

Original Research Article

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Effect of Ethanol on Physicochemical Properties of Micellar Casein Concentrate

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ABSTRACT

Casein (CN) is major milk protein which exists in milk in form of micelles of size ranging from 50 to 500nm. The CN micelles were harvested using microfiltration (MF) with 0.1 μm membrane in their native state. The CN micelles harvested using MF are called micellar CN concentrate (MCC). MCC harvested was treated by ethanol at rate varying from 10 to 40 % and strength varying from 10 to 80%. This treatment caused disintegration as well as aggregation. More pronounced results were observed in case of 60 – 80 % alcohol strengths added at rate of 40 %. Aggregated network of CN micelles was distinct in the transmission electron micrograph. The magnitude of Zeta potential decreased towards negative side in this treatment as aggregation occurred. The zeta potential varied from -16.2 mV to -8.2 mV in case of buffalo MCC when it was treated with 20 to 80 % alcohol strength at the rate of 40%. This value varied from -17 mV to -10 mV in case of cow MCC. PSD of skim milk showed that aggregation was observed at higher strengths only and disintegration was seen to a very less extent. In case of MCC disintegration was seen at 60 – 80 % alcohol strengths and aggregation was evident at 40 to 80 % (maximum at 80 %). This study would be helpful in manipulating physicochemical properties of CN micelles in different food systems.

Keywords

Micellar CN concentrate (MCC), CN (CN), Z-average (Z-avg), Microfiltration (MF), Whey proteins (WP)

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Introduction

Major milk protein is CN which occurs mainly as micelles. Casein (CN) Micelles are source of Calcium, phosphate and protein. CN micelles comprise of calcium, magnesium, phosphate, and citrate. The native form of CN micelles can be maintained when these are harvested by use of microfiltration (MF). The CN micelles obtained in a concentrated form by process of MF are known as Micellar CN Concentrate (MCC) (Elizabeth, 2015). CN micelles have structure that is best explained

by internal structure model consisting of kappa CN hairy layer on the surface and alpha and beta CN on inside (Phadungath, 2005). It has colloidal calcium phosphate forming bridges inside the micelle (Phadungath, 2005). The structure of CN micelles can be modified with change in environmental conditions (pH, ionic strength and solvent quality) around it (Fox *et al.*, 2005). Various researchers have studied the change in size, zeta potential and absorbance of CN micelles on change in the environmental conditions. Change in solvent quality i.e., addition of ethanol caused

disturbance in outer stabilizing layer of the CN micelles. The upper layer of CN micelles which majorly consists of κ -CN, which stabilizes the CN micelles. Various researchers have observed that this layer collapses on treatment of CN micelles with ethanol. Connell *et al.*, (2001) and Post *et al.*, (1982) attributed the aggregation of milk on addition of alcohol towards removal of stearic stabilization.

Transmission Electron Micrography (TEM) is important analytical technique to view CN micelles at different magnifications. The structure of CN micelles affects the properties of various dairy products like dahi and cheese.

Modification in physicochemical properties of CN micelles can be helpful in its incorporation to various food systems. The treatment with alcohol and various physicochemical changes in structure of CN micelles would be helpful in incorporation of MCC as protein source in various foods. This would also be helpful in stabilizing various products like cream liquor. Manipulation of textural properties of various food systems can be done by varying alcohol strength and rate of addition.

Materials and Methods

Chemicals and reagents

Ethanol was obtained from Sigma Aldrich Ltd.

Experimental equipment

Freeze dryer (Labconco corporation, Kansas City, MO), Particle size analyzer (Malvern Instruments Ltd., U. K.), Kubota centrifuge (Tokyo, Japan), Transmission Electron Microscope (JEOL-JEM 2100F model), Manual Hollow Fiber Ultra Filtration assembly (QSM-03S model, M/s. GE Healthcare, Gurgaon, Haryana)

Sample collection

Milk sample of cow (sahiwal) and Buffalo (Murrah) was collected from Livestock Research Centre, NDRI.

Microfiltration of skim milk

The skim milk was micro filtered 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 at 200- 250 rpm and average transmembrane pressure was maintained below 5 kPa.

Determination of particle size of CN micelles in skim milk and MCC

The mean particle diameter, particle size distribution, Z- average, zeta-potential and Poly Dispersity Index (PDI) of the samples were observed 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 CN 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.

Determination of zeta potential of CN micelles in skim milk and MCC

The electrical charge on the CN 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.

Transmission electron microscopy analysis of CN micelles in skim milk and MCC

The TEM analysis for the samples was done at Advanced Research Instrumentation Facility, Jawahar Lal Nehru University, New Delhi.

JEOL-JEM 2100F model of field emission electron microscope was used to view the samples.

Sample preparation was done by stating the reconstituted samples in uranyl oxalate. Images were obtained at 10 thousand, 30 thousand and 1 lakh magnification value.

Results and discussion

Microfiltration of skim milk

Buffalo and cow skim milk were subjected to MF to obtain retentate at five fold level of concentration.

This retentate was subjected to different alcohol strengths at various rates to observe the changes in physicochemical properties of CN micelles.

Effect of different alcohol concentrations on

Fig.1 Effect of different alcohol concentrations on particle size distribution of cow micellar

CN micelles in skim milk and MCC

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 both species. It was observed that significant changes occurred only at higher strengths and higher rate of addition (40 %). From the particle size distributions (Fig. 1, 2, 3, 4), it was evident that alcohol has the ability to cause disintegration as well as aggregation. The formation of large sized particle at 60, 70 and 80 % strength alcohols added at 20 and 40 % was observed. There was appearance of CN 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 milks (Fig. 1, 2, 3, 4). The Z- average was not the true representative of the aggregation and dissociation that is occurring in the sample.

In the buffalo as well as cow MCC smaller as well as large sized aggregates of CN micelles are formed but there is no major change in case of Z-average (Fig. 1 and 3). The PSD curves for cow and buffalo 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 CN micelles. Coagulation of CN micelles in milk may also be induced by the addition of ethanol (Davies *et al.*, 198).

Solvent conditions that lead to the collapse are often similar to those leading to aggregation of CN micelles (Horne *et al.*, 1981). The action of the ethanol in micellar aggregation may be explained by collapsing of the hairy structure which leads to removal of the stearic stabilizing component from the system (Post *et al.*, 1982).

casein concentrate

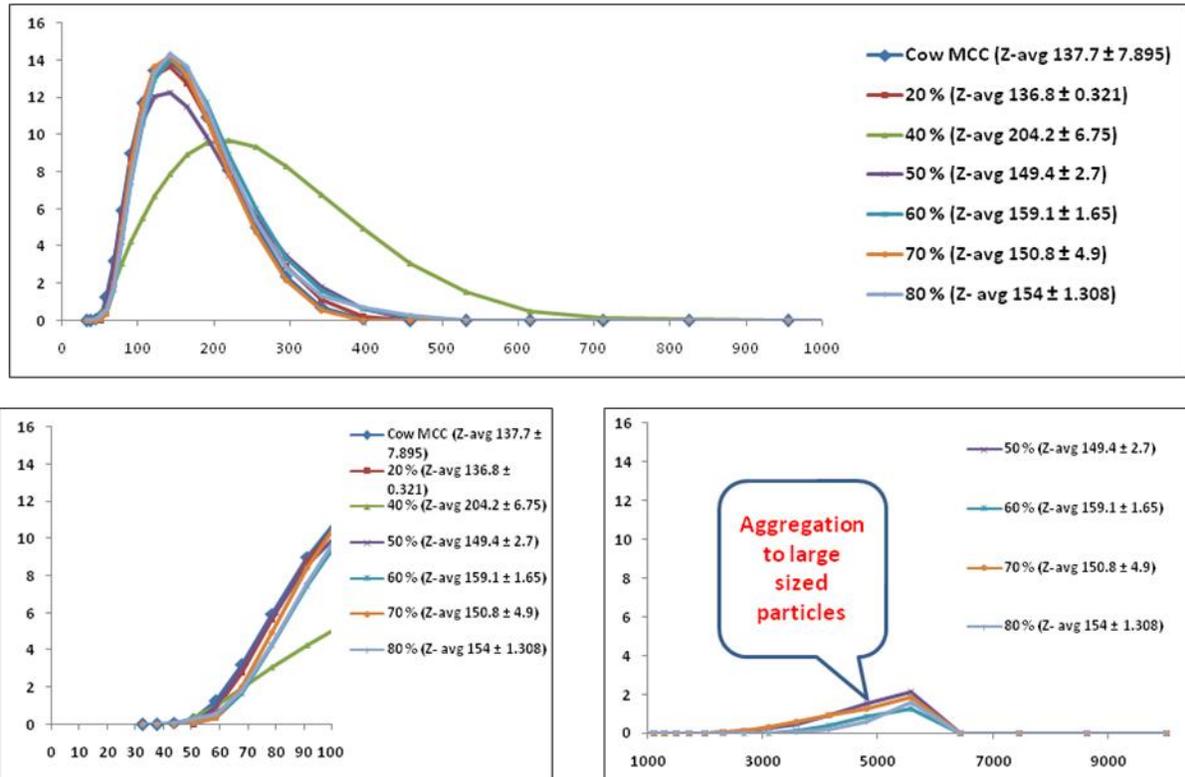


Fig.2 (a) Effect of different alcohol concentrations on particle size distribution of cow skin milk

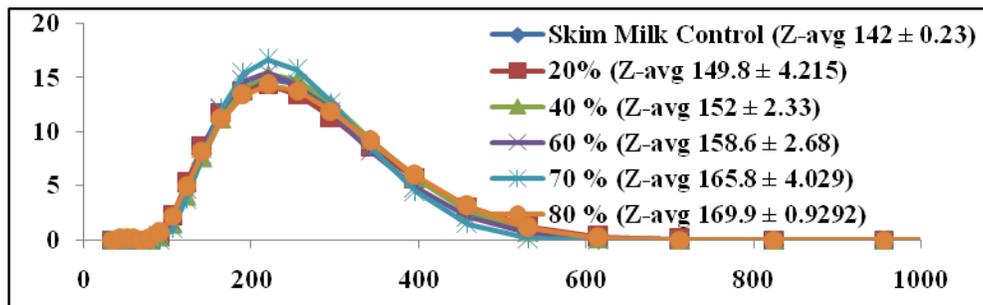


Fig.2 (b) Effect of different alcohol concentrations on particle size distribution of cow skin milk

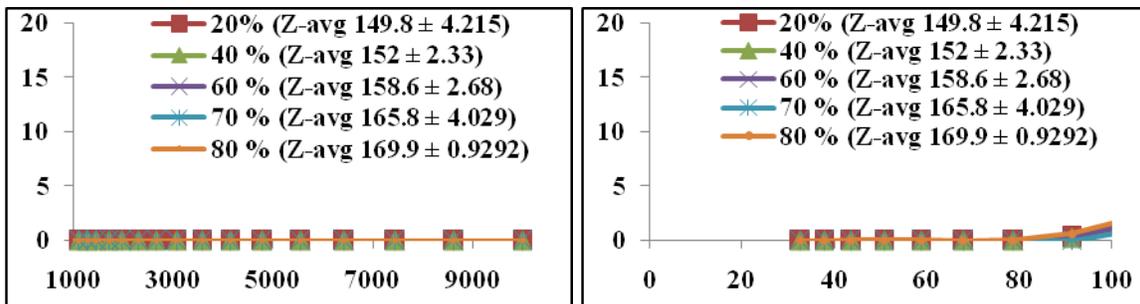


Fig.3 Effect of different alcohol concentrations on particle size distribution of buffalo micellar

casein concentrate

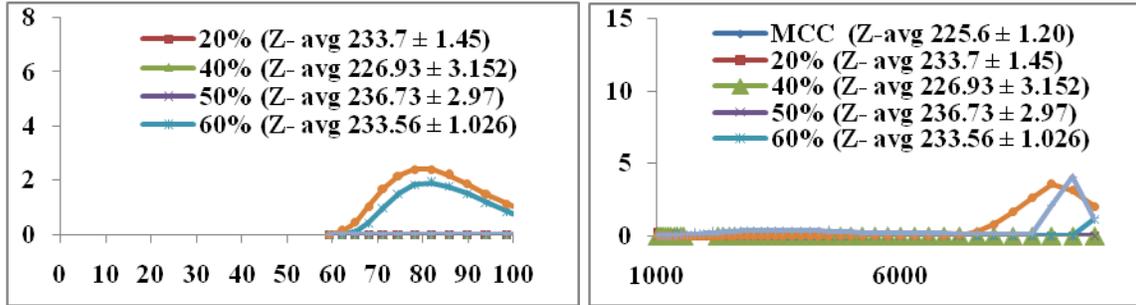


Fig.4 (a) Effect of different alcohol concentrations on particle size distribution of buffalo skim milk

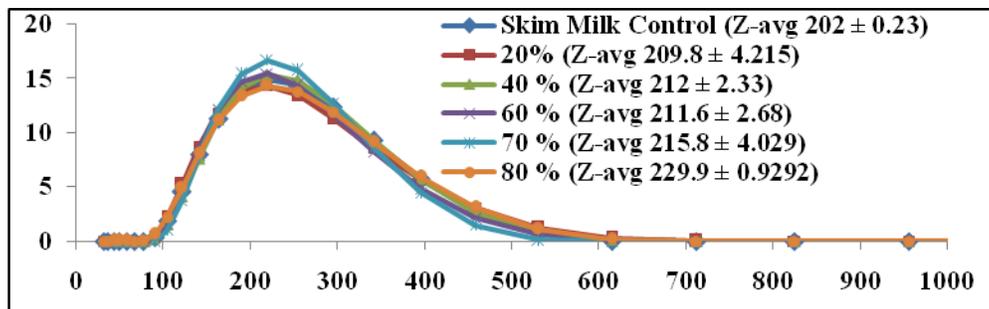


Fig.4 (b) Effect of different alcohol concentrations on particle size distribution of buffalo skim milk

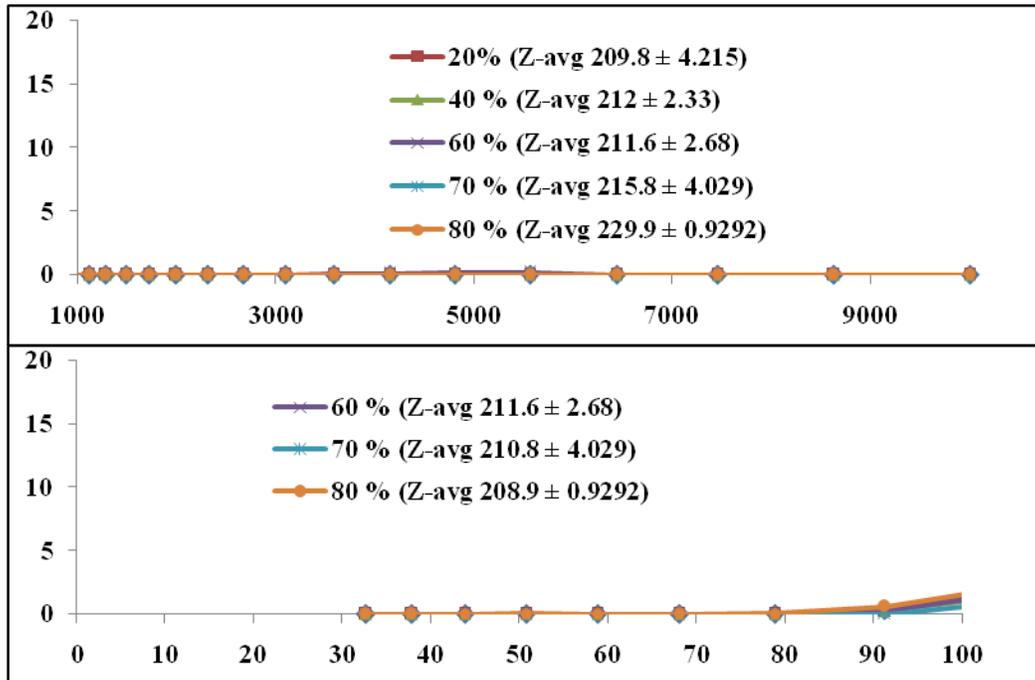


Fig.5 Effect of different alcohol concentrations on zeta potential of buffalo skim milk

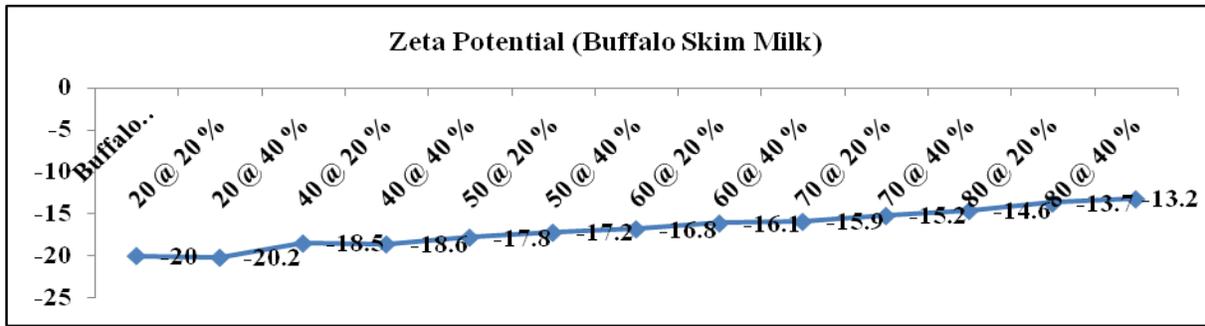


Fig.6 Effect of different alcohol concentrations on zeta potential of cow skim milk

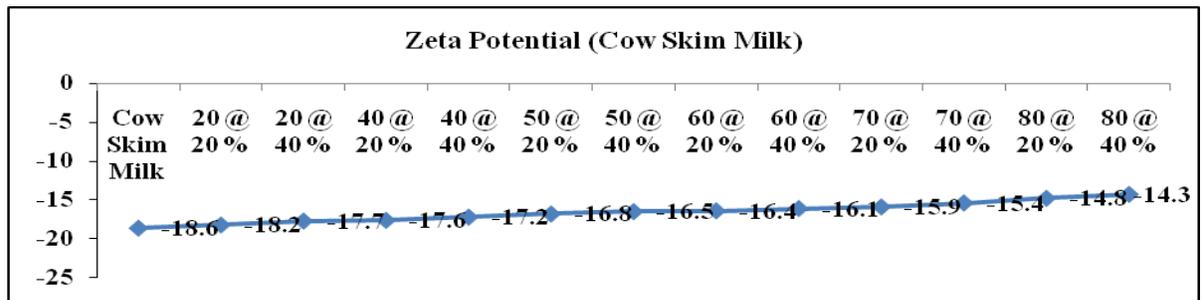


Fig.7 Effect of different alcohol concentrations on zeta potential of buffalo micellar casein concentrate

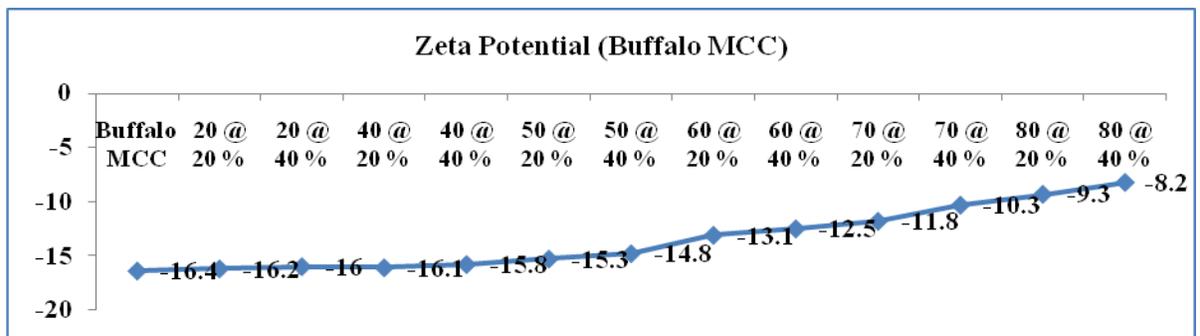


Fig.8 Effect of different alcohol concentrations on zeta potential of cow micellar casein concentrate

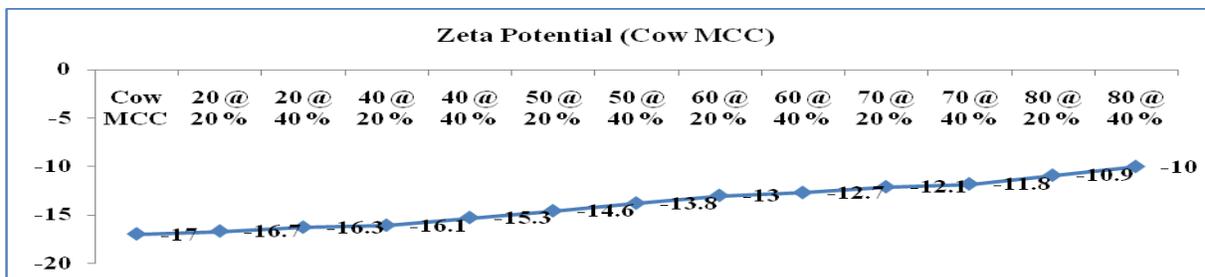


Plate.1 (a) Buffalo MCC at 10,000X

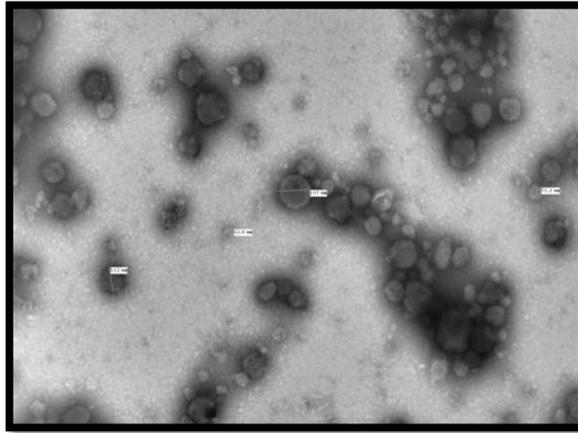


Plate.1 (b) Buffalo MCC at 30,000X

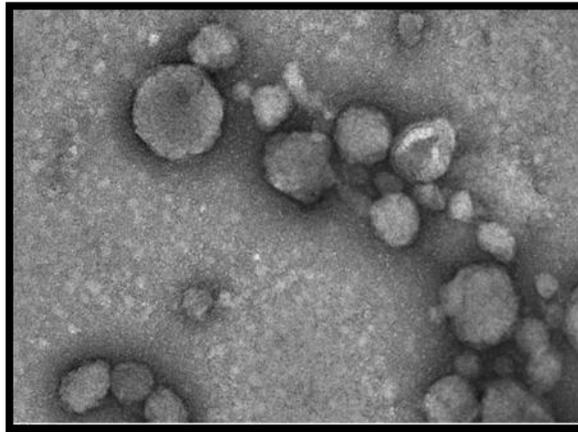


Plate.2 (a) Effect of Alcohol on Buffalo Micellar Casein Concentrate viewed through TEM at 10,000X

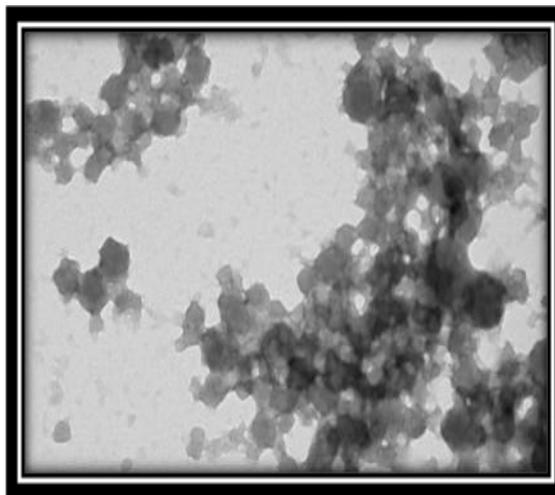
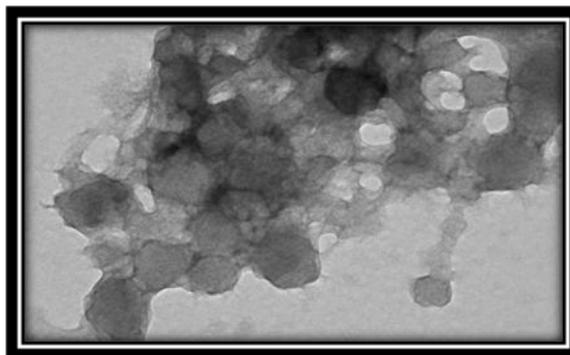


Plate.2 (b) Effect of

Alcohol on Buffalo

Micellar Casein Concentrate viewed through TEM at 30,000X



Effect of alcohol treatment on zeta potential on CN micelles in skim milk and MCC

Zeta potential analysis showed that there was a decrease in magnitude of zeta potential towards negative side. The Zeta Potential varied from -16.2 mV to -8.2 mV in case of buffalo MCC when it was treated with 20 to 80 % alcohol strength at the rate of 40% (Fig. 7). This value varied from -17 mV to -10 mV in case of cow MCC mV (Fig. 8) with the same treatments which was in accordance with the (Payens *et al.*, 1979). In case of buffalo skim milk the variation was from -20mV to -13.2 mV (Fig. 5) and from -18.2 to -14.3 mV in case of cow skim milk (Fig. 6). The zeta potential of CN micelles is attributed to charge of double layer (Fox *et al.*, 2015). Due to treatment with alcohol the charge on CN micelles decreases as κ -CN layer is removed (Fox *et al.*, 2015). Hence as the strength and rate of addition of alcohol is increased the value of zeta potential decreases towards negative side.

TEM analysis of CN micelles in skim milk and MCC

TEM micrograph showed aggregated CN micelles when buffalo MCC treated with 70 % ethanol added at the rate 40% (Plate 1). It could be clearly seen in the TEM images that the identity of CN micelles is lost. CN

micelles appear as aggregated networks. Due to loss of stearic stabilization of CN micelles, these tend to collapse together to form a network (Plate 2 and 3) and the native conformation of CN micelles is lost (Plate 1).

The skim of both buffalo (murrh) and cow (sahiwal) was subjected to MF to obtain retentate (MCC) at five fold concentration which was further treated by ethanol of various strengths and rate of additions.

PSD of skim milk showed that aggregation was observed at higher strengths only and disintegration was seen to a very less extent. In case of MCC disintegration was seen at 60 – 80 % alcohol strengths and aggregation was evident at 40 to 80 % (maximum at 80 %). The Zeta Potential varied from -16.2 mV to -8.2 mV in case of buffalo MCC when it was treated with 20 to 80 % alcohol strength at the rate of 40%.

This value varied from -17 mV to -10 mV in case of cow MCC. TEM micrographs of 70 % alcohol treated buffalo MCC showed aggregation and distortion of structure of CN micelles at different magnifications. Hence these observations can be used to vary physicochemical properties of CN micelles in various food systems and hence to manipulate the textural properties.

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