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## ELECTROPHORETIC MOBILITY AND CHROMATROGRAPHIC PATTERNS OF CASEINS OF STORED BUFFALO MILK CONCENTRATES

*Studies were conducted to elucidate the status of protein fractions obtained from freshly prepared and stored (30°C and 37°C) sterilized buffalo milk concentrates by electrophoretic mobility on polyacrylamide gel and and by elution profile from chromatography through sephadex G-100 column. The results indicated the distinct changes in aggregation and disaggregation of proteins through the appearance and disappearance of different peaks in elution profile on sehadex column.*

*At high total solids more coalescence of proteins took place initially in stored sample at 30°C and 37°C storage in contrast to fragmentation of protein at lower total solids (25% T.S.). Aggregation of proteins was faster at storage temperature of 37°C compared at 30°C. However, polyacrylamid gel electrophoresis gave rise to only two bands upto third month of storage both at 30°C and 37°C but at the end of sixth month, one band was noticed with reduced mobility for 30% and 35% T.S. concentrate. Thus these results also confirm the aggregation of proteins during storage.*

### INTRODUCTION

Interaction of casein with whey proteins and also self aggregation of whey proteins takes place in the manufacture of milk concentrates. During storage of the milk concentrate both aggregation and fragmentation of proteins have been reported by earlier workers (Wilson *et al.*, 1963; Morr, 1969; Harwalkar and Vreeman, 1978; Snoermen *et al.*, 1979). A progressive decrease in non protein nitrogen was observed. The change could be because of reactivation of proteolytic enzyme. During storage of UHT sterilized milk concentrate, major changes in beta-casein fraction alongwith a concomitant in alpha-fraction was observed (Whitney *et al.*, 1977). Changes in casein during the storage of UHT sterilized milk was studied by Corradini (1975) using starch gel electrophoresis and indicated a decrease in relative concentration of alpha-S<sub>1</sub>, alpha-S<sub>2</sub>, beta and kappa-casein fraction and corresponding increase in gamma-casein and para kappa-casein fraction. Harwalhar and Vreeman (1978) studied the changes in casein obtained from stored UHT sterilized concentrated milk containing phosphate and observed that the increase in

gamma casein was less as compared to UHT sterilized milk concentrate without any stabilizer. They also noticed an increase in proteose peptone and a decrease in alpha -S<sub>2</sub>-casein. To study the changes in proteins in detail, gel filtration technique was also used by scientists in recent past (Majumdar *et al.*, 1970; Morr *et al.*, 1964) and they resolved skim milk into three fractions using sephadex G-100. Heat treatment at 88°C/10 minutes reduced the number of peaks to two. Paulina and Markh (1979) passed six months old condensed milk through gel and obtained 25 fractions that strongly absorbed UV light. These contained complex product of melanoidin which was not observed in fresh condensed milk. Cheeseman and knight (1974) studied the nature of casein aggregates in heated and stored milk by gel filtration on sepharose 6B. They observed that the increase in the length of storage period of UHT milk caused more assemblage.

### METERIALS AND METHDOS

**Concentrated milk:** Concentrated milks of 25, 30 and 35% total solids (T.S.), having mixed stabilizer (disodium hydrogen phosphate: trisodium citrate: Kappacarrageenan:2:1:0.015) Concentration of 0.05, 0.10 and 0.15% respectively were manufactured by the

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method of Prasad (1985).

**Storage of samples:** Samples were stored in two laboratory incubators at temperatures of  $30 \pm 1^\circ\text{C}$  and  $37 \pm 1^\circ\text{C}$  separately. Upto third month the samples were drawn at an interval of one month and then at the last after six months.

**Reconstitution of samples:** Samples were reconstituted to 15% T.S. so that the reconstituted product had 6% fat and 9.0% S.N.F.

**Fractionation of casein:** On litre of standardised buffalo milk (6.0% fat and 9.0% S.N.F.) and reconstituted products were taken in separate beakers of 2 litres capacity. Two hundred sixty four grams of ammonium sulphate was gradually added to each beaker over a period of 40 minutes with constant stirring at  $20^\circ\text{C}$ . The precipitated casein and fat were allowed to stand for one hour at  $4^\circ\text{-}5^\circ\text{C}$ . Clear whey was then obtained by filtration through whatman no. 1 filter paper. The mixture of casein and fat which remained on filter paper was redispersed in one litre of distilled water and 244 gm of ammonium sulphate was added gradually in the same manner stated above. After allowing to stand for one hour at  $4^\circ\text{-}5^\circ\text{C}$ , the contents were filtered separately through whatman no.1 filter paper. The casein samples obtained on filter papers were dispersed in double the quantity of acetone in separate beakers and the acetone-lipid-casein mixture was mixed in an electrical mixer for 2

minutes. The acetone-lipid and casein mixture was then filtered through whatman 1 filter paper. The casein thus obtained on filter papers were dispersed in diethyl ether to remove fat completely and finally filtered again through Whatman no. 1 filter paper. The samples of casein thus obtained were dispersed in little quantity of water and freeze dried.

**Analytical methods:** Polyacrylamide gel electrophoresis (PAGE): Polyacrylamide gel electrophoresis was carried out in an E-C vertical cell according to the method of de Jong (1975). Chromatography of casein on sephadex G-100: The method suggested by Yoguchi and Tarassuk (1967), incorporating the modifications adopted by Gupta (1975) was used.

## RESULT AND DISCUSSIONS

Electrophoretic pattern of casein samples, fractionated from raw milk (A), 25% (B), 30% (C) and 35% (D) T.S. fresh concentrates on polyacrylamide gel electrophoresis (PAGE) have been shown in plate I. The elution profile on sephadex G-100 can be noticed in Fig. 1. a, 1.b, 1.c and 1.d for raw milk, 25%, 30% and 35% T.S. Concentrate while the elution volume in ml and relative percent proportions of different fractions have been shown in Table. 1 The results obtained at manufacture serves as control comparing the changes which has taken place during storage.

TABLE 1  
Elution volume (A) and relative proportion of area under various fractions (B) of casein from raw buffalo milk and concentrates obtained through chromatography on sephadex G-100 (2.6x60 cm) column.

Fractions	Raw milk		25% T.S. concentrate		30% T.S. concentrate		35% T.S. concentrate	
	(A)	(B)	(A)	(B)	(A)	(B)	(A)	(B)
I	15	17.3	25	35.5	45	47.4	40	62.5
II	70	78.2	10	42.1	115	52.6	100	37.5
III	45	4.5	50	9.5	-	-	-	-
IV	-	-	15	1.5	-	-	-	-
V	-	-	50	1.3	-	-	-	-

Casein patterns of stored milk concentrates

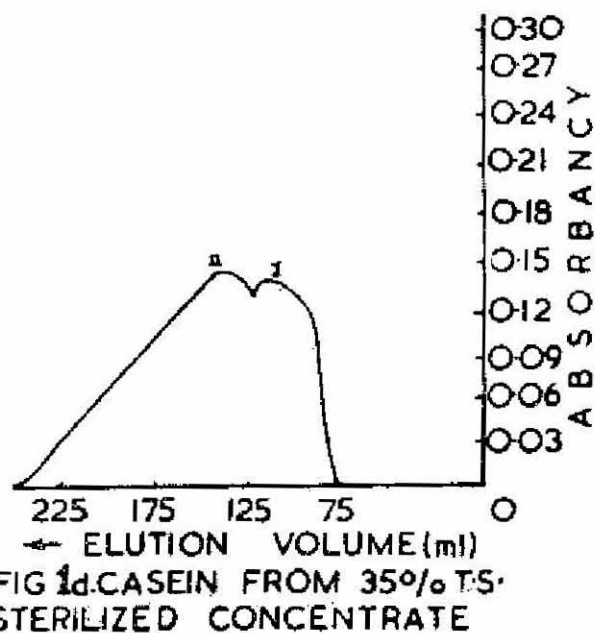
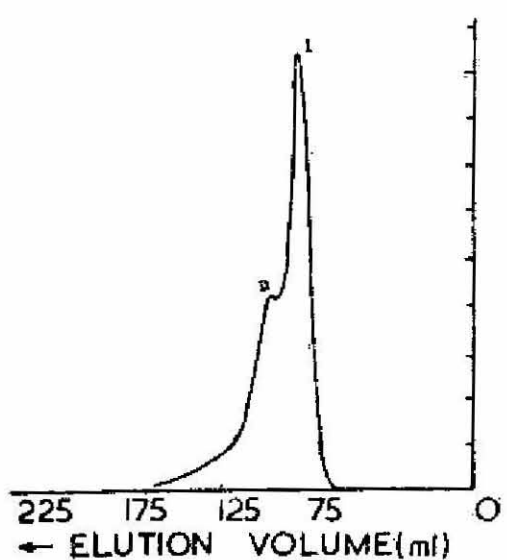
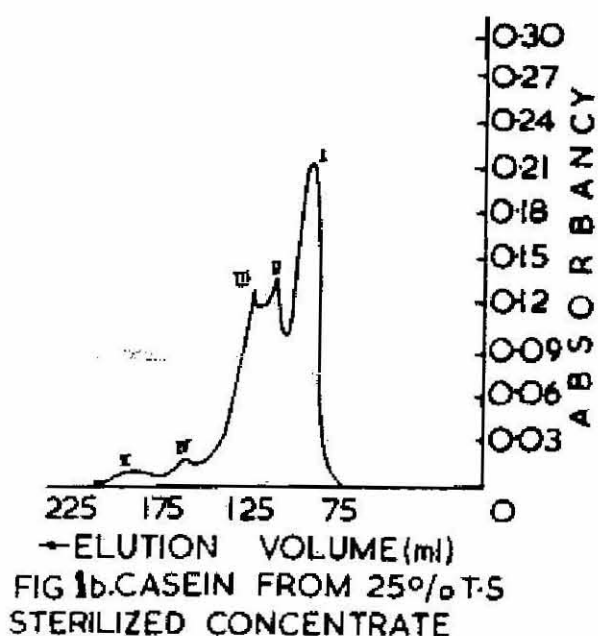
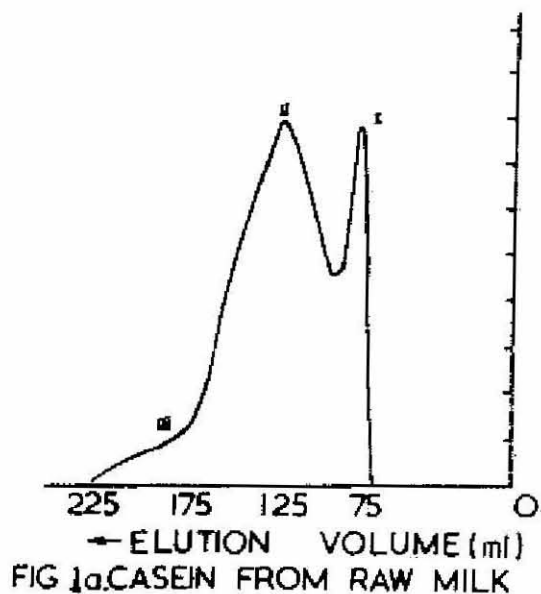


FIG 1. ELUTION PROFILE OF CASEIN OBTAINED BY FRACTIONATION AND CHROMATOGRAPHED ON SEPHADEX G-100 COLUMN (2.6X60 Cms) USING IMIDAZOLE-HCl BUFFER pH 7.6 WITH 6.6M UREA

**Changes at  $30 \pm 1^\circ \text{C}$  in 25% T.S. concentrate :** Electrophoretic mobility of casein samples from 25% T.S. concentrates stored for one month, three months and six months have been shown in Plate 2, 3, 4 (B). No difference in mobility was observed up to third month but at the end of sixth month, reduced mobility was noticed. The elution profile from chromatography on sephadex G-100 has revealed a better picture of changes. It has been shown in Fig 2.a, 2.b., 2.c and 2.d for first, second, third and sixth month of storage period respectively. Table 2 shows the elution volumes in ml and relative proportion of concentrations of different eluted fractions on the completion of first, second, third and six month of storage period. At the termination of the first month of storage, the peak size of fraction I reduced and fraction II also fragmented into different small fraction (Fig 1.b and fig 2.a). The small fractions could be the dissociation product of casein as well as interacted product of casein and whey proteins. At the end of second month, only two fractions appeared which may

be because of coalescence of minor fractions during storage. It is evident from Table 2 that the concentration of both the fractions increased. On the completion of third month a slight increase in relative concentration of fraction I had taken place but fraction II had fragmented into two smaller fractions with different relative concentration. At the termination of sixth month, the peak size of fraction I was reduced considerably whereas concentration of fraction II increased. Again fractions III of Fig 2.c seemed to have fragmented into two fractions that is fraction III and IV (Fig 5.d.). Paulina and Markh (1980) also observed aggregation of casein micelles in condensed milk which finally resulted into number of small fractions on long storage. The smaller peaks coming at the extremity could be due to fragmentation of amino sugar complex during storage. Cheeseman and Knight (1974) also reported fragmentation of protein during first few months which finally aggregated on further storage of concentrated milk.

TABLE 2

Elution volume (A) and relative proportion of area under various fractions (B) of casein from 25 percent total solids sterilized buffalo milk concentrate stored at  $30 \pm 1^\circ \text{C}$  obtained through chromatography on sephadex G-100 (2.6x60 cm) column.

Fractions	Storage period							
	1st month		2nd month		3rd month		6 month	
	A	B	A	B	A	B	A	B
I	28	17.6	30	40.6	30	43.0	40	21.2
II	15	32.2	45	59.4	40	48.6	65	67.9
III	10	18.2	-	-	25	8.3	25	8.0
IV	40	13.4	-	-	-	-	15	2.9
V	40	10.7	-	-	-	-	-	-
VI	10	4.0	-	-	-	-	-	-
VII	35	3.9	-	-	-	-	-	-

**Changes during storage at  $37 \pm 1^\circ \text{C}$  in 25% T.S. concentrate:** The electrophoretic mobility of casein samples of 25% T.S. concentrate for the period of first, third and sixth month have been shown in plate 2, 3 & 4 (c). The electrophoretic behaviour remained more or less similar to what had been observed for storage temperature of  $30^\circ \text{C}$ . The elution profile of casein

obtained from 25% T.S. concentrate stored at  $37^\circ \text{C}$  have shown in Fig 3.a, 3.b, 3.c and 3.d for a storage period of first, second, third and sixth months respectively. The relative concentration of protein fractions and their respective elution volumes in ml have been shown in table 3. From the Fig 3.a, it can be observed that there are two major fractions (fraction I and II)

Casein patterns of stored milk concentrates

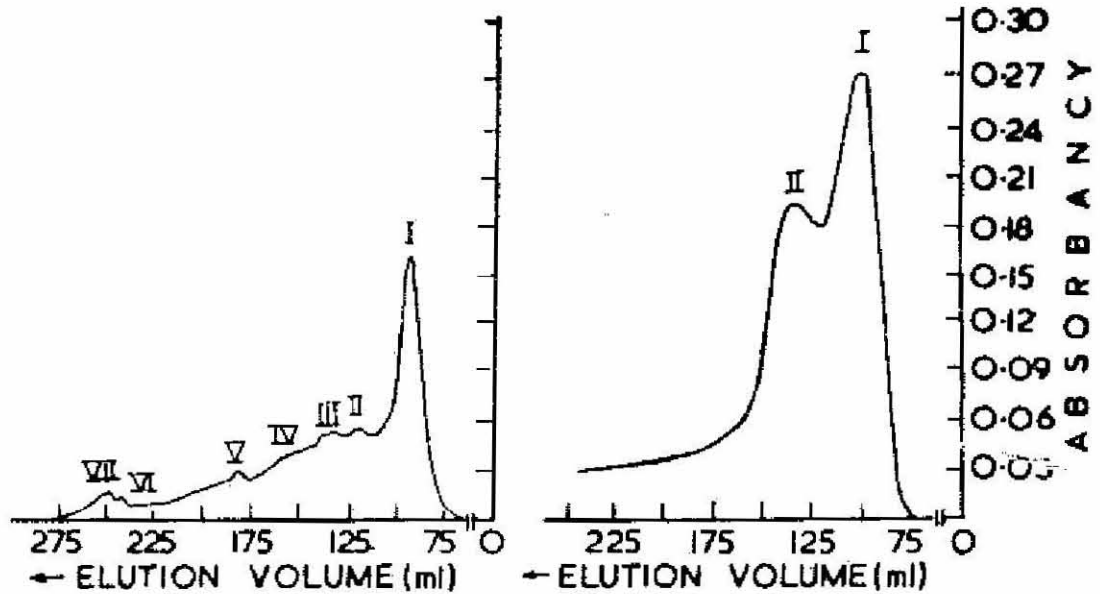


FIG. 2a AFTER 1 MONTH STORAGE      FIG. 2b AFTER 2 MONTH STORAGE

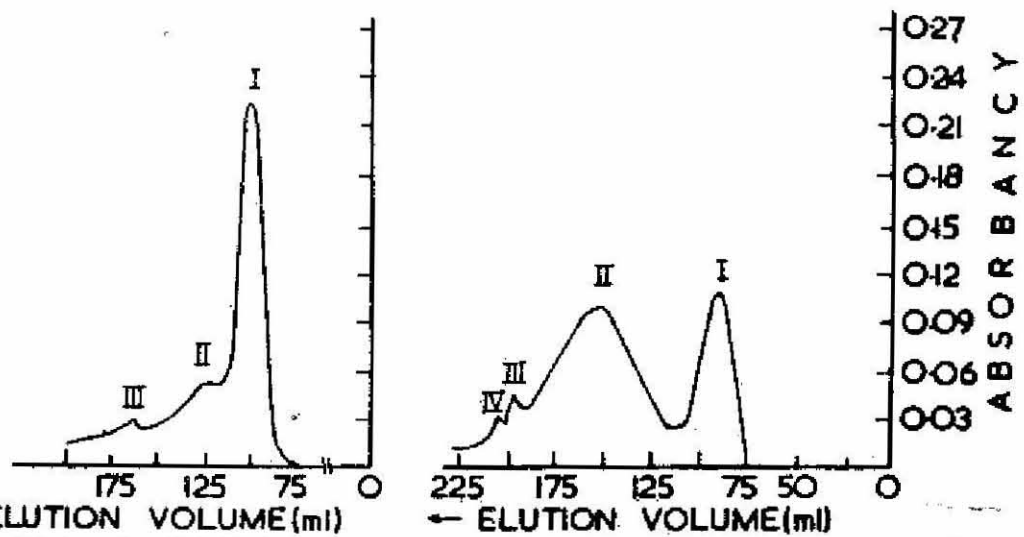


FIG. 2c AFTER 3 MONTH STORAGE      FIG. 2d AFTER 6 MONTH STORAGE

FIG 2 ELUTION PROFILE OF CASEIN OBTAINED BY FRACTIONATION FROM 25% TS CONCENTRATE STORED AT  $30 \pm 1^\circ\text{C}$  CHROMATOGRAPHED ON SEPHADEX G-100 (2.6 60 cms) COLUMN USING IMIDAZOLE-HCl BUFFER, pH 7.6 WITH 6.6M UREA

of casein obtained at the end of one month storage. On the termination of second month the relative concentration of both the fractions (fraction I and II) increased (Table 3) while the fraction III of fig 3.b appeared to be amalgamation of peaks III, IV and V

of fig 3.a. At the completion of third month the relative concentration of fraction I reduced to 11.0% while second fraction increased to 54.37% and another four small fractions appeared having relative proportions of 8.48, 7.95, 9.28 and 8.76 (Table 3).

**TABLE 3**  
Elution volume (A) and relative proportion of area under various fractions (B) of casein from 25 percent total solids sterilized buffalo milk concentrate during storage at  $37 \pm 1^\circ\text{C}$  obtained through chromatography on sephadex G-100 (2.6x60 cm) column.

Fractions	Storage period							
	1st month		2nd month		3rd month		6th month	
	(A)	(B)	(A)	(B)	(A)	(B)	(A)	(B)
I	25	43.2	45	44.5	35	11.1	70	74.3
II	15	20.0	35	26.7	30	54.4	50	25.7
III	15	23.3	75	28.8	20	8.4	-	-
IV	60	12.7	-	-	15	7.9	-	-
V	15	0.8	-	-	5	9.4	-	-
VI	-	-	-	-	35	8.8	-	-

This showed that the fragmentation of protein had taken place during storage. The smaller fractions at this stage could have resulted because of amino sugar complex with the formation of carbonyl compounds (Paulina and Markh; 1980). At the extremity of storage all these fractions were observed to have assembled to form two peaks with relative percent concentration of 74.26 and 25.74 with elution volumes of 70.0 and 50.0 ml respectively (Table 3). The coalescence of the protein at the end of sixth month under the present study appears to be similar to reports of Wilson (1971) and Cheeseman and knight (1974) who also observed the aggregation of proteins during long storage of concentrated milk.

**Changes during storage at  $30^\circ \pm 1^\circ\text{C}$  IN 30% T.S. concentrate:** Like 25% T.S. concentrate stored at  $30^\circ\text{C}$ , not much changes were observed in electrophoretic mobility of casein obtained from 30% T.S. concentrates up to third month. At the termination of storage, only one fraction was detected in this case. The mobility remained almost similar (Plate 2, 3, 4-D). Gel filtration profile of fractionated casein showed two peaks on the completion of first month of stor-

age (Fig 4.a). At the end of second month also two peaks were observed with a little change in percent composition of peak I (Table 4). After storage of three months two peaks were noticed with a marked increase and a marked decrease in relative percent concentration of peak I and II respectively (Table 4). At the completion of sixth month fragmentation of fraction II (Fig 4.c) took place, resulting into higher relative concentration of first fraction and formation of new fraction III (Fig 4.d, Table 4). The elution profile and the relative concentration of fractions indicated that aggregation of fraction I had taken place with fraction II and also fragmentation of fraction II (Fig 4.c) took place.

**Changes during storage at  $37 \pm 1^\circ\text{C}$  in 30% T.S. concentrate:** The pattern of electrophoretic mobility was observed to be similar to that of storage temperature of  $30^\circ\text{C}$  (Plate 2, 3.4-E). But the elution profile on sephadex gave a different picture as seen in fig. 5 for different storage period. At the end of first month of storage, one large and three smaller fractions were obtained (Fig 5.a) which coalesced to form one large fraction and one small fraction on the termination of

Casein patterns of stored milk concentrates

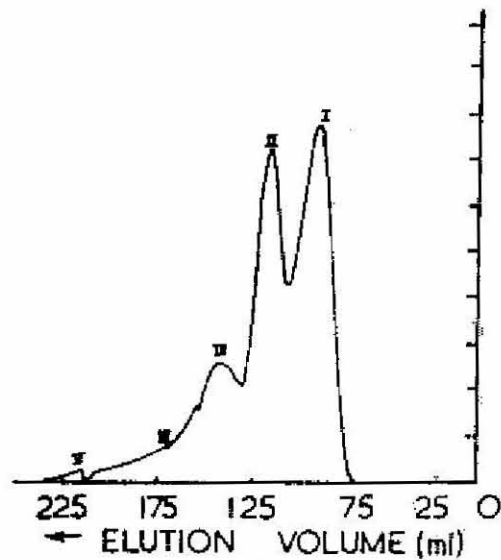


FIG 3a-AFTER 1 MONTH STORAGE

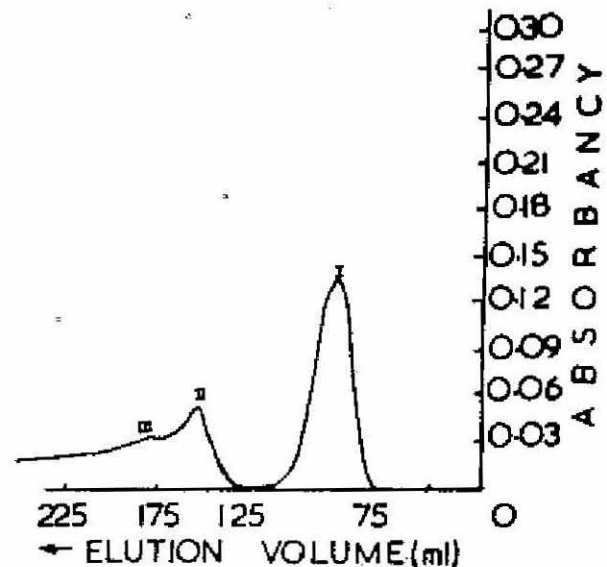


FIG 3b-AFTER 2 MONTH STORAGE

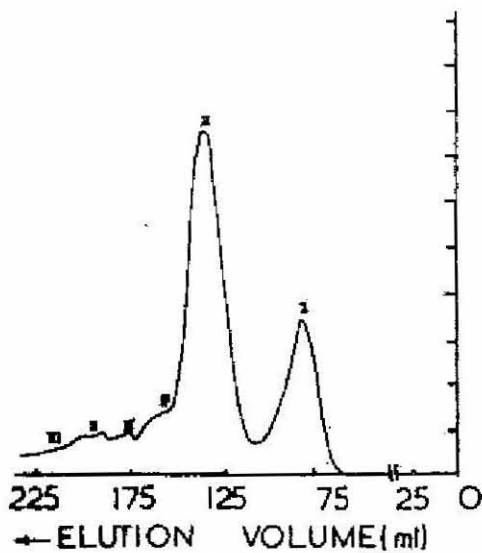


FIG 3c-AFTER 3 MONTH STORAGE

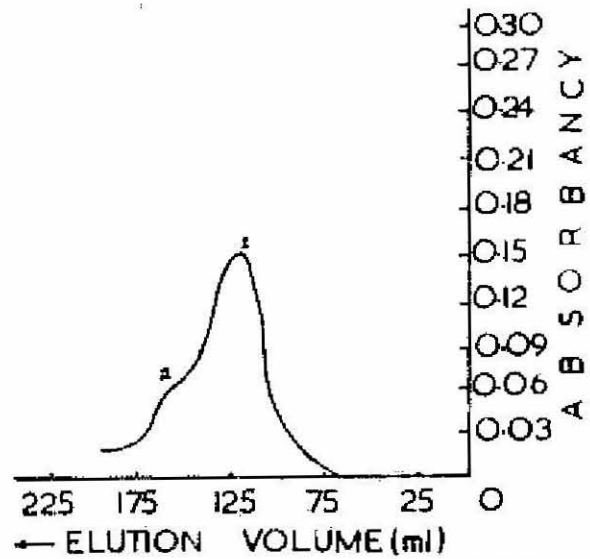


FIG 3d-AFTER 6 MONTH STORAGE

FIG 3 ELUTION PROFILE OF CASEIN OBTAINED BY FRACTIONATION FROM 25% T-S CONCENTRATE STORED AT  $37 \pm 1^\circ \text{C}$  CHROMATOGRAPHED ON SEPHADEX G-100 (2.6X60 cms) COLUMN USING IMIDAZOLE-HCl BUFFER pH 7.6 WITH 6.6M UREA

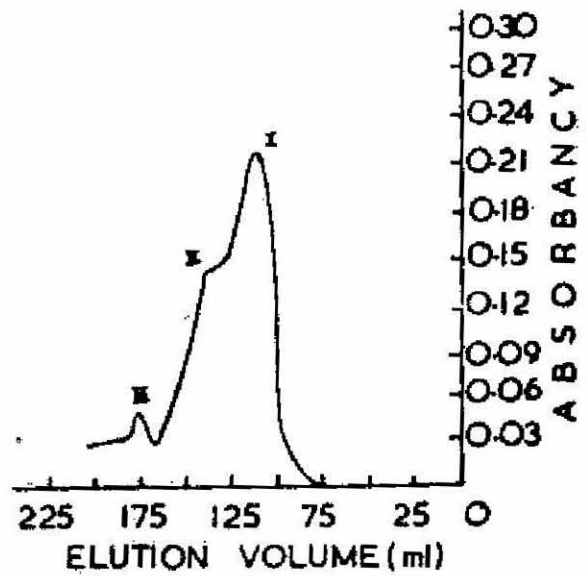
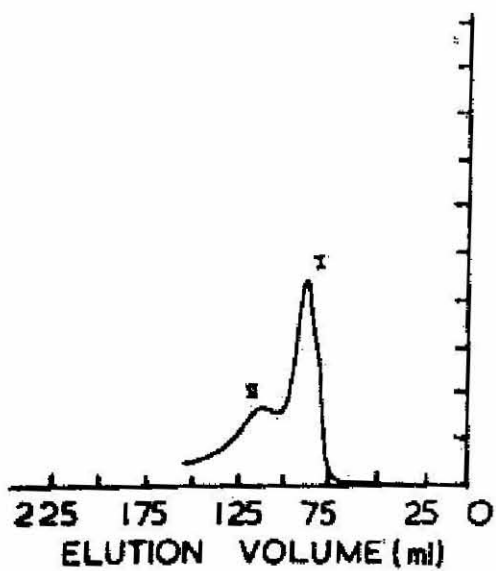
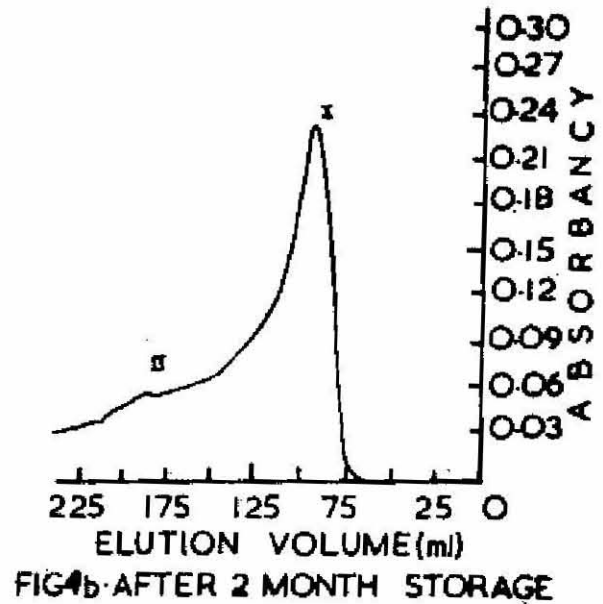
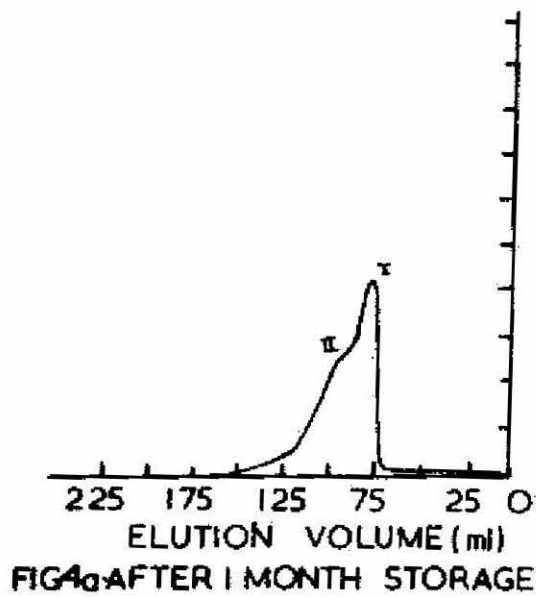


FIG4 ELUTION PROFILE OF CASEIN OBTAINED BY FRACTIONATION FROM 30% TS. CONCENTRATE STORED AT 30°C CHROMATO — GRAPHED ON SEPHADEX G-100(2.6X60cms) COLUMN USING IMIDAZOLE-HCl BUFFER pH 7.6 WITH 6.6M UREA

*Casein patterns of stored milk concentrates*

**TABLE 4**

**Elution volume (A) and relative proportions of area under various fractions (B) of casein from 30 percent total solids sterilized buffalo milk concentrate during storage at  $30 \pm 1^\circ\text{C}$  obtained through chromatography on sephadex G-100 (2.6 x 60 cm) column.**

Fractions	Storage period							
	1st month		2nd month		3rd month		6 month	
	(A)	(B)	(A)	(B)	(A)	(B)	(A)	(B)
I	25	61.7	110	69.4	25	49.2	60	75.0
II	100	38.3	40	30.6	50	50.8	35	18.8
III	-	-	-	-	-	-	15	6.2

second month of storage. On the completion of third month also two fractions were obtained with a slight increase in relative percent concentration of fraction I. But at the termination of six month of storage, drastic fragmentation of both the fractions were noted which resulted into nine smaller fractions. The relative percent concentrations and elution volumes obtained throughout the storage period have been listed

in Table 5. The results thus suggested that at higher total solids concentration more coalescence of proteins took place initially in stored samples both at  $30^\circ$  and  $37^\circ$  storage in contrast to fragmentation of proteins at lower concentration (25% T.S. concentrate). Wilson (1971) and Schmidt (1975) also reported the formation of large protein particles during storage of sterile milk concentrates of higher total solids.

**TABLE 5**

**Elution volume (A) and relative proportion of area under various fractions (B) of casein from 30 percent total solids sterilized buffalo milk concentrate during storage at  $37 \pm 1^\circ\text{C}$  obtained through chromatography on sephadex G-100 (2.6 x 60 cm) column.**

Fraction	Storage period							
	1st month		2nd month		3rd month		6th month	
	(A)	(B)	(A)	(B)	(A)	(B)	(A)	(B)
I	40	65.5	75	86.7	50	87	10	5.0
II	50	22.0	75	13.3	50	12.9	15	23.5
III	25	9.4	-	-	-	-	20	17.3
IV	25	3.0	-	-	-	-	10	16.5
V	-	-	-	-	-	-	20	14.3
VI	-	-	-	-	-	-	25	8.6
VII	-	-	-	-	-	-	10	8.0
VIII	-	-	-	-	-	-	15	4.1
IX	-	-	-	-	-	-	25	2.6

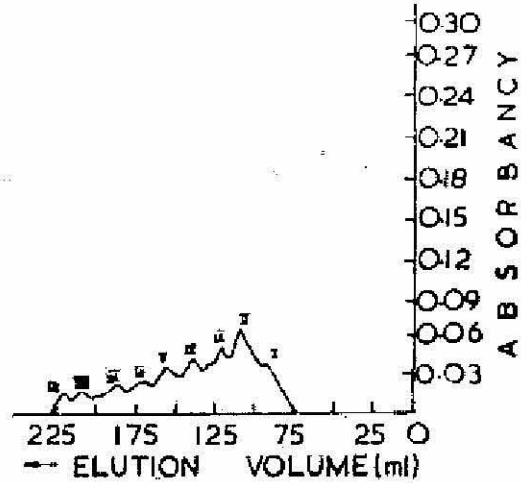
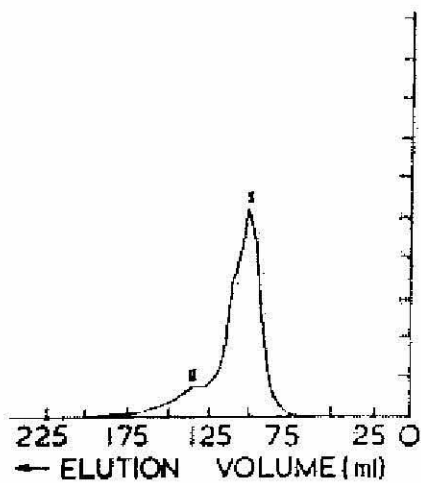
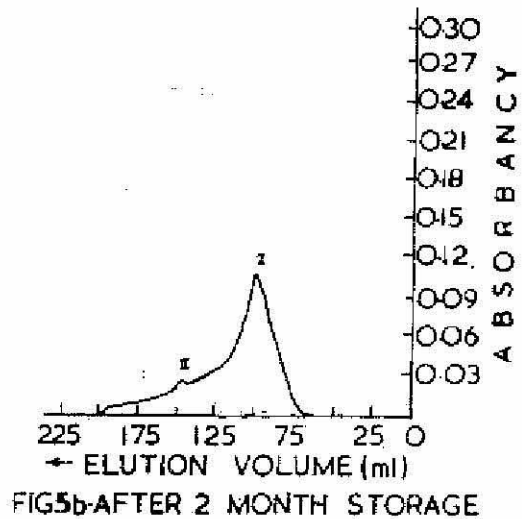
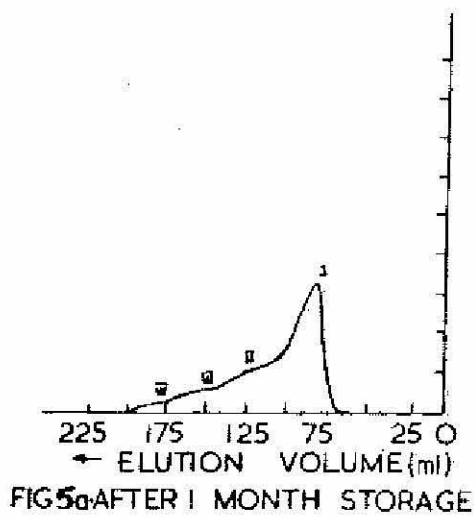


FIG 5. ELUTION PROFILE OF CASEIN OBTAINED BY FRACTIONATION FROM 30% T.S. CONCENTRATE STORED AT 37±1°C CHROMATOGRAPHED ON SEPHADEX G-100 (2.6X60 Cms) COLUMN USING IMIDAZOLE-HCl BUFFER pH 7.6 USING 6.6M UREA

*Casein patterns of stored milk concentrates \**

**Changes during storage at  $30 \pm 1^\circ\text{C}$  in 35% T.S. concentrate:** The electrophoretic pattern of casein samples obtained from 35% T.S. concentrate stored for one month, three months and six months are being shown in plate (2,3,4,-F). The pattern of mobility remained similar to 30% T.S. concentrate stored at this temperature for the similar period indicating not much of change occurring due to different total solids concentration. Table 6 shows the elution volumes in ml and percent concentrations of different fractions

obtained after chromatography from the stored samples. On the completion of first month seven peaks were obtained (Fig 6.a) which reduced to four at the end of second month. On the termination of third month the number of peaks further reduced to two and the number of peaks remained same at the end of sixth month also. The results showed that the fragmentation of casein fraction had taken place up to the end of first month which coalesced during further storage period.

**TABLE 6**

**Elution volume (A) and relative proportions of area under various fractions (B) of casein from 35 percent total solids sterilized buffalo milk concentrate during storage at  $30 \pm 1^\circ\text{C}$  obtained through chromatography on sephadex G-100 (2.6x60 cm) column.**

Fractions	Storage period							
	1st month		2nd month		3rd month		6 month	
	(A)	(B)	(A)	(B)	(A)	(B)	(A)	(B)
I	20	7.5	65	38.9	35	68.7	25	27.0
II	45	40.3	45	32.0	65	31.3	85	73.0
III	15	11.7	40	27.0	—	—	—	—
IV	15	25.0	30	2.2	—	—	—	—
V	5	9.1	—	—	—	—	—	—
VI	10	3.2	—	—	—	—	—	—
VII	20	3.0	—	—	—	—	—	—

**Changes during storage at  $37 \pm 1^\circ\text{C}$  in 35% T.S. concentrate:** The electrophoretic pattern remained the same as what had been observed for storage at  $30^\circ\text{C}$  (Plate 2,3, 4-G), indicating not much of change occurring due to different storage temperature and total solids concentration. The elution profile of chromatography has been shown in Fig. 7. The elution volumes in ml and relative percent concentrations have been shown in Table 7 for the entire storage period. From the chromatographic profile, it is seen that on the completion of first month only two fractions were obtained as compared to seven fractions noticed for the sample stored at  $30^\circ\text{C}$ . From the Fig 1, d it can be seen that aggregation had taken place in fresh product of 35% T.S. The two fractions appearing after one month storage fragmented into three fractions at the

end of second month. On the termination of third month once again two peaks were obtained. And finally at the extremity of storage four peaks were obtained. As indicated from Table 1 and 7 that fragmentation of first fraction took place at the end of first month which interacted with second fraction increasing the relative concentration from 37.54 to 51.29%. At the termination of second month, the second fraction fragmented and interacted with first fraction, showing a relative concentration of 68.73%. Finally at the extremity of storage both the fractions fragmented resulting into four fractions.

The heat treatment given to milk during processing may affect the micellar state of casein. The changes in body and texture and viscosity of concen-

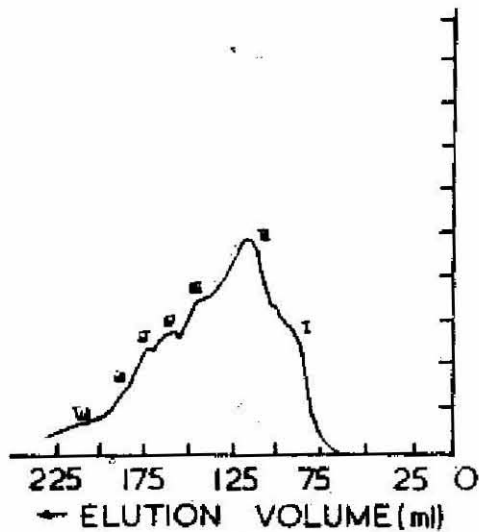


FIG 6a. AFTER 1 MONTH STORAGE

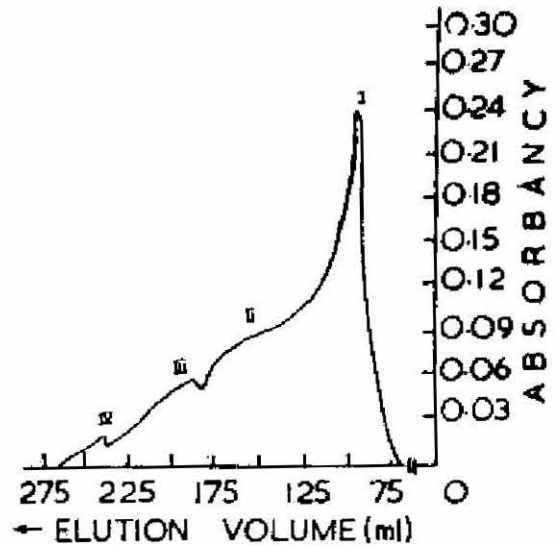


FIG 6b. AFTER 2 MONTH STORAGE

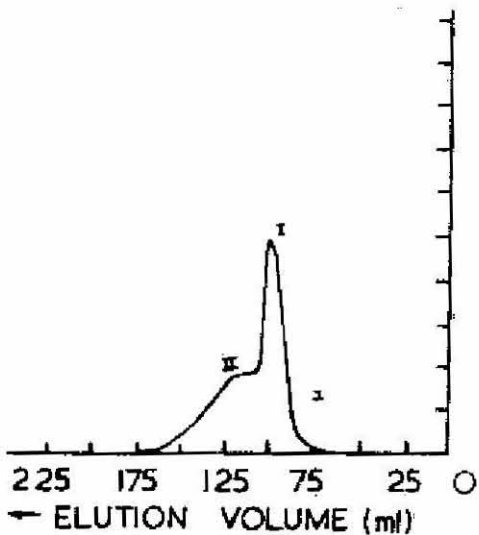


FIG 6c. AFTER 3 MONTH STORAGE

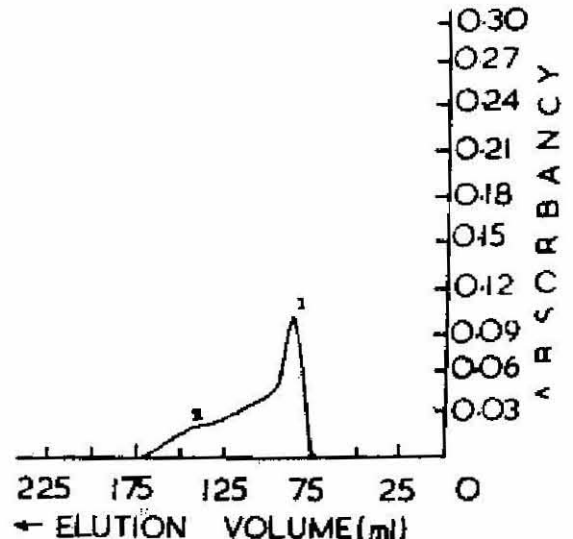


FIG 6d. AFTER 6 MONTH STORAGE

FIG 6 ELUTION PROFILE OF CASEIN OBTAINED BY FRACTIONATION FROM 35% T.S. CONCENTRATE STORED AT 30°C CHROMATOGRAPHED ON SEPHADEX G-100 (2.6X60cms) COLUMN USING IMIDAZOLE HCl BUFFER pH 7.6 WITH 6.6M UREA

Casein patterns of stored milk concentrates

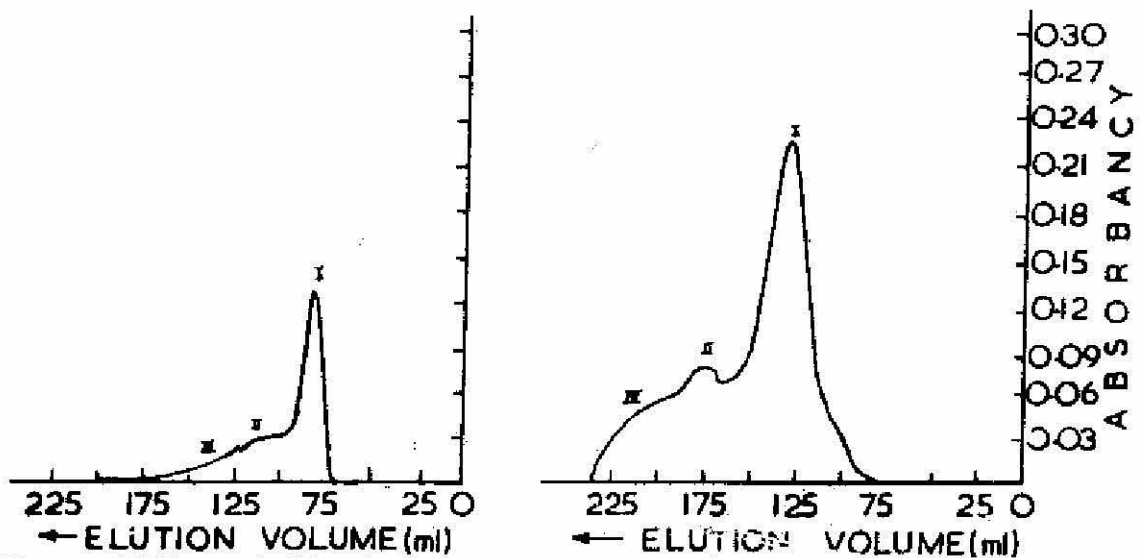
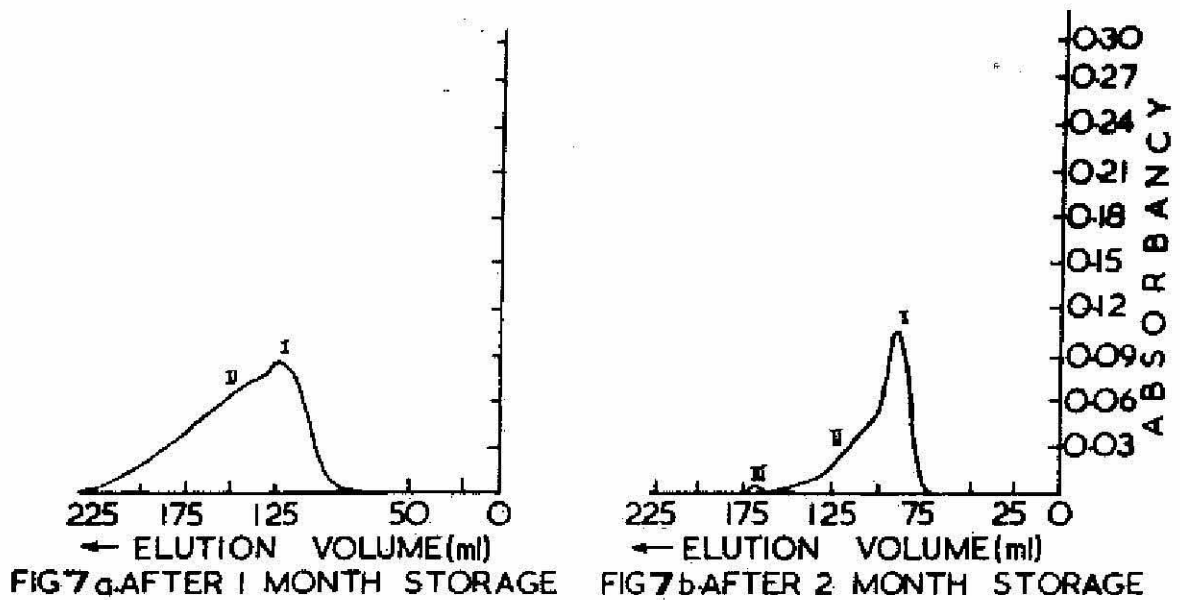


FIG 7. ELUTION PROFILE OF CASEIN OBTAINED BY FRACTIONATION FROM 35% TS CONCENTRATE STORED AT  $37 \pm 1^\circ\text{C}$  CHROMATOGRAPHED ON SEPHADEX G-100 (2.6 X 60 CM) COLUMN USING IMIDAZOLE HCl BUFFER pH 7.6 WITH 6.6M UREA

TABLE 7

Elution volume (A) and relative proportions of area under various fractions (B) of casein from 35 percent total solids sterilized buffalo milk concentrate during storage at  $37 \pm 1^\circ\text{C}$  obtained through chromatography on sephadex G-100 (2.6 x 60 cm) column.

Fractions	Storage period							
	1st month		2nd month		3rd month		6th month	
	(A)	(B)	(A)	(B)	(A)	(B)	(A)	(B)
I	30	48.7	35	63.6	25	68.7	20	8.0
II	95	51.3	60	34.8	60	31.3	60	10.2
III	-	-	10	1.6	-	-	25	61.1
IV	-	-	-	-	-	-	60	20.7

trated sterilized milk are important to be considered during storage. Their aggregation and disaggregation may lead to thickening or thinning, gelation; sedimentation etc. Very little information is available to show the influence of severe heat treatment on the state of whole casein in concentrated products. The present study which is preliminary in nature was to establish the necessity for going in depth of this problem. The present investigation has amply revealed that there are changes occurring in the states of casein fractionated from different sterilized concentrated buffalo milk which were stored at  $30^\circ\text{C}$  and  $37^\circ\text{C}$  for different periods.

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