Effect of pH on turbidity, size, viscosity and the shape of sodium caseinate aggregates with light scattering and rheometry

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Abstract The characterization of sodium caseinate solutions as a function of pH was determined using titration with HCL through turbidimetry in different concentrations (0.03 wt.%, 0.045 wt.%, 0.06 wt.%, 0.09 wt.%, 0.2 wt.%, and 0.3 wt.%). Additionally, the coupling of slow in situ acidification of the solution and rheometry was utilized to gain deeper insights into pH-induced structural transitions during the self assembly process and particle size distribution analysis have been used to determine the behavior of sodium caseinate solutions in different pHs. The formation of aggregates during the acidification process was clearly visualized using microscopy. Surprisingly the viscosity of sodium caseinate solution at pH 4.64 was maximum and decreased by lowering pH. Particle size analysis confirmed the onset of big aggregates on decreasing pH but further acidification led to formation of smaller aggregates. A small concentration effect on pI was seen where at sodium caseinate levels of 0.03 wt.% the pI occurred at 4.29, where at sodium caseinate levels of 0.30 wt.% pI value was 4.64.

Keywords Sodium caseinate · Spectrophotometry · Viscosity · Aggregation · Particle size

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Introduction

Assemblies of proteins in a fluid regarding their emulsifying, foaming, and gelling properties influence the macroscopic properties of food products. Thus, investigating the physico-chemical characteristics, morphology and mutual interactions of protein assemblies play a key role in the engineering of food materials. One of the proteins that is important because of its structure formation properties is casein (Dickinson et al. 1997; Ruis et al. 2007).

Due to its excellent nutritional and functional properties, sodium caseinate has been widely used in the formation and stabilization of food emulsions. It is prepared from coagulated casein micelles, through subsequently washing and neutralizing with NaOH (Dalgleish 1997; Damodaran and Paraf 1997; Fox 2003). Various products (for instance, yoghurt, acidified dairy drinks and cheese) contain casein molecules as one of their main building blocks. Caseins build different colloidal structures, which lead to differences in product characteristics that could be changed by changing the process treatment during structural build-up. This will modify the interaction between casein blocks and therefore results in different structural properties (Ruis et al. 2007).

The individual casein molecules interact with each other and form associate structures with a radius of approximately 10 nm (Chu et al. 1995), which are called aggregates. Several studies described effect of temperature and pH on this system (sodium caseinate solutions) stability. Adjustment of the pH towards the isoelectric point causes a decrease of the repulsive interaction, resulting in destabilization of the dispersion as soon as the pH drops below approximately 5 (Lucey et al. 1997; Braga et al. 2006). However, as far as we are aware, no systematic characterization has been made to characterize sodium caseinate dilute solutions as a function of pH. The aim of the present study was to characterize sodium caseinate by spectrophotometry as a

function of the concentration over a wide range of pH. In addition, to gain deeper understandings of pH-induced structural transitions during the assembly process, rheometry and particle size analysis were utilized also these structural transitions were visualized using microscopy.

Materials and methods

Materials

Caseinate sodium salt, from bovine milk (lot 100M0130V) was purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA) and used.

Preparation of sodium caseinate stock dispersions

Aqueous dispersion of sodium caseinate of known amounts of biopolymer powders were prepared by dissolving biopolymers in distilled water at room temperature for 2 h. Different total biopolymer concentrations (0.03 wt.%, 0.045 wt.%, 0.06 wt.%, 0.09 wt.%, 0.225 wt.%, and 0.3 wt.%) were prepared.

Spectrophotometry

Absorbance measurements were carried out with a SP-300 PLUS Optima UV–VIS spectrophotometer (Tokyo, Japan) at a wavelength of 400 nm. The samples were placed in a 1 cm path length cuvette, and the absorbance (Abs) was measured at 22 °C.

For time-dependent spectrophotometric measurements, a volume of 3 ml of the sodium caseinate solutions of desired pH value were placed in the cuvette, and the absorbance was monitored for 30 min with a measurement step of 60 s.

Simultaneous particle size analysis and light microscopic observation

The particle size distribution of the samples was determined by laser diffraction, using a Cilas 1090 particle size analyzer (Orleans, France) equipped with a 5 mw He/Ne (635 nm) laser beam. The sample was added to the measuring unit, containing distilled water at a pH similar to the pH of the sample. Particle size measurements were reported as: D_{0.1}, D_{0.5} and D_{0.9} on a surface basis that is the size of particle below which 10 %, 50 %, and 90 %, respectively, of the sample particles lie.

The diameter on surface is also known as diameter D[2,1].

Its mathematical formula is as follows: $D[2,1] = \frac{\sum n_i d_i^2}{\sum n_i d_i}$ in which:

n_i Number of particles of class “i”

d_i Diameter of class “i”

The volume mean diameter, D[4,3], was automatically calculated using the software provided with the apparatus: $D[4,3] = \frac{\sum n_i d_i^4}{\sum n_i d_i^3}$ in which:

n_i Number of particles of class “i”

d_i Diameter of class “i”

The span is the distribution width of particles in dispersion, which was calculated using the following equation:

$$Span = \frac{(D_{0.9} - D_{0.1})}{D_{0.5}}$$

The particle size analyzer was equipped with a commercially available Cilas 1190 expert shape attachment (Orleans, France). Microscopic observations were performed simultaneously with particle size measurements and the images were digitally recorded at a magnitude of 20×.

Rheological measurements

Rheological measurements were performed with a Physica MCR 301 rheometer (Anton-Paar GmbH, Graz, Austria) using a concentric cylinder geometry with a radius ratio of 1.0846 at 22 °C. The viscosity evolution of the samples was obtained at a constant shear rates (5 s⁻¹) as a function of time over 80 min; simultaneously, the monitoring of pH was carried out for the same sample once a minute during the mentioned period of time at 22 °C (Hasandokht et al. 2012).

Results and discussion

Effect of pH and concentration on sodium caseinate-sodium caseinate interactions

In current experiment turbidimetric measurements were used to investigate aggregation process of the sodium caseinate solution because any significant variation of absorbance reveals the appearance of aggregates by modifying the light properties of the system (Schmitt et al. 1999).

The effect of pH (7.00–2.50) and sodium caseinate concentration (0.03–0.3 wt.%) (at levels corresponding to those used in the mixed systems) on the absorbance of sodium caseinate solutions was investigated. As illustrated in Fig. 1, the absorbance of sodium caseinate was pH-dependent. The same results were obtained in other studies (O’Kennedy et al. 2006; Ruis et al. 2007; HadjSadok et al. 2008; Ru et al. 2012). At all concentrations, significant absorbance was found over a pH range between 5.50 and 2.50, with a broad peak occurring between pH 4.64 and 4.30. However, the maximum absorbance at that peak was found to increase

Fig. 1 Evolution of absorbance of sodium caseinate as a function of pH for different concentrations of sodium caseinate: Δ 0.300 wt.%, $\square$ 0.225 wt.%, $\circ$ 0.090 wt.%, $\diamond$ 0.060 wt.%, $\times$ 0.030 wt.%. Each point is the average of three independent experiments. Standard errors were within ± 5 % of reported averages

from 1.221 at 0.030 wt.% sodium caseinate, to 3 at 0.300 wt.% sodium caseinate. The rise in absorbance during the pH acid titration is supposed to be attributed to an increase in protein–protein aggregation associated with reduced charge repulsion between neighboring protein molecules near the protein's isoelectric point. The sudden decrease in absorbance after reaching the maximum turbidity is assumed to be a result of large-scale aggregation and subsequent precipitation of the caseinate samples around their pI (Guzey and McClements 2006; Ye et al. 2006). At solution pH $>$ pI, proteins assume a net negative charge, whereas at pH $<$ pI proteins assume a net positive charge. A small concentration effect on pI was seen where at sodium caseinate levels of 0.030 wt.%, the pI occurred at 4.29, where at sodium caseinate levels of 0.300 wt.%, pI value was 4.64. Small deviations in the reported pI values for different sodium caseinate concentrations may be the result of different levels of exposed charged amino acids on the surface, a consequence of increased protein–protein aggregation at higher concentrations.

Stability of sodium caseinate as a function of pH

The stability of sodium caseinate solution (0.300 wt.%) as a function of pH at various pH values (4.99, 4.80, 4.70, 4.30 and 3.67) was investigated by monitoring the turbidity of the mixtures in 30 min Fig. 2.

As we expected the stability of sodium caseinate solution is totally dependent on pH. When the pH is higher than pI of sodium caseinate (pH: 4.99, 4.80 and 4.70) the absorbance values in 30 min was constant that showed the stability of the solution whereas at pH values lower than pI of sodium

Fig. 2 Time-dependent profiles of absorbance of sodium caseinate at various pH values obtained from 0.3 wt.% sodium caseinate: Δ pH: 4.99, $\square$ pH: 4.80, $\circ$ pH: 4.70, $\diamond$ pH: 4.3, $\times$ pH: 3.67. Each point is the average of three independent experiments. Standard errors were within ± 5 % of reported averages

caseinate the absorbance values decreased which explains the instability of system because of coarsening of sodium caseinate aggregates and the phase separation of system. The system at pH 4.3 was more instable in comparison with the other systems even pH 3.67 because of rapid decrease of the absorbance value in min, which could be due to formation of larger and denser aggregates which sediment faster. At pH 3.67 the instability in the system can be observed while it is less than pH 4.3. It is postulated that at this pH the aggregates are smaller so the sedimentation of aggregates is slower.

Particle size analysis of sodium caseinate at different pH values

The particle size measurements indicate that the sodium caseinate tended to form large aggregates when the pH reduced to 4 (at pH 5, $d[2,1]=4.88 \mu\text{m}$ to pH 4, $d[2,1]=35.66 \mu\text{m}$) (Table 1). Moreover, further acidification led to smaller particles (at pH 3.6 $d[2,1]$ is $1.96 \mu\text{m}$). It has been revealed that

Table 1 Size distribution of particles formed in 0.045 wt.% sodium caseinate solutions at 4 specific pH values

pH	Sodium caseinate					
	$d(0.1) \mu\text{m}$	$d(0.5) \mu\text{m}$	$d(0.9) \mu\text{m}$	$D[2, 1] \mu\text{m}$	$D[3, 4] \mu\text{m}$	Span
5	0.04	0.16	0.38	4.88	57.67	2.12
4.2	0.32	13.95	44.45	18.27	34.93	3.16
4	2.08	32.49	71.51	35.66	55.34	2.136
3.6	0.11	0.98	4.45	1.96	5.50	4.42

Standard errors were within ± 5 % of reported averages

lowering pH from 5 to 4 leads to aggregation of caseinate so the contribution of the large particles was observed (O'Kennedy and Mounsey 2006; Ruis et al. 2007). At pH 4, which is the isoelectric point of caseinate, electrostatic repulsion was decreased hence extensive aggregation of caseinate occurred. However, more decrease in pH developed more positive charges on caseinate and due to the repulsion forces; sodium caseinate existed in the form of individual molecules again. This phenomena changed large aggregates to small ones. For this reason, as the particle measurements show, at pH 3.6 the particles are smaller. Sodium caseinate is present in the form of individual casein molecules if electrostatic interaction is strong, i.e. at low ionic strength and far from the isoelectric point (HadjSadok et al. 2008).

Microscopic observation of sodium caseinate at different pH values

The microscopic images also confirm the particle size measurements. As it is illustrated in Fig. 3 in higher pH values the aggregates were small Fig. 3a whereas in the lower pH values after pI of sodium caseinate large aggregates were produced Fig. 3c. Further acidification resulted in converting large aggregates into small ones. In addition these microscopic images are in agreement with studies stating that sodium caseinate forms small star-like aggregates in aqueous solution (Pitkowski et al. 2008).

Monitoring effect of pH on sodium caseinate by rheometry

Figure 4 illustrates the evolution of viscosity of the sodium caseinate solution as a function of pH.

Understanding the structural transition of sodium caseinate upon acidification can be obtained from rheological measurements as the rheology of dispersions is influenced by particle size (Schmitt et al. 1998; Balaghi et al. 2010; Gorji et al. 2011). Therefore we investigated the viscosity as a function of pH.

From pH 5.5 downward, viscosity gradually increased reaching a maximum at around pH 4.64, which is in good agreement with the pH of the maximum absorbance value. It is suggested that upon acidification a gradual growth of the sodium caseinate aggregates occurred which is responsible for

Fig. 4 Evolution of viscosity (sodium caseinate 0.3 wt.%) as a function of pH at a constant shear rate of 5 1/s. Each point is the average of three independent experiments. Standard errors were within ± 5 % of reported averages

the increase in viscosity. A sudden decrease of viscosity could be due to the phase separation of the system the same as absorbance profile. It can be assumed that the decrease in viscosity is due to the sedimentation of sodium caseinate aggregates thus the instrument showed the viscosity of the upper phase (water). This method (the coupling of slow in situ acidification of the sodium caseinate and rheometry) can help to attain deeper insights into the structural transitions during the acidification process.

Conclusions

Findings from this study describe the effects of pH and bio-polymer concentration on the self-assembly of sodium caseinate. The formation of sodium caseinate–sodium caseinate aggregates correlates well with the pH decrease. Aggregation process of sodium caseinate dilute solutions can be monitored using different methods such as rheometry, spectrophotometry and particle size analysis, which confirm the onset of big aggregates on decreasing pH. Surprisingly the viscosity of sodium caseinate solution at pH 4.64 was maximum and it decreased by lowering pH.

Fig. 3 Light microscopical images of sodium caseinate (0.045 wt%) solution at pH :a 5, b 4.2, c 4, d 3.6 (magnification 20 $\times$)

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