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Comparison of pH-dependent sonodisruption of re-assembled casein micelles by 35 and 130 kHz ultrasounds

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ABSTRACT

Sonodisruption behavior of re-assembled casein micelles was compared at two ultrasound frequencies (35 and 130 kHz) by turbidity measurement and laser-diffraction based particle size analysis. Sonochemical ultrasound (130 kHz) was more effective than power ultrasound (35 kHz) in micelle disruption. This was attributed to the higher strain rates generated upon implosion of cavities, as well as the liberation of more free radicals to the surrounding medium. The higher the pH of solution, the more effective was the ultrasonic disruption due to a looser expanded assembly of particles at higher pH values. Sonochemical ultrasound decreased the consistency coefficient of casein solutions and increased their flow index except at a pH value of 6.35, while power ultrasound did not affect the flow behavior of solutions across the whole pH range.

1. Introduction

Sonochemical and power ultrasound effects derive principally from acoustic cavitations (Suslick, 1990). This phenomenon is the violent collapse of microbubbles generated in the fluid exposed to high intensity ultrasound (Ciawi et al., 2006). A pure liquid would require very high power levels to initiate cavitation, which is not achieved by normal ultrasonic equipments. However, most normal liquids contain discontinuities, such as gas bubbles or dust motes, which act as weak spots and allow bubbles to form (Mason et al., 2005). Bubble nucleation is followed by successive rarefaction (expansion) and compression cycles. Rarefaction is due to the diffusion of gases and vapors into the bubble as a consequence of concentration gradients between the bubble interior and its surrounding liquid. Compression occurs when negative pressure inside the cavity is reduced and reaches atmospheric pressure. The bubbles now will start to lose a portion of their gas content and shrink due to surface tension (Zheng and Sun, 2006). Once the bubble is compressed, the boundary surface area available for diffusion is decreased. Therefore, the amount of gas that is expelled is less than the amount taken up during the rarefaction phase of the cycle. Consequently, bubbles grow bigger over each cycle until they reach an equilibrium size particular for the applied frequency (Mason and Paniwnyk, 2003). Ultimately some bubbles almost adiabatically implode (Ciawi et al., 2006) due to their dreadful internal pressures producing hot spots with temperatures of roughly 5000 K (Jeffries et al., 1992) and generating shear forces in the surrounding bulk liquid (Gogate, 2008). For perspective, within the liquid sonicated by an inexpensive apparatus, one can create the temperatures of the Sun's surface, the pressure of deep oceanic trenches, and the cooling rate of molten metal splatted onto a liquid-helium-cooled surface! (Suslick, 1990).

It is well known that ultrasound effects are strongly influenced by acoustic frequency. Jambrak et al. (2008) compared the ultrasonic treatment of whey protein suspensions with 500 kHz bath, 20 kHz probe and 40 kHz bath. They found that the 20 kHz probe had major effects in changing whey proteins' functional properties, such as solubility and foam formation ability. However, they did not apply the same acoustic intensity or power at different frequencies and, hence, their findings are a consequence of both frequency and intensity changes. The most effective degradation of dextran was found by using an ultrasonic frequency of 35 kHz compared with the other higher frequencies 500, 800 and 1600 kHz (Portenlänger and Heusinger, 1997). This report also lacked a comparison at the same acoustic intensity. The sonochemical hydroxylation of phenol was carried out at three different frequencies 20, 358 and 1062 kHz but at a constant acoustic intensity. Sonication of phenol at 20 kHz gave no significant effect but soni-

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cation at higher frequencies, especially at 358 kHz, caused significant hydroxylation of phenol (Ashokkumar et al., 2008).

Recently we showed that shear forces, generated by bubble implosion and/or microstreaming can break re-assembled casein micelles into smaller structures. At a higher pH, there was a greater decrease in particle diameter (Madadlou et al., 2009a). In the present study, we compare the sonodisruption phenomenon of re-assembled casein micelles at two different frequencies (35 kHz and 130 kHz) but at the same acoustic power.

2. Materials and methods

2.1. Materials and ultrasound equipment

Casein, methanol and phosphate buffer pH 7.0 (di-sodium hydrogen phosphate/potassium dihydrogen phosphate) were purchased from Merck (Darmstadt, Germany). Sodium tetraborate, sodium dodecyl sulfate (SDS), *o*-phthaldialdehyde (OPA) and β-mercaptoethanol were from Sigma–Aldrich (Dorset, UK). A double frequency ultrasonic bath model TI-H 10 (Elmasonics, Singen, Germany) of internal dimensions 30 × 24 × 15 cm provided continuous ultrasound (either 35 or 130 kHz) using four disc transducers with a maximum nominal power of 200 W. The power output could be set from 10% up to 100% by adjustment of the power level.

2.2. Sample preparation and sonoprocessing

Samples were prepared and sonicated as previously described (Madadlou et al., 2009a). Casein solution (3%) was prepared by adding 12 g casein powder to 0.5 M phosphate buffer (prepared by deionized water), stirring it at 4600 rpm for 60 min at room temperature and making it up to the final volume of 400 mL; sodium azide (100 mg L^{-1}) was added to prevent microbial growth. The solution was stored at 4 °C for 10 h to allow complete hydration. Solution (100 mL) was transferred to a 250 mL Erlenmeyer at its original pH value (6.35 ± 0.05). The pH value of the remainder was then adjusted to 8.0 ± 0.05, 9.7 ± 0.05 and 11.4 ± 0.05 by slow addition of sodium hydroxide solution to well-stirred solution. Each time, immediately after the desired pH was reached, 100 mL solution was transferred to a 250 mL Erlenmeyer. Very strong sodium hydroxide (20 M) was used for increasing pH value in order to prevent solution dilution.

The Erlenmeyers were placed in the center of the bath and rested for 1 h to equilibrate with the surrounding water (30 °C). They were then sonicated for 6 h, during which samples temperature was maintained at 30.5 ± 1.5 °C by tap water circulation around the containers in the bath (except for power measurements). The rise in temperature of casein solution during 120 s was used to calculate the actual power dissipated to the solution from the following equation (Jambrak et al., 2008):

$$P = mC_p\left(\frac{dT}{dt}\right) \quad (1)$$

where P is power (W), C_p is the specific heat capacity of the water (4.18 J $°C^{-1}$ g^{-1}), m is the mass of water (grams), dT/dt is the temperature difference over the 120 s sonication time. The actual powers dissipated to solutions were 0.0 and 4.1 W for control and sonicated samples, respectively. Electric power level was set at 70% for 35 kHz ultrasound to obtain an actual power of 4.1 W and set at 100% for 130 kHz ultrasound to obtain the same amplitude of actual power.

2.3. Turbidity measurements, particle analysis and flow behavior

Turbidity measurements were carried out as described previously (Madadlou et al., 2009a). The mean particle diameter and size distribution of re-assembled casein micelles were measured with a laser-diffraction based particle size analyzer (Malvern Master Sizer Hydro 2000 S, Malvern Instruments Ltd., Malvern, UK). The experiments were carried out as described previously (Madadlou et al., 2009b). In this instrument, the angular dependence of the obstruction of a continuous laser beam by particles is used for size measurement (Banavara et al., 2003; Thanasukarn et al., 2006). Experiments were performed on 5-fold diluted solutions over the range 20 nm to 15 μm. Just before measurements, samples were diluted with deionized water having the same pH of sample (adjusted by sodium hydroxide addition) to prevent their foaming during stirring and pumping in the instrument. Water was used as the dispersant with a refractive index of 1.330. Particle characteristics of solutions are reported by volume-weighted mean ($D_{4,3}$), specific surface area and span. The latter is an index of particles polydispersity (Tan and Nakajima, 2005) as expressed by

$$\text{Span} = (d_{0.9} - d_{0.1})/d_{0.5} \quad (2)$$

where $d_{0.9}$, $d_{0.1}$ and $d_{0.5}$ are the diameters at 90%, 10% and 50% cumulative volume of particles, respectively. Viscosity measurements were performed as described previously (Madadlou et al., 2009b). Measurements were performed using a rotational viscometer (model DV-II+ pro Brookfield Engineering Lab. Instruments, Middleboro, MA, USA). Samples were sheared from a rate 56.43 s^{-1} up to 140.03 s^{-1} and the relevant shear stresses (N m^{-2}) were recorded at 14 time intervals of 6 s. Data were collected by Rheocalc version 3.1-1 Demo application and analyzed by Curve Expert version 1.34 (Microsoft Corporation). Consistency (k) and flow (n) indices were calculated by fitting shear rate and shear stress data on power law model.

2.4. Primary amines content

The content of primary amines was measured to follow the probable lysis of peptide bond as a consequence of sonication as described previously (Madadlou et al., 2009a).

2.5. Statistical analysis

The experiment was replicated 3 times in a complete randomized design. The effect of pH value and ultrasonic treatment on tested parameters was determined by analysis of variance (ANOVA) using GraphPad Prism 5.0 for windows version 5.00 (GraphPad Software Inc.). Two-way ANOVA at 5% significant level ($\alpha = 0.05$) was carried out to assess whether the different treatments conducted result to statistically different variables evaluated.

3. Results

Ultrasonic experiments were conducted for 6 h at 2 differing frequencies of 35 and 130 kHz at constant acoustic power. Fig. 1 demonstrates the effect of ultrasonic frequency on the turbidity of casein solutions and diameter of re-assembled micelles. For control samples, increasing the pH value reduced their turbidity and increased particle diameter. The mechanism underlying the expansion of casein particles is discussed elsewhere and based on the electrostatic repulsion between casein molecules in micelles and the increased solvent quality of serum phase (Madadlou et al., 2009b). It is evident from Fig. 1 that sonication resulted in a decrease in the turbidity of solution and diameter of particles at any given pH value. The magnitude of decrease was greater for samples treated by 130 kHz ultrasound. The decrease in diameter of particles and consequently increase in the specific surface area (results not shown) available for light scattering, most likely ac-