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Ankita Hooda

M.Tech (DC) & B.Tech (DT),
NDRI, Karnal, Haryana, India

Dr. Bimlesh Mann

Principal Scientist & HOD,
Dairy Chemistry Division,
NDRI, Karnal, Haryana, India

Dr. Rajan Sharma

Principal Scientist, NDRI,
Karnal, Haryana, India

Dr. Rajesh Bajaj

Principal Scientist DC Division,
NDRI, Karnal, Haryana, India

Munmun Sen

Ph.D Scholar, DC Division,
NDRI, Karnal, Haryana, India

Sulaxana Singh

Ph.D Scholar, DC Division,
NDRI, Karnal, Haryana, India

Suvarthan Ranvir

Ph.D Scholar, DC Division,
NDRI, Karnal, Haryana, India

Correspondence

Dr. Bimlesh Mann

Principal Scientist & HOD,
Dairy Chemistry Division,
NDRI, Karnal, Haryana, India

Characterization of Micellar Casein concentrate prepared from goat milk

Ankita Hooda, Dr. Bimlesh Mann, Dr. Rajan Sharma, Dr. Rajesh Bajaj, Munmun Sen, Sulaxana Singh and Suvarthan Ranvir

Abstract

Casein (CN) Micelles are nano-capsules created by nature to deliver nutrients, such as Calcium, phosphate and protein, to the neonate. Variation in pH, presence of solvents, and level of different salts affects the stability & physicochemical properties of CN micelles. CN Micelles so harvested by microfiltration (MF) are known as Micellar Casein Concentrate (MCC). The average particle size of Goat Skim Milk (200 ± 3.61 nm) and that of Goat MCC (198.2 ± 4.2 nm) which indicates that the diameter of CN micelles remains unaltered during process of MF. The magnitude of Zeta Potential decreased towards the negative side from -19.2 mV in goat skim milk to -16.6 mV in goat MCC. The magnitude of zeta potential increased towards negative side during increase of pH. Sodium Hexa Meta Phosphate, Tri sodium citrate, Di sodium Hydrogen Phosphate and Sodium Di Hydrogen Phosphate had dissociating effect on CN micelles which followed the order SHMP>TSC>Na₂HPO₄>NaH₂PO₄. The effect of Alcohol (strength varying from 10 to 80 caused disintegration as well as aggregation. These studies would be helpful in using MCC as protein source and as a functional ingredient in different food systems.

Keywords: Micellar casein concentrate, microfiltration, colloidal calcium phosphate, casein micelles

1. Introduction

Casein (CN) Micelles contain 94% protein and 6% colloidal calcium phosphate (CCP) on a dry basis, which is comprised of calcium, magnesium, phosphate, and citrate. The nature and structure of casein micelle have been studied extensively because it plays important role in determining the stability of milk and milk product.

The Dual binding (Internal structure) model of CN is widely accepted (Phadungth, 2005) and suggests that two types of bonding: attractive hydrophobic (driving force for formation of casein micelles) and electrostatic repulsions (responsible for limiting growth of polymers) are responsible for CN micelle structure. Calcium phosphate nanoclusters or CCP are considered one of linkages between CN micelles and neutralizing agent of negative charge of phosphoserine residues. K-CN does not have any phospho serine residue and hence can only bind hydrophobically, so it acts as chain terminator.

CN micelles has unique techno functional and bi functional characteristics that can be explored for use of casein in different food systems as functional ingredient.

The stability of casein micelles is affected by change in pH, addition of solvents and ionic strength. These conditions affect the structure of casein micelles and affect their stability and functional properties. Hence manipulation of these conditions would be beneficial in changing structure of casein micelles. This characteristic of casein micelles can make it a potential ingredient in food systems as a source of protein as well as a functional ingredient.

As size of casein micelles vary from 50 nm to 500 nm these could be harvested from skim milk by membrane technology. Microfiltration (MF) processing membrane (pore size 0.1 to 1 micrometer) is suitable for harvesting CN micelles from skim milk Level of concentration can be controlled to avoid physical changes in state of casein micelles. The method of MF is also economical to be used at industrial scale as compared to ultracentrifugation which is another method by which casein micelles can be separated from milk in the native state. Hence MF is most favourable, adjustable and economical method for separation of CN micelles in their native state from milk.

Retentate from the MF of skim milk consists mainly of micellar CN; and hence known as Micellar Casein Concentrate (MCC). MCC would have a lower concentration of lactose, SP, NPN and serum phase minerals compared to Skim Milk. MCC is expected to exhibit different properties as compared to skim milk because it has lower concentration of NPN, serum proteins, soluble mineral and lactose. MCC also exhibits higher heat stability and shelf life. So, MCC could easily be used in different food systems as protein source and functional ingredient.

Further, this study would help to harness casein micelles that will be useful for the nano-encapsulation of hydrophobic nutraceutical (for enrichment of low or non fat food products), therapeutic and cosmetic compounds. By rationalizing effects of pH, ionic strength, solvents on the casein micelles, will help in manipulating the textural properties of products based on these. Moreover solubility of Milk Protein Concentrate are dependant CN micelles, hence manipulating the conditions could increase solubility of MPC.

2. Materials and Methods

2.1 Materials

Sodium Hydroxide, Hydrochloric Acid, Ethanol, Tri Sodium Citrate, Sodium Hexa Meta Phosphate, Disodium Hydrogen Phosphate, Sodium dihydrogen Phosphate were obtained from

Sigma Aldrich Ltd. Copper Sulphate, Boric Acid, Sodium Carbonate were obtained from Himedia Pvt. Ltd.

2.2 Experimental equipment

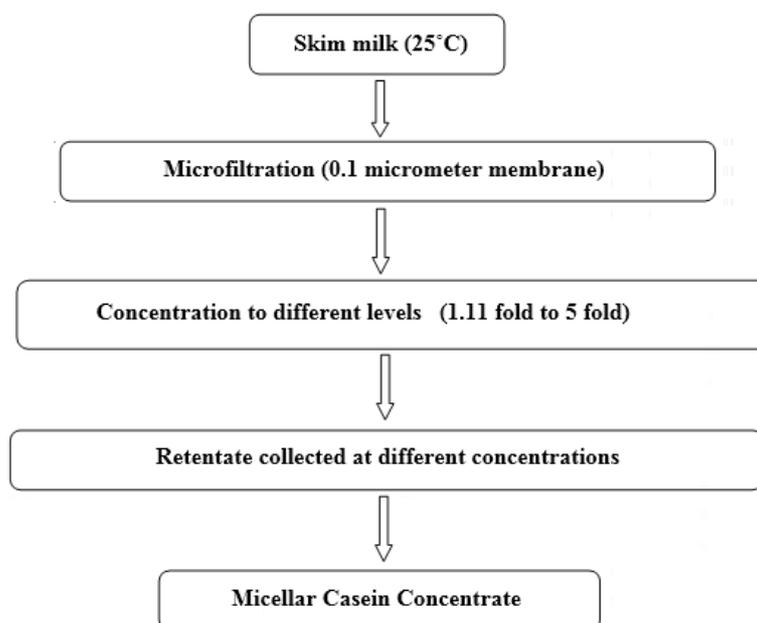
Freeze dryer (Labconco corporation, Kansas City, MO), Particle size analyzer (Malvern Instruments Ltd., U. K.), Kubota centrifuge (Tokyo, Japan), pH meter (PHAN, Lab India, India), Atomic Absorption Spectrophotometer (Shimadzu, Japan), Manual Hollow Fiber Ultra Filtration assembly (QSM-03S model, M/s. GE Healthcare, Gurgaon, Haryana)

2.3 Sample Collection

Milk sample Goat was collected from Livestock Research Centre, NDRI. The whole milk was centrifuged immediately at 2 ° C at 3500 rpm for 45 minutes. The obtained skim milk was subjected to microfiltration.

2.4 Concentration of Skim Milk by Microfiltration

The skim milk obtained was micro filtered. MF was done in Manual Hollow Fiber Ultra Filtration assembly (QSM-03S model, M/s. GE Healthcare, Gurgaon, Haryana) using a Hollow Fiber membrane Cartridges of 0.1 micrometer pore size (M/s GE Healthcare Bio-Sciences Ltd., Hong Kong). Pump flow rate was adjusted at 200- 250 rpm and average transmembrane pressure maintained below 5 kPa.



2.5 Determination of particle size distribution

The mean particle diameter, particle size distribution, Z-average, zeta-potential and Poly Dispersity Index (PDI) of the samples were measured by using Malvern Nanoparticle Analyzer. The experiments were carried out on the 50 times diluted freshly prepared samples. A He-Ne laser was used, set at an angle of 90°, with the wavelength of the laser beam being 633 nm following the procedure of other researchers (Gastaldi *et al.*, 2003). The viscosity and refraction index of water were 0.8872 cP and 1.330, respectively. For each sample, the light scattering measurements were carried out at 25°C, and casein micelle size and poly dispersity index (PDI) were determined. Three replicate measurements were performed for each sample. The size measurements using dynamic light scattering are based on the scattering of light by moving particles.

Hence, the samples must be sufficiently diluted to avoid multiple scattering casein micelles. The duration between dilution and size measurement has also varied amongst researches, some preferring to make immediate measurements, while others left the samples for up to 30 min before making measurements. Although the duration between dilution and making measurements has been reported to not affect measurements, in this study the samples were kept for 15 min at room temperature before measurement, following the procedure described 8 Renan and others. A preliminary trial on the relationship between the measurement of casein micelles and the dilution factor was conducted. It was observed that a 50-fold dilution with Milli-Q water at the same pH was optimal for the measurement. Disposable four-side plain cuvettes were used under an operating temperature of 25°C and humidity 85%. The lower and upper size limits for this instrument is 0.3 nm and 8 µm respectively.

2.6 Determination of zeta potential

The electrical charge on the casein micelles in the skim milk, MCC, MCC treated with different environmental conditions was determined using Malvern Nanoparticle Analyzer in the form of zeta potential. The experiments were carried out on the 50 times diluted freshly prepared samples. It is based on the principle of Laser Doppler electrophoresis. In this method, sample particles suspended in a solvent are irradiated with laser light and an electric field is applied. When the frequency shift at angle θ is measured once the electric field is applied, the following relationship between particle motion velocity (V) and mobility ($U=V/E$) is formed. The analyzer uses a heterodyne optical system to observe particle motion velocity and calculate electrical mobility from the resulting frequency intensity distribution.

The zeta potential is determined by measuring the velocity of the droplet when in an applied electric field. The measurement was carried out under holder at 25°C and electric voltage 3.9 V. A parallel plate electrode (0.45 cm² square platinum plates with a 0.4 cm gap) was inserted and the cuvette was placed in a temperature-controlled holder at 25°C. Measurements were carried out in triplicate for each sample and results were reported in mV. The samples can be diluted to required concentration before measurement.

2.7 Mineral Estimation

Calcium was estimated by dry digestion method of AOAC (2005) using Atomic absorption spectroscopy.

2.7.1. Cleaning of glass ware

All glassware used for AAS analysis were washed properly by nitric acid and then washed with triple distilled water.

2.7.2. Reagents

A. Triacid solution

100 mL Triacid solution was prepared by adding HNO₃, HClO₃, H₂SO₄ in ratio 3:2:1.

B. Standard Mineral Solution Preparation

Stock solution of calcium (1000ppm) was prepared in 100ml volumetric flask. This stock solution was diluted to get standards of desired concentration.

2.7.3 Sample Preparation

100mg of sample was weighed in a silica crucible and transferred to a muffle furnace at 550°C/8h. Crucible was then taken out of furnace and 10ml of triple acid (nitric acid: perchloro acetic acid: sulphuric acid in the ratio 3:2:1) was added and heated on hot plate for complete dissolution. It was then diluted 100 times with triple distilled water.

2.7.4 Calcium estimation

Sample prepared by dry ashing was suitably diluted and analyzed by atomic absorption spectrophotometer (Shimadzu AAS, AA-7000, Japan) at wavelength λ_{\max} 422.7 nm. A standard curve was prepared by taking standard calcium solution in concentration ranges from 2 ppm to 10 ppm (Fig. 1) and the calcium content of the samples was calculated from it.

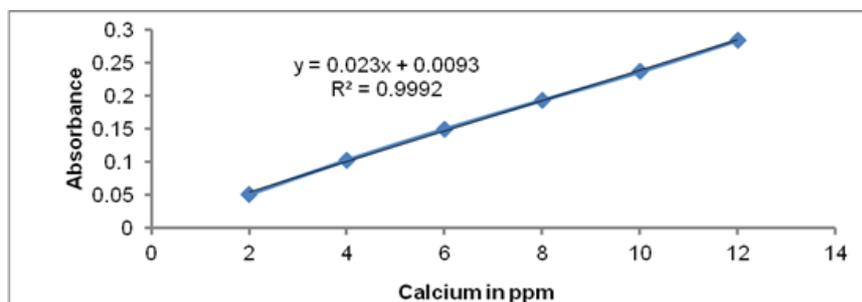


Fig 1: Standard curve for calcium estimation

2.8 Effect of pH, ionic strength, ethanol concentration

2.8.1 Micellar casein concentrate and skim milk was subjected to change in pH by addition of 1 N NaOH for basic pH, and 1 N HCl was used for decreasing pH. The effect of change in pH were analyzed by particle size analyzer as per method given in 3.4, the change in zeta potential was measured as per method given in 3.5, the change in micellar structure was seen through TEM as per method in 3.9.

2.8.2 Different Salts were added at the rate of 0 to 2 % (w/v) in micellar casein concentrate and skim milk. The effect of change in pH were analyzed by particle size analyzer as per method given in 3.4, the change in zeta potential was measured as per method given in 3.5, the change in micellar structure was seen through TEM as per method in 3.9.

2.8.3 Ethanol (strength of 10 – 80%) was added at the rate of 10 to 40 % in MCC and skim milk.

3. Results and Discussion

3.1 Microfiltration of Skim Milk

Goat Skim milk was subjected to microfiltration. Firstly membrane was washed 3-4 times with distilled water at 45-

50° C, to make it free of ethanol. Then one liter of skim milk was filled in the jar, attached to assembly. Pump flow rate was adjusted at 200- 250 rpm and average transmembrane pressure maintained below 5 kPa. Permeate was collected in a beaker while maintaining the retentate knob closed. Retentate withdrawn at regular interval when desired level of concentration was achieved (900 ml, 800 ml, 700 ml, 600 ml, 500 ml, 400 ml, 300 ml and 200 ml). Retentate withdrawn at regular interval when one to five fold concentration was achieved. Elizabeth Hurt (2015) used different conditions of flux, temperature and feed conditions to optimize the process of MF were used. This procedure by Pouliot *et al.*, 1996 allowed the preparation of native phosphocaseinate containing about 79% protein with casein to total protein nitrogen ratio over 0.91.

3.2 Effect of Concentration on Particle Size Distribution of CN Micelles in Goat Skim Milk and MCC

The retentate collected at various concentrations were subjected to different analytical techniques to find the effect of concentration, on aggregation behavior of casein micelle. Retentate of all levels and skim milk were primarily analyzed

for particle size distribution by Malvern zetasizer. There were no larger size particles observed and PSD of retentate were similar to skim milk. The average particle size of goat skim milk (200 ± 3.61 nm) and that of goat MCC (198.2 ± 4.2 nm) which indicates that the diameter of casein micelles remains unaltered during process of MF (Fig. 2). Hence it was concluded that MF of skim milk to different levels of

concentration caused no aggregation of CN micelles and also no disintegration of CN micelles has taken place. It is also clear that CN micelles were stable in the MCC and were in their native state. The average diameter size of CN micelles (Z-average) did not vary with progressive concentrations of retentate and also the physicochemical characteristics of casein micelles remain unchanged.

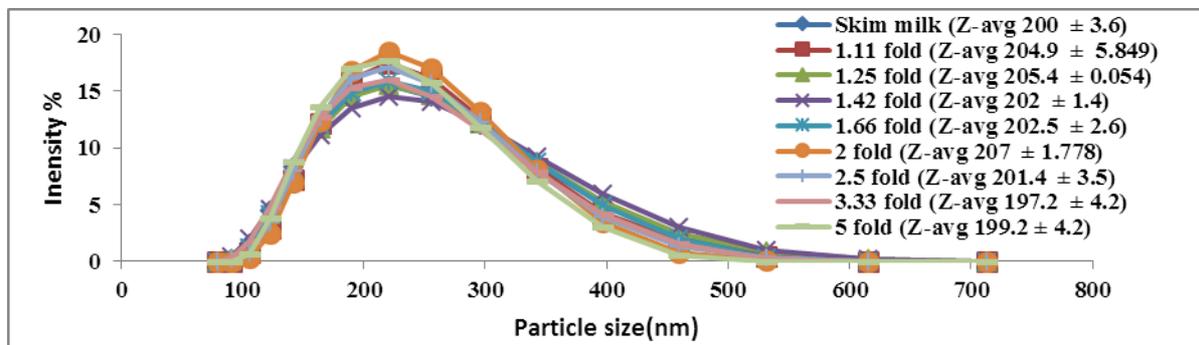


Fig 2: Particle Size distribution of goat skim milk at different concentration level

3.3 Effect of Concentration on Zeta Potential of Skim Milk and Micellar Casein Concentrate

The retentate and skim milk were analyzed for zeta potential by Malvern zeta sizer. With the progressive concentration of skim milk, slight decrease in zeta potential observed (Fig. 3). Slight decrease in zeta potential observed may be due to

decrease in charge of double layer because after concentration by MF, the CN micelles get nearer and concentrated (Payens, 1966). The magnitude of Zeta Potential of goat skim milk decreased from -19.2 mV to -17.2 mV in five fold concentrated MCC (Fig. 3). Wade (1995) also found the Zeta Potential of skim milk at natural pH was -18 mV.

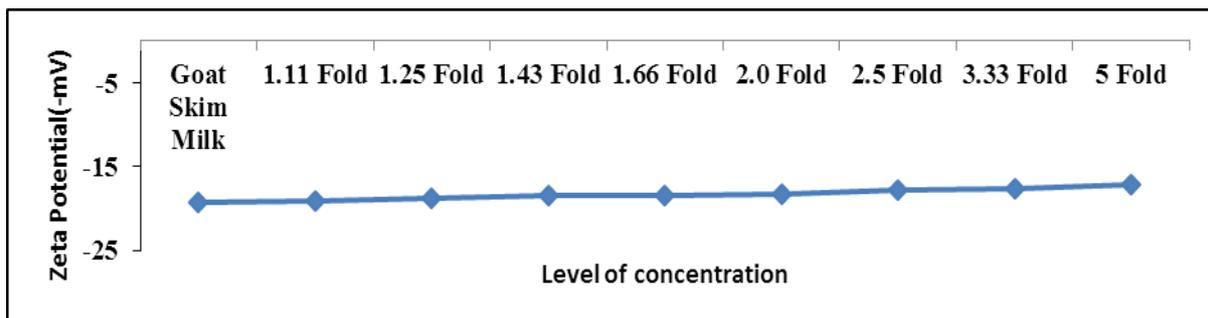


Fig 3: Zeta potential of goat skim milk at different concentration level of concentration

3.4 Effect of Concentration on Turbidity of Skim Milk and MCC

Turbidity analysis of skim milk and retentate at different concentrations were carried out as per method given by Liu *et al.*, 2008 [21] by observing absorbance at 450 nm using ELISA plate reader. It was observed (Fig. 4) that turbidity increased from lower to higher concentration level. Turbidity of milk or the white color of milk is due to casein micelles. Hence on

concentrating the skim milk the casein micelles get concentrated and the turbidity value increases. This was clearly evident from turbidity values which increased with level of concentration for all the three types of milk. The value of absorbance in case of Goat skim milk was 0.129 ± 0.009 which increased to 0.162 ± 0.002 in five fold concentrated MCC.

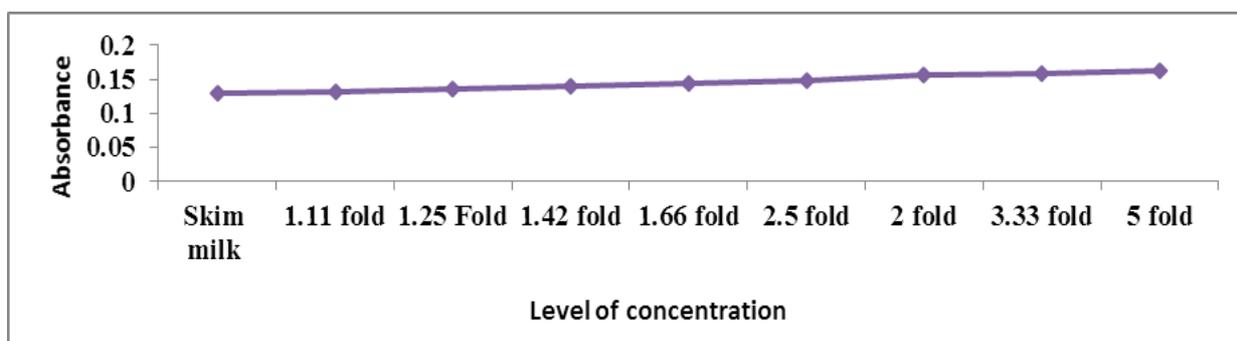


Fig 4: Absorbance (turbidity) of goat skim milk at different concentration levels

3.5 Effect of Concentration on Total Calcium content of Skim Milk and MCC

Total Calcium Concentration was estimate in skim milk and retentate at different level of concentration using Atomic absorption spectroscopy to see the effect of progressive concentration. It was found that the Ca concentration (ppm) decreased from skim milk to retentate upto a certain level but then it showed slight increase up to the 5 fold concentration level in goat milk (Fig. 5). Total calcium includes colloidal and soluble calcium (Fox *et al.*, 2015) ^[9]. On concentrating,

most of soluble calcium is removed in permeate and this may be the reason the concentration of calcium decreases initially (Elizabeth, 2015). After that due to concentration effect, soluble calcium starts moving towards colloidal phase (Fox *et al.*, 2015)^[9]. Hence all the soluble calcium is not removed and some of it is converted to the colloidal (CCP) calcium phosphate form. The total calcium in case of goat skim milk was 910 ± 17.39 ppm, 668 ± 21.27 ppm in case of 1.66 fold concentrated retentate and 690 ± 22.05 ppm in five fold concentrated retentate (Fig. 5).

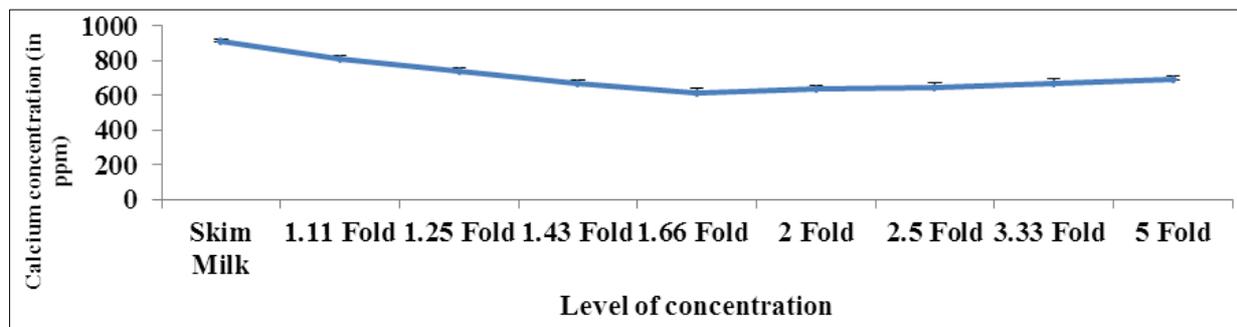


Fig 5: Variation in total calcium concentration in goat milk during microfiltration

3.6 Compositional Analysis of skim milk and MCC

Protein content of skim milk and MCC was estimated by International Dairy Federation (block digestion) method for determination of total nitrogen content. Total solid and ash content were also determined in skim milk and 5 fold concentrated MCC. The protein content in goat skim milk was $3.57 \pm 0.04\%$ while in MCC was $14.42 \pm 0.27\%$ (Table 1).

Table 1: Composition of skim milk and micellar casein concentrate of buffalo, cow and goat

| | Goat Skim Milk | Goat MCC |
|-----------------|------------------|------------------|
| Protein (%) | 2.8 ± 0.81 | 13.06 ± 0.42 |
| Total Solid (%) | 7.5 ± 0.14 | 11.84 ± 0.62 |
| Ash (%) | 0.78 ± 0.081 | 1.26 ± 0.042 |

3.7 Effect of pH and additives on physico-chemical properties of MCC and skim milk

The physicochemical properties and stability of CN micelles are changed as the environmental conditions change. It is essential to find out the variation in the structure, stability and properties of casein micelles with change in pH, ionic strength and solvent addition so that these characteristics could be used further for incorporation of casein in various food systems. These studies would also be helpful in predicting the behavior of casein micelles in various foods where it is used as a functional ingredient and as a protein source.

3.7.1 Effect of pH on Goat skim milk and MCC

Change in pH modifies the internal and external structure of micelles thus changing its properties and its stability. The behavior of casein micelles to acidic and basic side is entirely different. The MCC prepared was diluted to skim milk level and subjected to different pH conditions. Variable pH was adjusted using 1 N NaOH to basic side, and 1 N HCl to the acidic side. When the pH of MCC and skim milk was increased to basic side, the sample tends to become transparent from white. There was a decrease in turbidity as

pH increases towards basic side in the skim milk as well as MCC. Some studies have observed same phenomenon that by increasing in milk pH up to 11.0 appear to decrease milk turbidity (Vaia *et al.*, 2008 and Madadlou *et al.*, 2009) ^[23]. As the turbidity is generated by the light scattering properties of CM, the increased transparency of milk could reflect a decrease in size of casein micelles. It was evident that on moving towards the basic side there was formation of casein micelles of average diameter less than 100 nm. The particle size distribution revealed that with increase in pH towards the basic side, the graph of particle size distribution widened on both the side (Fig. 6). It is due to the reason that this high pH caused the precipitation of serum calcium phosphate onto CN micelles, which is shown by the low level of ionic calcium and non micellar calcium in the serum phase. These conditions tend to weaken the cohesive interaction in the micelles, leading to the breakage of the hydrophobic bonds amongst caseins which eventually dissociate the casein micelles (Sinaga *et al.*, 2016). On the other hand in the acidic side there was appearance of small flakes initially, which gradually became larger and finally the sample coagulated with separation of curd and liquid part. The magnitude of Zeta potential changed from -16.22 mV to -35.4 mV in goat MCC (Fig. 7). Effects were more pronounced in MCC as compared to skim milk due to concentration effect. When we move towards acidic side casein micelles precipitated out and whey was separated. This process started earlier in case of MCC. The pH at which goat MCC becomes transparent was 11.72 (Plate 2). The zeta potential varied in case of goat skim milk from -19.2 mV to -32.4 mV (Fig. 8). Goat skim milk also turns transparent but to lesser extent than MCC (Plate 1). A decrease in the milk natural pH affects the internal structure of the CM, as well as their external surface layers (Sinaga *et al.*, 2016). The PSD at acidic side, initially the average diameter of the casein micelles decreased and then there was appearance of larger aggregated particles on the PSD (Fig. 6).

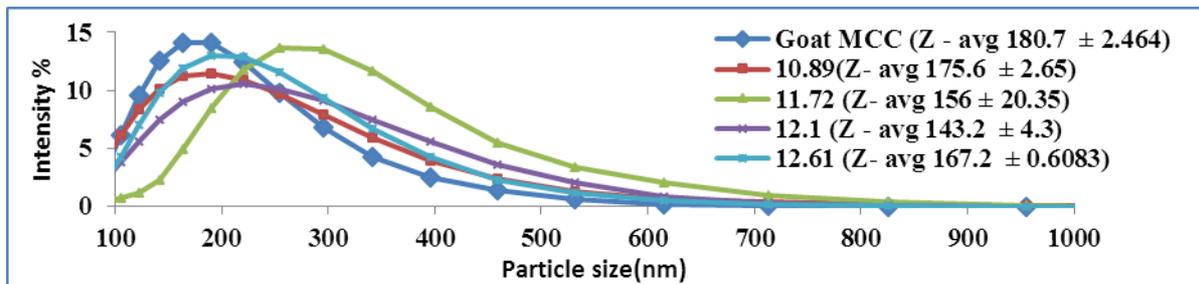


Fig 6(a): Effect of pH on particle size distribution of goat micellar casein concentrate

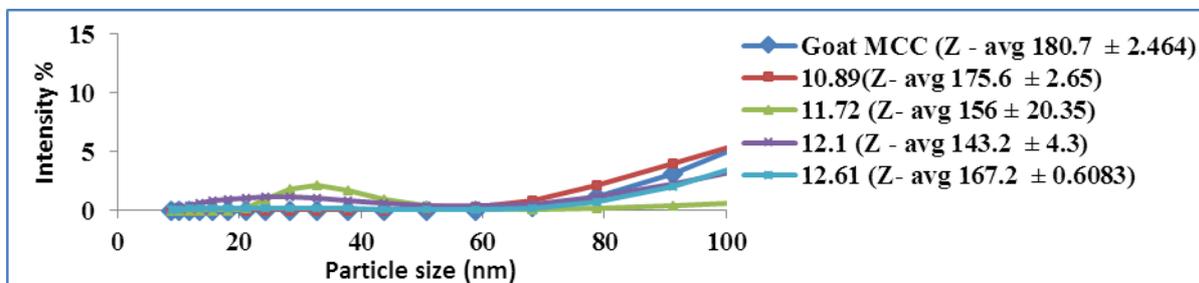


Fig 6(b): Effect of pH on particle size distribution of goat micellar casein concentrate

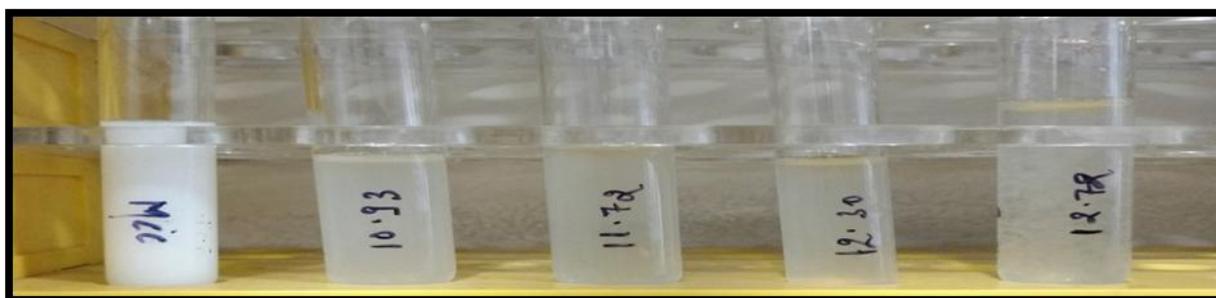


Plate 1. Effect of pH at basic side on goat micellar casein concentrate

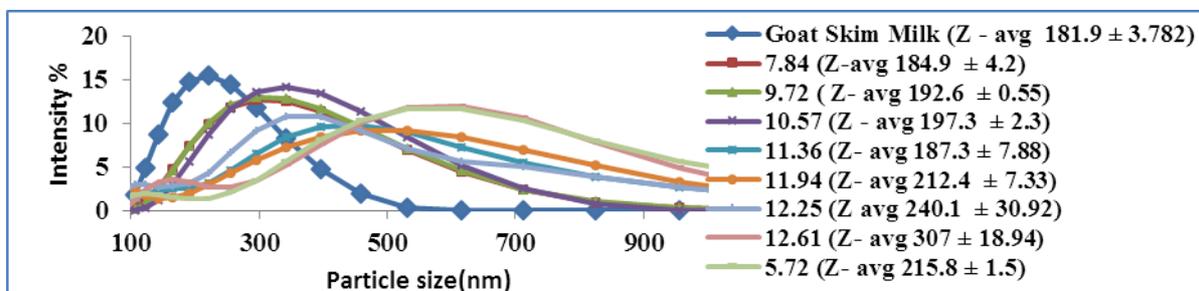


Fig 7(a): Effect of different pH on particle size distribution of goat skim milk

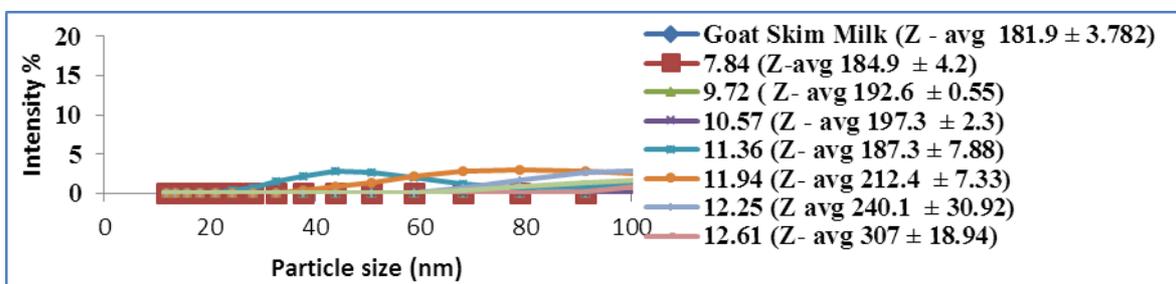


Fig 7(b): Effect of different pH on particle size distribution of goat skim milk

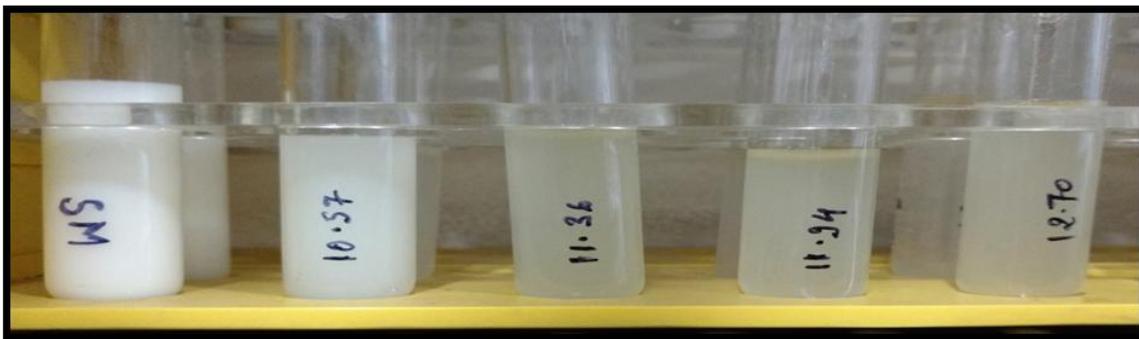


Plate 2: Effect of different pH on Particle Size Distribution of Goat Skim Milk

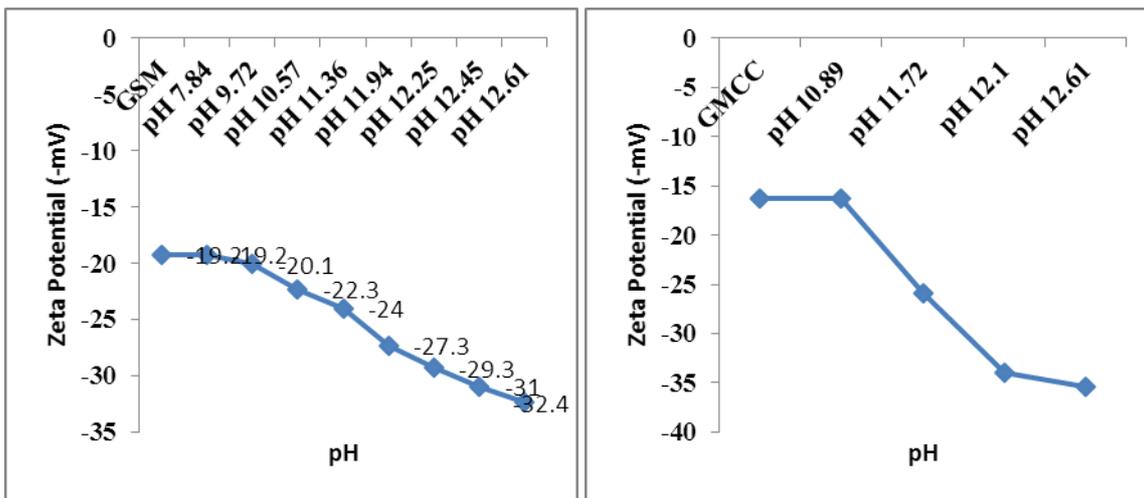


Fig 8: Effect of change in pH on zeta potential of goat skim milk and goat MCC

3.8 Effect of Different Alcohol Concentrations on MCC & Skim Milks

Ethanol has the ability to cause conformational changes in structure of CN micelles. The effect of addition of alcohol (10-80%) at the rate of 10-40% was studied on skim milk and MCC of goat milk (Fig. 9). It was observed that significant changes occurred only at higher strengths and higher rate of addition was chosen as 40%. From the PSD, it was evident that alcohol has the ability to cause disintegration as well as aggregation or formation of large sized particle. 60, 70 and 80% strength alcohols added at 20 and 40% level caused disintegration as well as association. There was appearance of casein micelles of less than 100 nm size as well more than

1000nm size when this combination of alcohol was used to treat the MCC and skim milk. The PSD curve of Goat MCC widen as the strength and concentration of ethanol added is increased. Connell et al., 2001 added ethanol of strength 65% (w/w) in skim milk and found that mixtures of milk and ethanol became transparent on heating, which suggests dissociation of casein micelles. Coagulation of casein micelles in milk may also be induced by the addition of ethanol (Davies et al., 1988).

Zeta Potential analysis at different strengths and rate of additions showed that there was a decrease in magnitude of zeta potential in negative side. The zeta potential of Goat MCC varied from -16.2 mV to -4.44 mV (Fig. 10).

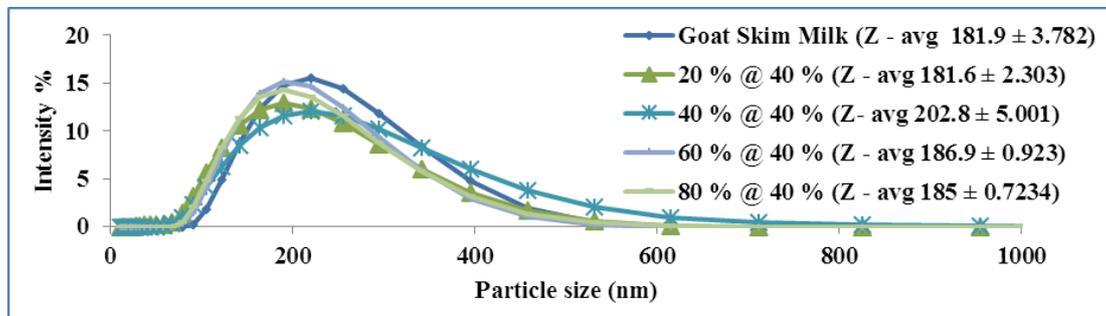


Fig 9(a): Effect of different alcohol concentrations on particle size distribution of goat skim milk

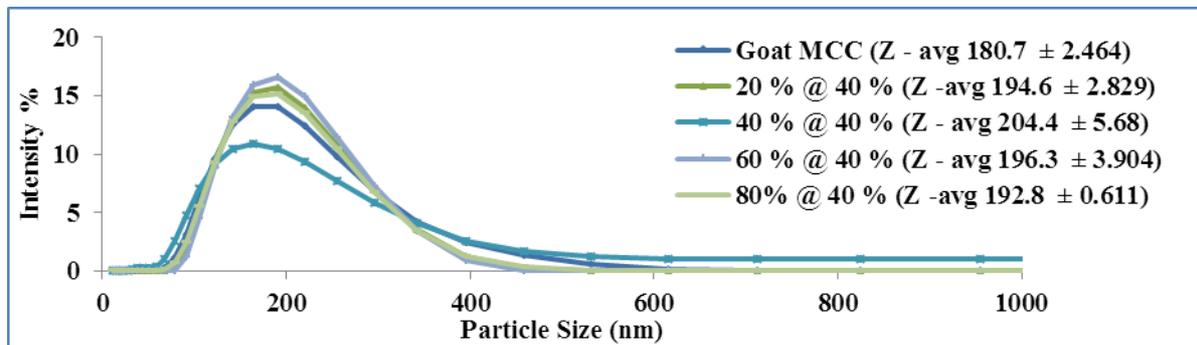


Fig 9 (b): Effect of different alcohol concentrations on particle size distribution of goat micellar casein concentrate

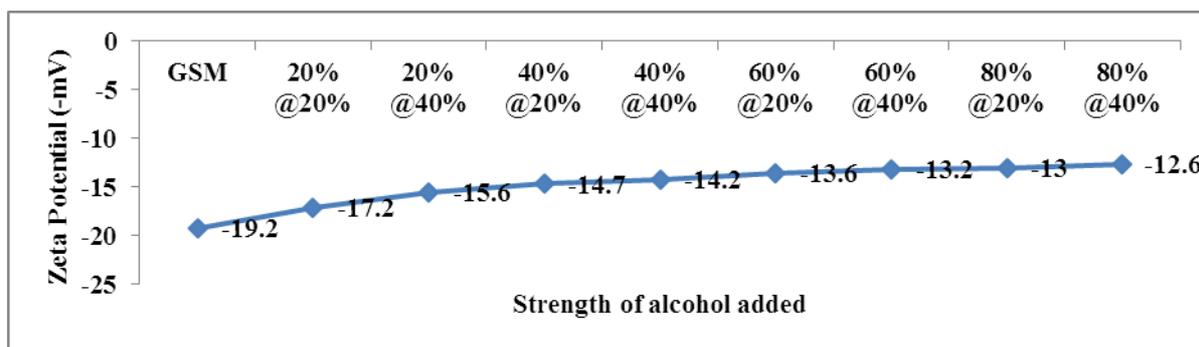


Fig 10 (a): Effect of different alcohol concentrations on zeta potential of goat skim milk

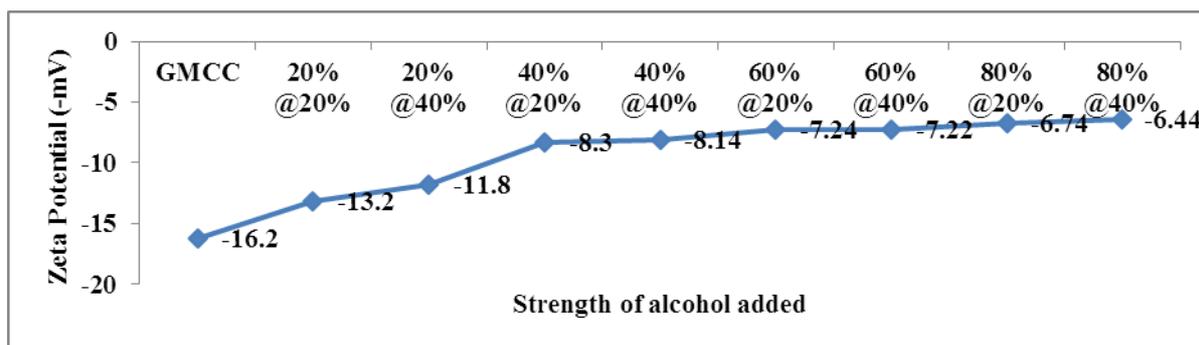


Fig 10(b): Effect of different alcohol concentrations on zeta potential of goat micellar casein concentrate

3.9 Effect of Salts on Goat MCC & Skim Milk

Four types of salts were added to skim milk and respective MCC diluted to skim milk level. Tri Sodium Citrate (TSC), Sodium Hexa Meta Phosphate (SHMP), Disodium Phosphate (DSP), Sodium dihydrogen phosphate (SDHP) were added at the rate of 0 to 2% (w/v) to the samples. The samples were analysed for the particle size. It was observed that there was appearance of peak below 100 nm in PSD of TSC added samples. There was also formation of larger particles on TSC addition. So was the case with SHMP. There was no evidence of significant association or dissociation of CN micelles on addition of DSP and SDP. The PSD of goat skim milk as well as MCC showed two peaks on addition of TSC and SHMP due to the dissociation of CN micelles (Fig. 13 and Fig. 14). The decrease in absorbance after increasing strength of addition of TSC and SHMP was observed [Fig. 11(c & d) and Fig. 12(c & d)].

It has been suggested that one part of the added phosphate reacted with ionic calcium (this form is directly available in the diffusible phase). As the diffusible phase is saturated in calcium phosphate (Holt, 1997), precipitation and/or combination of calcium phosphate with the casein micelle occurred. Udabage *et al.* (2000) [36] obtained similar results. It is observed that TSC and Na_2HPO_4 had a different impact on

turbidity. Calcium ions in the CN micelle are bound to the phosphoserine residues or are part of the CCP complexes. The added chelator competes with the phosphoserine residues and CCP in the CN micelle for the calcium ions. The chelators have a different affinity for calcium ions, which gives them the ability to release a different amount of CCP from the micelle (De Kort *et al.*, 2009; Upreti, Buhlmann, & Metzger, 2006). The effect on turbidity was also visually observed (Plate 3, 4, 5, 6, 7, 8, 9, 10).

Zeta Potential analysis of the samples showed that there was an increase in magnitude of zeta potential towards the negative side on addition of TSC, SHMP, DSP (Fig. 15). The change being maximum in case of SHMP and lowest in case of DSP (Fig. 15). In case of DSP the zeta potential showed no significant change, but a slight decrease in magnitude of zeta potential towards negative side. The values for Goat MCC -16.22 mV to -32.2 mV in case of SHMP, from -16.22 to -25.3 mV in case of TSC, from -16.22 to -17.5 mV in case of Na_2HPO_4 , and from -16.22 to -14.5 mV in case of NaH_2PO_4 (Fig. 15) The degenerating effect followed the order SHMP>TSC> Na_2HPO_4 > NaH_2PO_4 . The same order is observed in case of absorbance of 450 nm. SHMP showed cross linking as well as chelating effect. TSC and Na_2HPO_4 had only chelating effect.

The effect on zeta potential which increases in magnitude towards the negative side could be attributed to the dissociation of CN micelles. The release of calcium ions from

phosphoserine residues of the CN micelles also causes more of exposed negative charge.

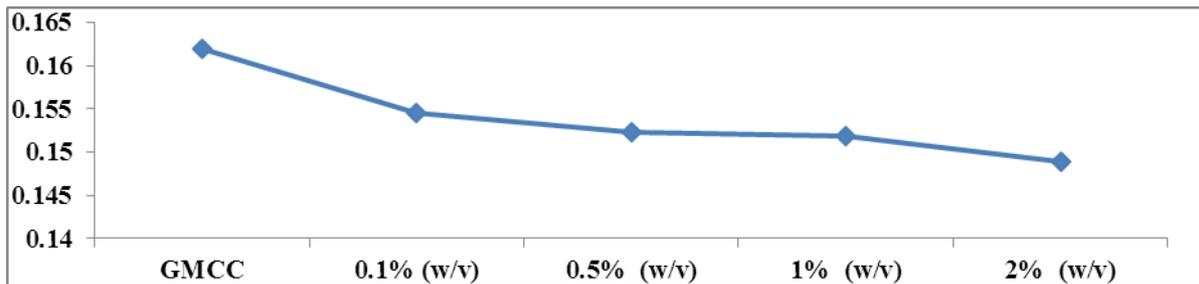


Fig 11(a): Absorbance at different concentration of sodium di-hydrogen phosphate on goat micellar casein concentrate

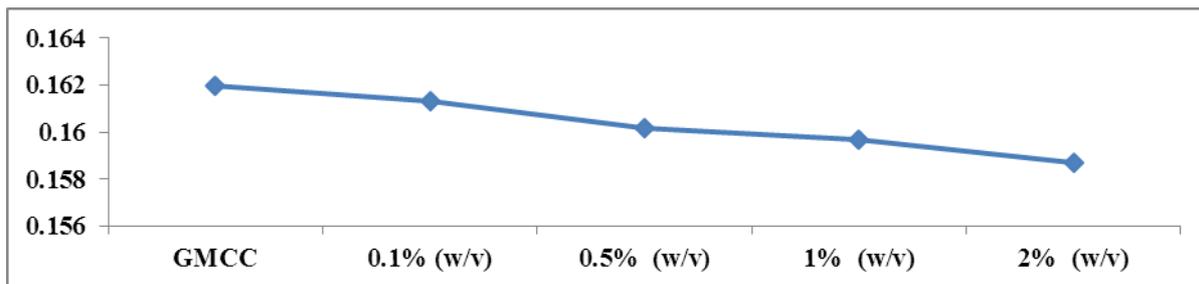


Fig 11(b): Absorbance at different concentration of di sodium hydrogen phosphate on goat micellar casein concentrate

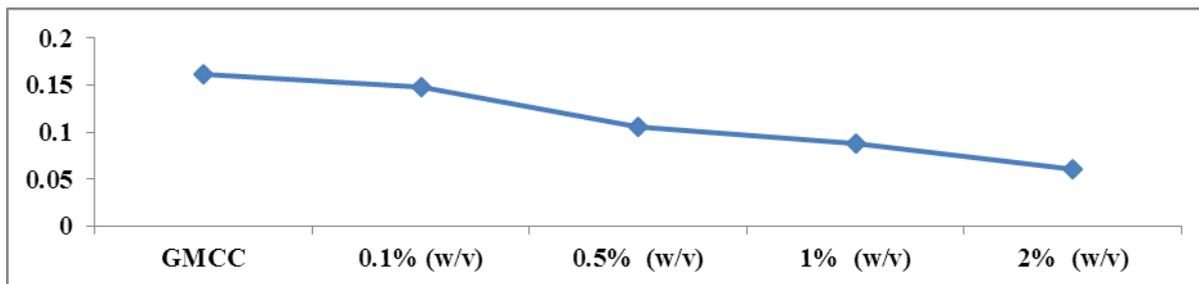


Fig 11(c): Absorbance at different concentration of tri sodium citrate on goat micellar casein concentrate

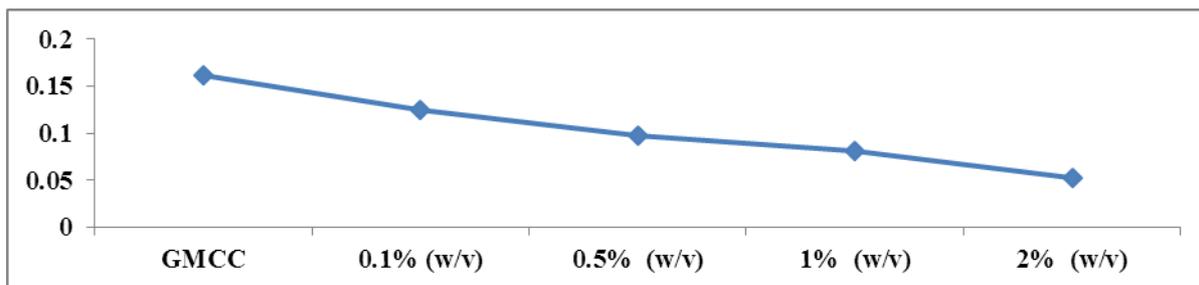


Fig 11(d): Absorbance at different concentration of sodium hexa meta phosphate on goat micellar casein concentrate

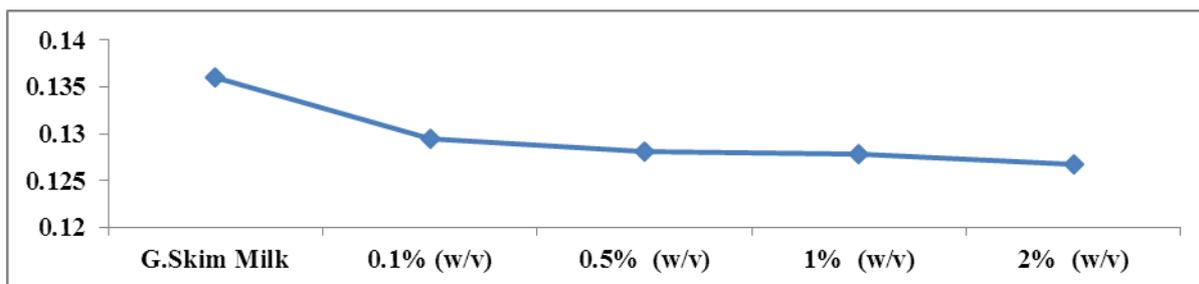


Fig 12(a) Absorbance at different concentration of sodium di hydrogen phosphate on goat skim milk

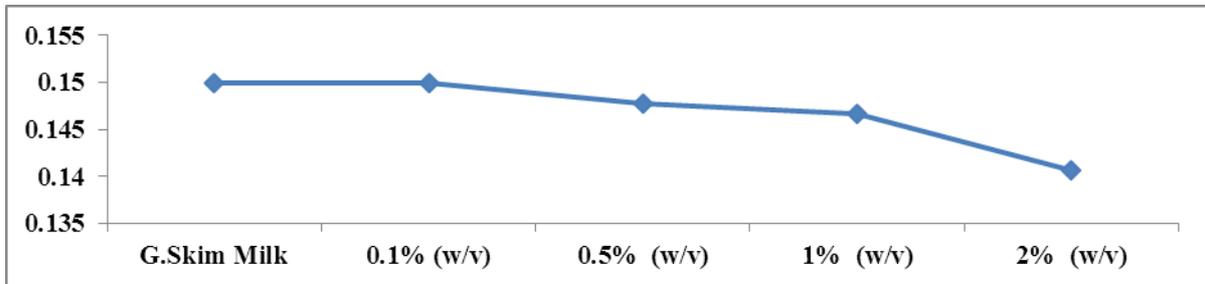


Fig 12(b): Absorbance at different concentration of di sodium hydrogen phosphate on goat skim milk

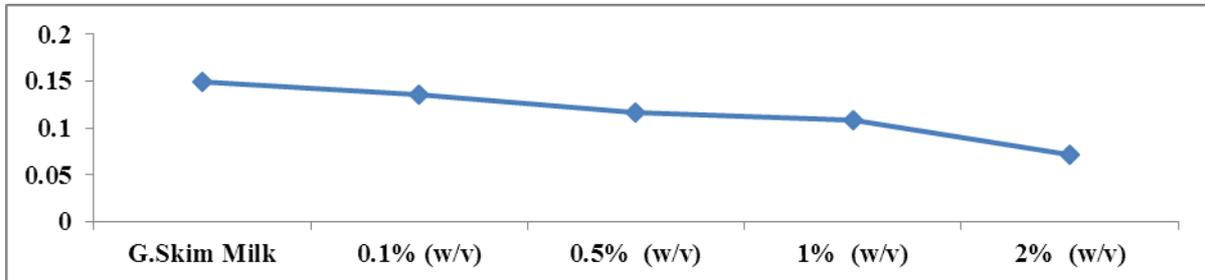


Fig 12(c): Absorbance at different concentration of tri sodium citrate on goat skim milk

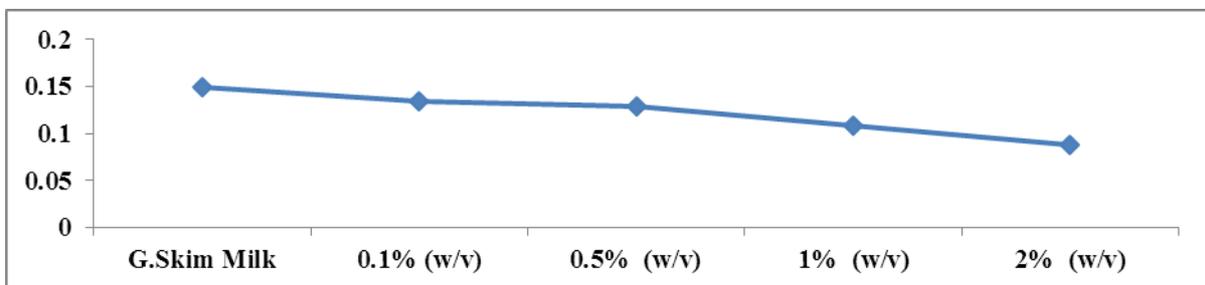


Fig 12(d): Absorbance at different concentration of sodium hexa meta phosphate on goat skim milk

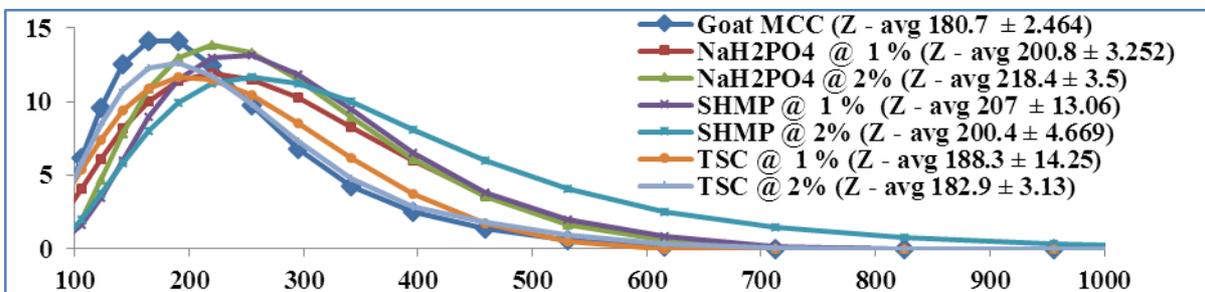


Fig 13(a): Effect of various salts at different concentrations on particle size distribution of goat micellar casein concentrate

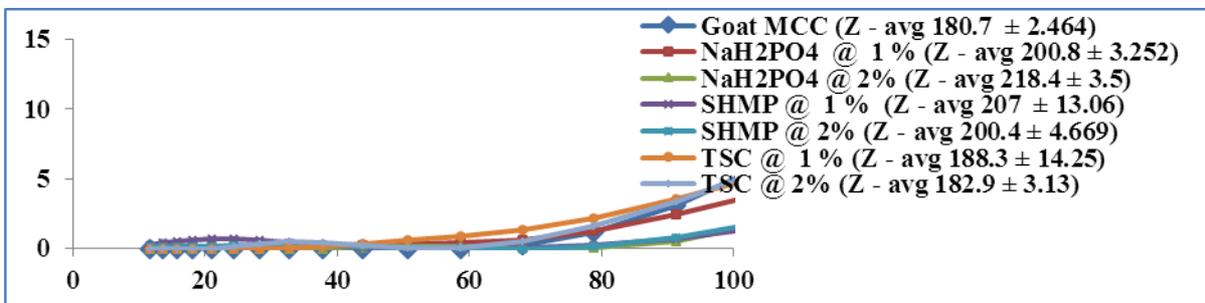


Fig 13(b): Effect of various salts at different concentrations on particle size distribution of goat micellar casein concentrate

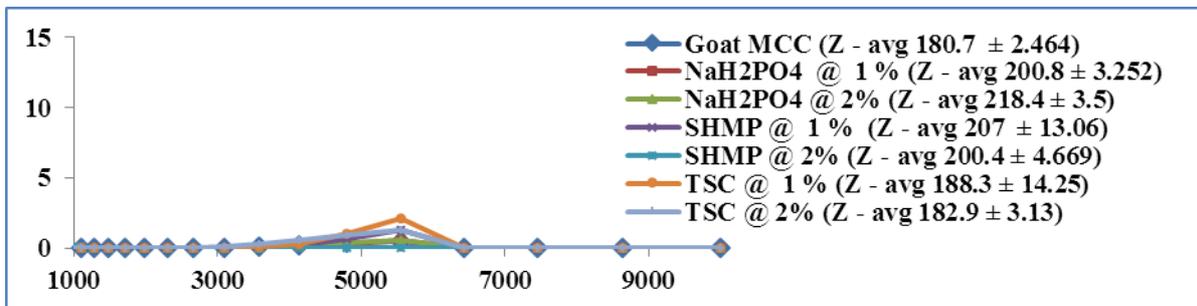


Fig 13(c): Effect of various salts at different concentrations on particle size distribution of goat micellar casein concentrate

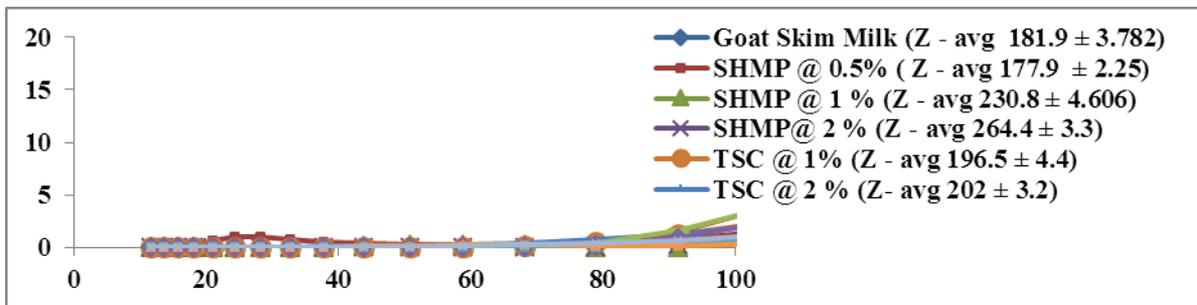


Fig 14(a): Effect of various salts at different concentrations on particle size distribution of goat skim milk

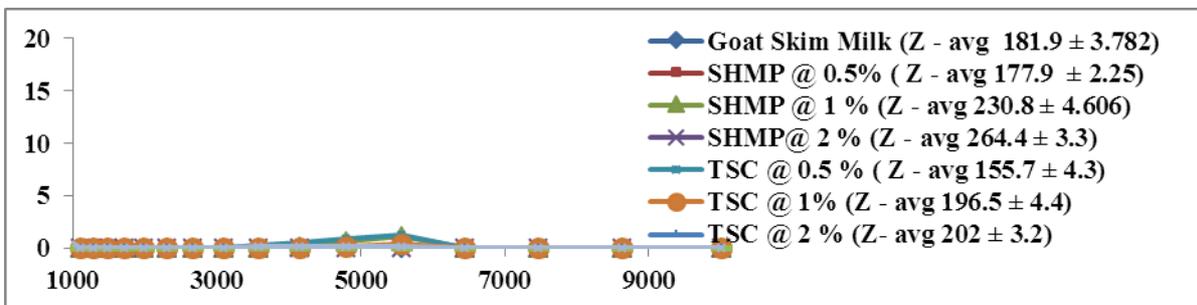


Fig 14(b): Effect of various salts at different concentrations on particle size distribution of goat skim milk

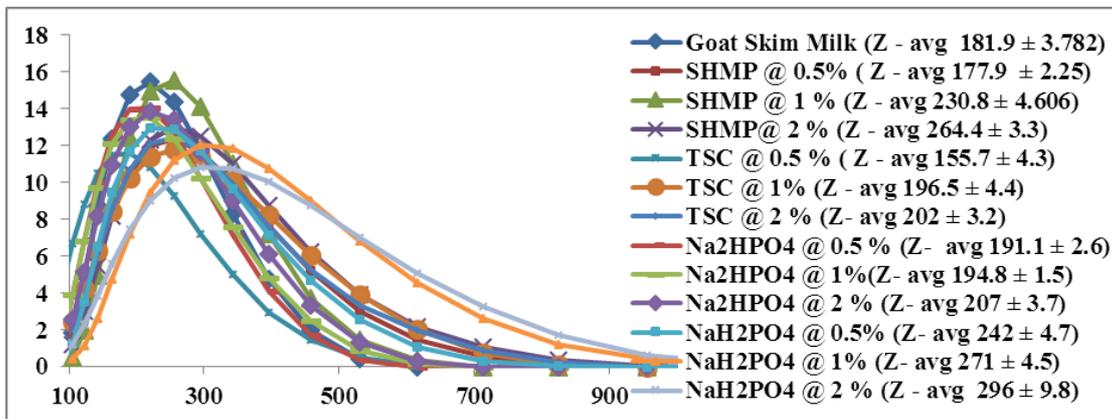


Fig 14(c): Effect of various salts at different concentrations on particle size distribution of goat skim milk



Plate 3. Effect of Na₂HPO₄ on goat micellar casein concentrate

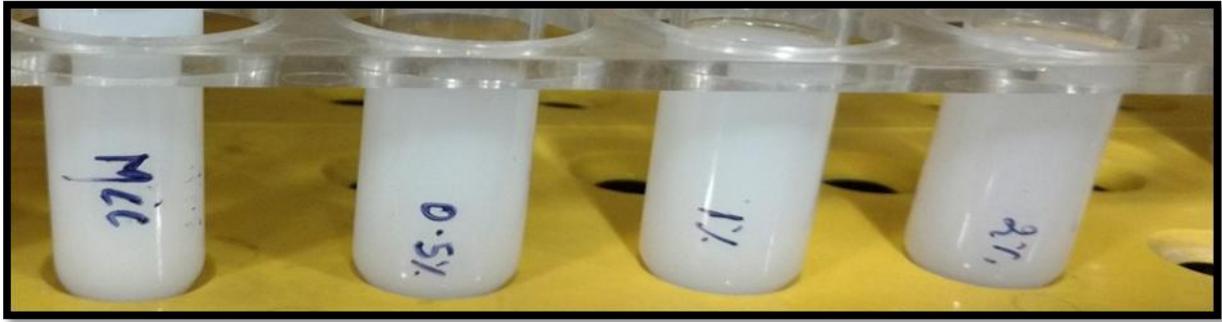


Plate 4: Effect of NaH_2PO_4 on goat MCC

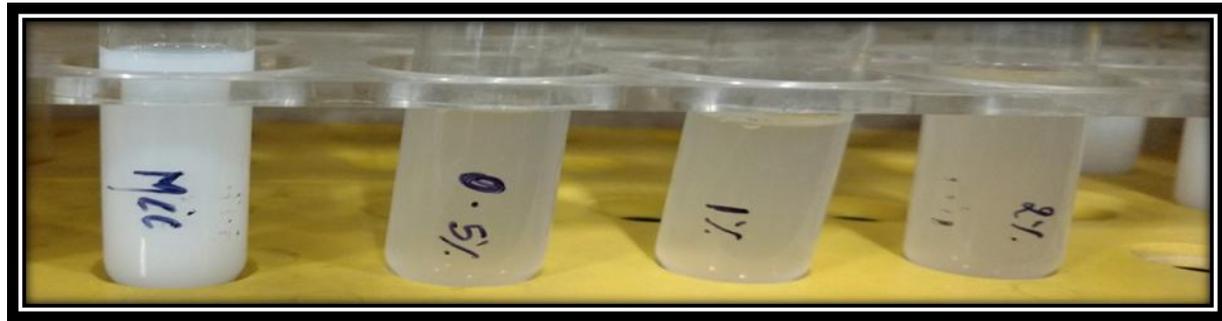


Plate 5: Effect of sodium hexa meta phosphate on goat MCC

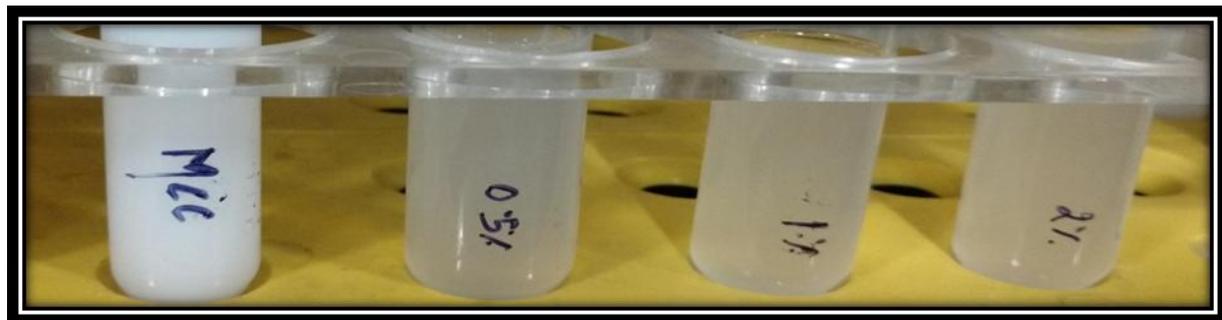


Plate 6: Effect of tri sodium citrate on goat MCC



Plate 7: Effect of Na_2HPO_4 on goat skim milk



Plate 8: Effect of NaH_2PO_4 on goat skim milk

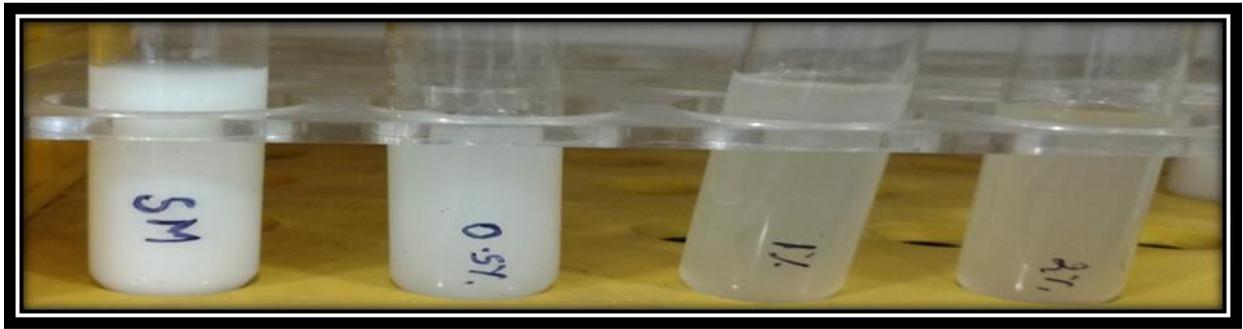


Plate 9: Effect of sodium hexa meta phosphate on goat skim milk

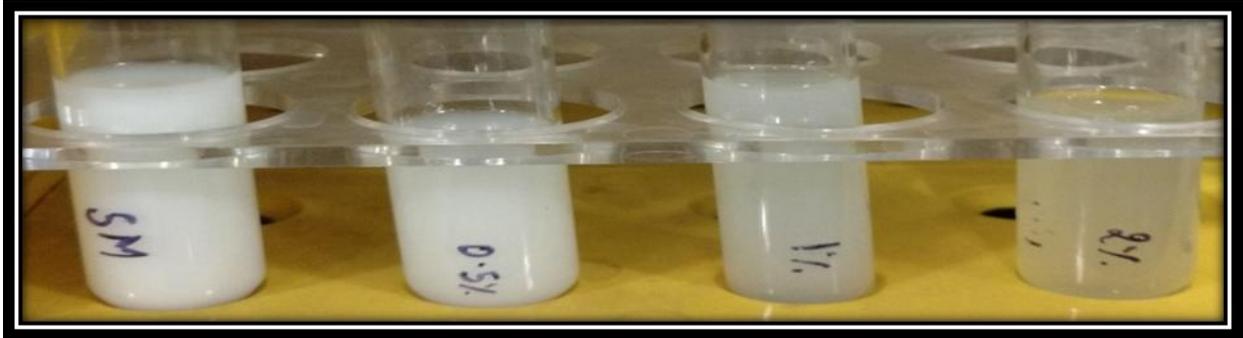
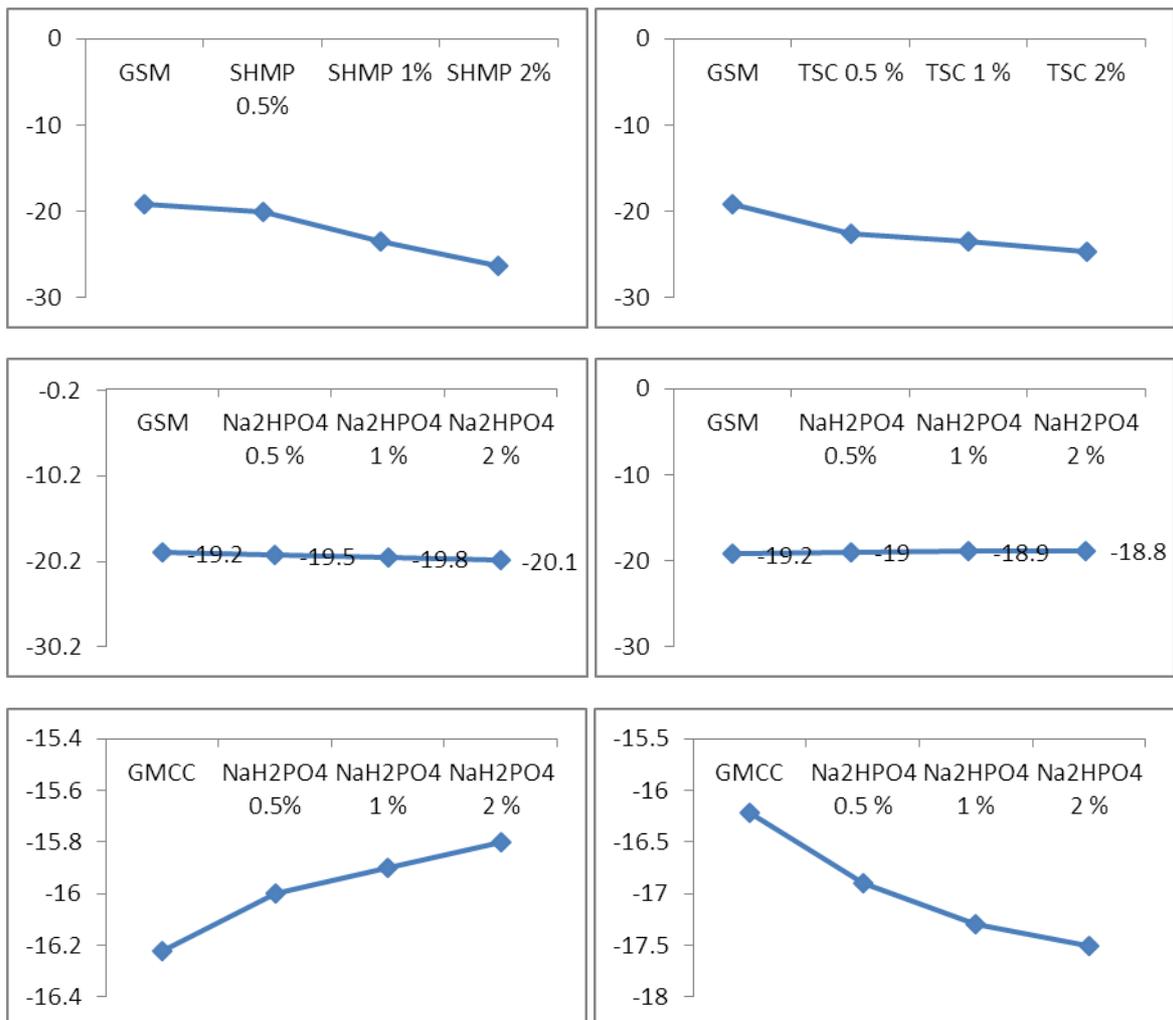


Plate 10: Effect of tri sodium citrate on goat skim milk



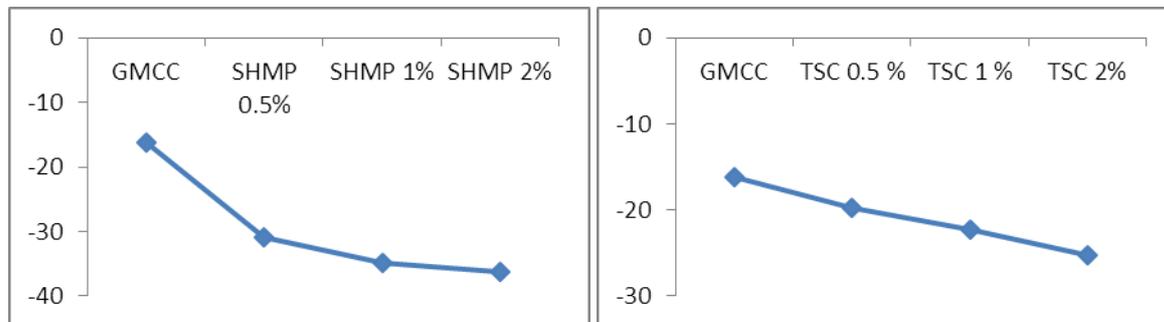


Fig 15: Effect of various salts at different concentrations on particle size distribution of goat skim milk and goat MCC (X-axis amount of salt, Y-axis Zeta potential)

4. Conclusion

The milk of Goat (Black Bengal) was subjected to MF. It is found that CN micelles maintain their integrity and stability during MF when concentrated to five fold concentration. The average diameter size of CN micelles (Z-average) did not vary with progressive concentrations of retentate and also the physicochemical characteristics of CN micelles remain unchanged. The average particle size of Goat Skim Milk (200 ± 3.61 nm) and that of Goat MCC (198.2 ± 4.2 nm), indicates that the diameter of CN micelles remains unaltered during process of MF. Zeta Potential decreased slightly, but no major change occurred. The magnitude of Zeta Potential decreased towards the negative side. This decrease was from -19.2 mV of goat skim milk to -16.6 mV in goat MCC. Turbidity value increased slightly during the process. AAS results of total calcium concentration at different levels of concentration showed that total calcium value decreased initially which is due to moving of soluble calcium into the permeate phase. But it was seen that after a certain concentration level the value rises gradually up to a certain extent. The total calcium concentration in goat, skim milk being 910 ± 17.39 ppm, 668 ± 21.27 ppm in case of 1.66 fold concentrated retentate and 690 ± 22.05 ppm in five fold concentrated retentate.

Modification of pH caused change in the internal and external structure of CN micelles present in MCC as well as skim Milk. The particle size distribution curve broadened in all three species on moving towards basic side. The magnitude of Zeta potential increases towards the negative side on increasing pH to basic side due to increasing negative charge on casein micelles as pH is increased away from its isoelectric pH.

Effect of ethanol on MCC and skim milk depends on strength and rate of its addition to the sample. Particle Size Distribution of skim milk showed that aggregation was observed at higher strengths only and disintegration was seen to a very less extent. The effect of salts varied with type and concentration of salt added. The magnitude of zeta potential increased towards negative side as the concentration of these salts was varied from 0 to 2 %. The degenerating effect followed the order SHMP>TSC>Na₂HPO₄>NaH₂PO₄. The same order is observed in case of absorbance at 450 nm. SHMP showed cross linking as well as chelating effect. TSC and Na₂HPO₄ had only chelating effect. The present study would help in using MCC as a functional ingredient and protein source for different food systems. The modification in structure of CN micelles at various pH, ionic strength and solvent concentration can make it a potentially important source for use as an encapsulant.

CN Micelles could be used to encapsulate hydrophobic nutrients like vitamin A in case of low fat products.

This study can be used as basis of various industrial applications of casein micelles as it would give an insight to their behaviour. Also MF is a very economical process which can be installed at larger scale in the industry and can be used to harvest the casein micelles in the native state without any aggregation, for its use as ingredient.

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