nature of sediment formation discloses that sediment formed during storage of less than 180 days was soft, flappy and could be redissolved by gentle shaking through inversion of the container. A comparison of the results of Table 129 for BEM and Table 147 for CEM shows that evaporated milk prepared from milk modified by addition of casein gave much less sediment as compared to evaporated milk prepared from milk alone. A further comparison between the results of these two tables indicates that sediment formation was more prominent in CEM than in BEM, at least after storage for 180 days, for both casein incorporated milk or only milk samples. Storing at low temperature showed distinct inhibition to sediment formation and there was almost no sediment in EM samples prepared from casein incorporated milk even subsequent to storage for 180 days. Although retardation of sediment formation by storing at lower temperature is well known (Mojonnier and Troy, 1922) presence of higher percentage of casein preventing formation of sediment has not been reported earlier. Precipitation of salts of calcium and magnesium with phosphate and citrate ions has been attributed to be the cause for sediment formation in evaporated milk, particularly on storing at higher temperature (Mojonnier and Troy, 1922; Sato, 1923). Addition of acid precipitated casein to milk has increased the ratio
of casein to the mineral concentrations in the evaporated milk prepared subsequently, and in consequence, the ability of the colloidal dispersed casein micelles to hold back the calcium and magnesium salts, formed from precipitation is understandable. Buffalo milk contains more of colloidal calcium and less of soluble calcium ions, while in cow milk the distribution of calcium is reverse to that of buffalo milk (Table 2). Consequently, the possibility of precipitation of calcium salts in CSM is more than that in BBM and the same has been reflected in relatively less sediment formation in BBM, as pointed out earlier.

Incorporation of citrate or phosphate ions has led to greater sediment formation during storage in both BBM and CSM excepting in one case (Tables 136 and 154). Such exception was observed in case of BBM prepared from buffalo milk to which both casein and citrate has been added, where this sample has shown no sediment formation at all after storage for 120 days at 37°C or at low temperature (Table 136). On the contrary addition of both casein and citrate to cow milk gave a CSM which showed distinctly greater sediment formation than CSM from cow milk with casein alone, even after 90 days storage at either 37°C or 4°C-6°C (Table 154). Here again the beneficial effect of citrate ions in conjunction with additional casein in the keeping quality of BBM prepared from such milk has been established. Addition of citrate ions to buffalo milk (Table 136) was found to improve the defect of sediment formation as compared to the addition of phosphate ions or a mixture of phosphate and
citrate ions. Such observations on the effect of citrate ions in retarding the growth of sediment in evaporated milk, particularly when casein is present in higher concentration, is contrary to earlier observations with evaporated milk prepared from milk from cow of Western breeds (Hunziker, 1949). Buffalo milk contains more calcium but less citrate in colloidal form as compared to cow milk (Table 2). Addition of citrate ions and subsequent heating operations subjected to milk in the course of conversion to sterilized evaporated milk might have helped more of citrate ions to be partitioned to the colloidal phase thereby binding with the calcium present in that phase and minimizing the destabilizing action of divalent calcium ions with respect to casein denaturation. As a consequence not only the separation of calcium salts in the form of sediment was prevented but also viscous property of the evaporated milk was improved. The insolubilization of calcium and separation as calcium salts lead to lowering in pH due to heating of milk (Jenness and Patten, 1959). BEM samples prepared from citrate incorporated milk have shown slight but persistent higher pH as compared to those prepared from milk alone and more so to those prepared from milk incorporated with phosphate ions (Tables 133 and 135). This may be an evidence to the possibility of maintaining the calcium in colloidal form and prevent it from being separated as either phosphate or citrate in case of BEM.

Fat separation

Separation of fat, or increased concentration of fat
in the top layer of evaporated milk, is one of the problems in the storage of the evaporated milk (Webb and Holm, 1939; Hall and Hedrick, 1966). Consequently, a semiquantitative measurement for fat separation was undertaken with all the samples of evaporated milk studied. Analysis of results from such study (Tables 130 and 137 for BEM, and Tables 146 and 155 for CBM) reveals that separation of fat in BEM is slightly more than that in corresponding CBM samples during identical periods of storage, particularly, in the samples stored at 37°C. It can be seen from earlier presented results and discussion that the BEM samples had relatively less viscosity than CBM samples under identical conditions. A high viscosity (having an optimum value) in evaporated milk is known to definitely retard fat separation in storage (Hunsiker, 1949). It was, therefore, expected that CBM samples with their higher viscosity would show fat separation to a lesser extent. Separation of fat in both BEM and CBM was observed to be comparatively less in samples prepared from milk incorporated with casein than in those prepared from untreated milk. Incorporation of casein caused a lowering in the proportion of fat with respect to solids—not-fat in evaporated milk, and consequently, had a retarding effect on fat separation. It was, however, a general observation in the present study that the separated fat column was not in the form of permanent fat separation, but could be redispersed in a sufficiently stable manner through gentle inversion of the containers.
The flavour of all the evaporated milk samples prepared was good, in other words, no more than very slight "cooked flavour" was detectable. Storage of the product caused a slight decrease in the cooked flavour, but not eliminating it completely. No "stable flavour" was detected in any of the samples even after storing for 180 days in the maximum. Presence of cooked flavour in a concentrated milk product to a moderate extent is no problem in this country; to the contrary the same has a preference to the Indian consumers.

Sterility of evaporated milk

Over and above the properties discussed in the foregoing paragraphs the most indispensably important quality of evaporated milk is its bacterial sterility (Hunziker, 1949). Consequently, all samples of evaporated milk prepared during the present study were subjected to bacteriological analysis immediately after the final stage of preparation (sterilization) and also after storage for a maximum period of 180 days. The representative results for one series of evaporated buffalo milk and another of evaporated cow milk (Table 160), of such bacteriological analysis indicate clearly the sterile nature of the evaporated milk samples prepared. Such results further assured the efficacy of the temperature-time combination used for sterilization, which, though not very common, was adopted on the basis of available resources.
Reconstitution of evaporated milk and physico-chemical properties of reconstituted milk

Evaporated milk on recombination with water in proper proportion gives reconstituted milk suitable for fluid consumption or other purposes. Changes in two of the basically important physico-chemical properties of such reconstituted whole milk, namely, viscosity and pH, have been studied with one series of samples each of BEM and CRM. Results from such studies are discussed in brief.

pH Reconstituted milk showed in general pH nearer to that of original raw milk (Table 142 for buffalo reconstituted milk, BRM, and Table 157 for cow reconstituted milk, CRM). BRM from evaporated milk stored at 37°C for 90 days had pH lower than that of original milk by 0.03 to 0.14 units of pH. BRM from evaporated milk kept at 4°-6°C showed a difference from original milk ranging from 0.00 to 0.08 units. The influence of addition of casein and citrate ions to milk processed for evaporation was found to be most beneficial, since in such cases variation in pH was minimum, by 0.00 and 0.03 units depending on storage at 4°-6°C and 37°C, respectively. Such observations support the possibility of retardation of precipitation of calcium salts in buffalo evaporated milk subsequent to preparation and also storage, as has been pointed out earlier, while discussing viscosity change and sediment formation in buffalo evaporated milk.

In comparison, addition of phosphate ions along with casein to milk gave evaporated milk which on reconstitution showed
much less pH than original milk, by 0.08 - 0.14 units. The lowering in pH of milk due to concentration has been contributed to precipitation of calcium phosphate (Jeness and Patton 1959). Addition of phosphate ions to milk is likely to accelerate such precipitation and thereby, causing greater fall in pH in evaporated milk which could not be restored totally by dilution of reconstituted milk. On the contrary, addition of citrate ions may lead to binding some of the calcium and preventing it from precipitation as calcium phosphate, thereby causing a lesser fall in pH in evaporated milk and a subsequent better recovery of pH in reconstituted milk to its original milk value.

As in the case of BRM the pH of CRM samples also was higher than that of corresponding evaporated milk samples and closer to the pH of original milk samples. In these samples also, superiority of storage at 4°C-6°C, in comparison to that at 37°C, has been established. Further, addition of citrate ions either with or without casein, to the original milk has caused the least variation in the pH of both evaporated milk and CRM therefrom as compared to the original cow milk samples. As in BRM incorporation of phosphate, on the other hand, effected a larger difference between the pH of original cow milk and reconstituted cow milk. All these observations substantiate the explanation offered earlier in case of pH change in BRM.

**Viscosity** Results from Table 141 for BRM and Table 156 for CRM disclose that reconstituted milk shows slightly higher viscosity than the original raw milk from which it
was prepared. Further the viscosity of reconstituted milk prepared from evaporated milk stored at 37°C was higher than that of reconstituted milk from evaporated milk stored at 4°-6°C, excepting in one series. Such exception was observed in cases of BRM from buffalo evaporated milk prepared from milk with incorporation of casein and minerals. In these samples viscosity of BRM from evaporated milk stored at low temperature was slightly higher than viscosity of BRM from evaporated milk stored at 37°C. Although, the difference was small, 0.09 to 0.18 units in relative viscosity, it was not negligible to be explained as experimental error. Vujicic and De Mana (1966) have shown that the percentage of soluble calcium in original milk is more than that in reconstituted milk. In other words, not all of the calcium ions shifted from soluble to colloidal state due to concentration of milk returned to soluble form on redilution of the concentrated milk. Addition of citrate or phosphate ions along with casein to the milk and subsequent storing of the evaporated milk at low temperature might have helped in maintaining the calcium in colloidal form and thus preventing its precipitation as salts, thereby, causing greater viscosity in the reconstituted milk. A relatively higher pH of reconstituted milk prepared from buffalo evaporated milk containing additional casein and minerals (Table 143) as pointed out earlier, lends support to such an explanation.