

Spectroscopic methods to determine in situ changes in dairy systems – ultrasonic and light scattering

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Abstract – Many of the techniques currently employed to study the aggregation of dairy systems are either invasive or not able to identify changes at the early stages of aggregation. Recently, diffusing wave spectroscopy (DWS) and ultrasonic spectroscopy (US) have shown promise as samples can be observed in situ without any preparation or disruption. With DWS measurements we can obtain information on size, spatial correlation between the colloidal particles and their movement. With high resolution ultrasonic spectrometers the velocity or attenuation of a sound wave propagating through the sample can be observed while physical chemical changes occur to the sample (for example, gelation, phase separation). DWS and US have been recently employed in the study of the interactions between proteins and other ingredients in milk-based systems. Although the changes observed during the measurements of complex systems such as milk are still to be fully understood, it is clear that these spectroscopic techniques present great potential to derive further information on the dynamics of change in aggregating systems.

ultrasonic spectroscopy / diffusing wave spectroscopy / colloidal aggregation

摘要 – 光谱法测定乳体系中原位变化-超声和光散射。目前许多用于研究乳体系凝聚作用的方法多是采用入侵式方法或者是不能测定出聚集作用早期变化的方法。近来,漫射光谱(DWS)和超声频谱(US)可以直接测定出样品的原位变化而不需要对样品进行任何制备和分解。采用漫射光谱法可以测定胶体微粒大小以及胶体微粒之间的空间运动关系。采用高分辨的超声频谱,可以观察到速度或者声波穿过样品后的衰减以及样品所发生的物理化学变化(如,凝胶作用、相分离)。漫射光谱和超声频谱已经应用于研究乳基质中蛋白质和其他成分的相互作用。尽管,这两种方法用于测定复杂食品体系(如,牛乳)的变化应需要进一步探讨,但是显然这两种光谱技术有可能应用于研究凝聚体系变化的动力学。

超声频谱 / 漫射光谱 / 胶体的聚集作用

Résumé – Méthodes spectroscopiques pour déterminer in situ les changements dans les systèmes laitiers : ultrasons et diffraction de la lumière. Beaucoup de techniques actuellement employées pour étudier l'agrégation des systèmes laitiers sont soit invasives, soit incapables d'identifier les changements au tout début de l'agrégation. Récemment, la spectroscopie de diffusion d'ondes (DWS) et la spectroscopie ultrasonique (US) se sont avérées prometteuses puisque les échantillons peuvent être observés in situ sans préparation ni destruction. Avec les mesures DWS, on peut obtenir des informations sur la taille, la corrélation spatiale entre les particules et leurs mouvements. Avec les spectromètres ultrasoniques à haute résolution, on peut observer la vitesse ou l'atténuation d'une onde

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sonore se propageant au travers de l'échantillon pendant que les changements physico-chimiques ont lieu dans l'échantillon (par exemple gélification, séparation de phase). La DWS et l'US ont été récemment employées dans l'étude des interactions entre protéines et autres ingrédients dans les systèmes à base de lait. Bien que les changements observés au cours de la mesure de systèmes complexes comme le lait ne soient pas encore complètement compris, il est clair que ces techniques spectroscopiques présentent un potentiel important pour obtenir des informations sur la dynamique des changements dans les systèmes s'agrégeant.

spectroscopie ultrasonique / spectroscopie DWS / agrégation

1. INTRODUCTION

Food systems often contain a multitude of components present in different aggregation states. To be able to control the final bulk properties of a product (for example, stability, microstructure and texture) it is of great importance to understand the molecular reactions occurring between the various components during processing and storage. In dairy systems, liquid to solid transitions (i.e. gelation) often occur under conditions where repulsive or attractive forces between the colloids dominate. These reactions can be controlled by changes in concentration, and the techniques routinely employed to observe molecular changes require extensive dilution of the samples or some type of sample preparation which can cause disruption of the structure. For instance, techniques that measure bulk properties (such as rheology) require (although minimal) shear forces to be applied to the sample, ultimately affecting the dynamically fragile stages preceding colloidal aggregation. Although there is a breath of work involving the gelation of milk either induced by the enzyme rennet or by acidification, the full dynamics of the interactions between the casein micelles, of about 100 nm of diameter, are not fully understood, as most results either focus on identifying the gelation point (by rheology) or by determining molecular changes to the micelles under extremely diluted conditions (for example, using traditional light scattering techniques). Oil-in-water emulsions are another dairy systems extensively

investigated using well-established experimental techniques. The aggregation and destabilization of protein stabilized oil droplets during pH changes or in the presence of polysaccharides are important mechanisms to understand their bulk properties and long term stability. As in the case of milk systems, most of the current knowledge has derived from studies of dilute systems.

Though the results obtained have been invaluable for our current understanding of the general mechanisms of gelation at the molecular level, there is still a lack of information regarding the aggregation behaviour in undisturbed, concentrated states. In recent years dairy research has started to profit from developments of technologies made for disciplines related to the material sciences, and techniques such as ultrasonic spectroscopy or diffusing wave spectroscopy are now applied to the study of dairy colloids. These techniques can be employed to observe turbid suspensions such as undiluted milk or heat-set protein gels, and this brings us a step closer to understanding the mechanisms of colloidal aggregation under more realistic conditions. Although the challenge still remains in developing a theoretical framework to be able to fully describe the kinetics of gel formation and the dynamics of change in these systems, methods such as DWS and US, often combined to oscillatory rheometry and confocal microscopy are being employed to observe the effect of processing parameters and ingredients interactions on the aggregation behaviour

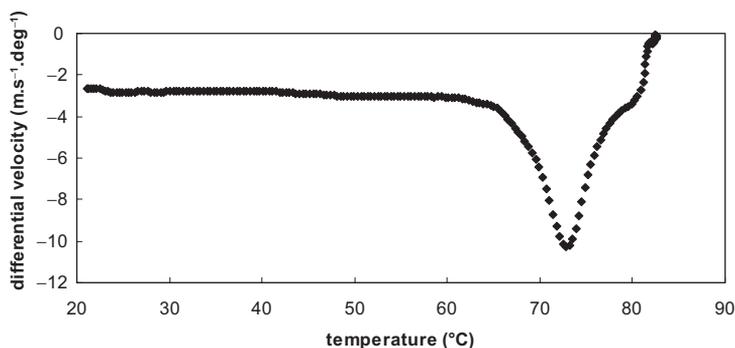


Figure 1. Temperature differential of the ultrasonic relative velocity (difference between the velocity of protein solution and the velocity measured in the reference cell containing buffer) for a 10% (w/v) β -lactoglobulin solution containing $0.1 \text{ mol}\cdot\text{L}^{-1}$ NaCl during heating from 20 to 85 °C at a rate of $0.4 \text{ }^\circ\text{C}\cdot\text{min}^{-1}$. Measurements carried out with a HRUS (Ultrasonic Scientific, Dublin) at 5 MHz.

and in the dynamics of gel formation in milk systems. In this manuscript we intend to discuss some examples of our research on the application of DWS and US to dairy systems.

2. ULTRASONIC SPECTROSCOPY

Ultrasonic measurements allow the observation of real-time changes in concentrated milk systems. As an ultrasonic wave propagates through the sample its intensity and phase change due to the presence of density and compressibility inhomogeneities along its path. The decrease in intensity of the sound wave is referred to as attenuation, while the phase is related to the velocity of the wave propagating through the system. The changes in velocity and attenuation depend on the physico-chemical properties of the sample and this has been employed to study changes in food systems. Velocity and attenuation of sound have been shown to be sensitive to composition, changes in the microstructure and/or phase separation in foods [13, 18].

Ultrasonic velocity is very sensitive to changes in temperature. It has been reported that sound speed in water changes

about $2.4 \text{ m}\cdot\text{s}^{-1}$ per degree centigrade ($^\circ\text{C}$) [13]. For this reason, temperature control is fundamental in measurements of ultrasonic velocity and with high resolution spectrometers the changes are often reported as relative to a reference sample [5]. Ultrasonic velocity is optimal for characterizing chemical reactions and changes in chemical composition that occur at a molecular level. For example, it has been reported that the addition of $0.8 \text{ mol}\cdot\text{L}^{-1}$ CaCl_2 to water can change the velocity of sound traveling through it by $45 \text{ m}\cdot\text{s}^{-1}$ [13]. During acid-induced gelation of casein micelles changes in ultrasonic velocity (as well as attenuation) are mostly related to the dissociation of micellar calcium phosphate in the milk serum [11, 16], and this is independent on the rate of acidification [11]. In dispersed systems, changes in velocity are caused by variations in the volume and adiabatic compressibility of the solutes as well as by changes in the hydration shells.

High resolution ultrasonic spectroscopy has been successfully applied to the observation of the heat-induced interactions of whey proteins [8, 19]. With US it is possible to observe changes in the aggregation in the stages preceding bulk gelation [8]. Figure 1 illustrates these changes

in velocity (measured as velocity relative to the velocity of a reference sample containing only buffer) during heating of β -lactoglobulin [8]. The relative ultrasonic velocity of the whey protein solutions decrease steadily with temperature, with a rapid change occurring at temperatures $>65^\circ\text{C}$, and this can be clearly observed in Figure 1 for heating of β -lactoglobulin in the presence of $0.1\text{ mol}\cdot\text{L}^{-1}$ NaCl. When compared to other analytical techniques that quantify the degree of aggregation of whey proteins (DSC, HPLC), in contrast to HPLC that quantifies the amount of residual native protein, DSC and ultrasonic velocity measurements can assess the extent of irreversible protein aggregation [19].

In general, in the studies of dairy colloids more information can be derived from measurements of attenuation of the ultrasonic wave than from its velocity [13, 18]. Attenuation is sensitive to changes in the nanometers to micrometers scale, and therefore it is much more suitable for use in colloidal systems, which fall within this size range [3]. It is also important to point out that the frequency range capability of the instrument in use is very significant, as the wider the range, the more information can be obtained from the acoustic attenuation frequency spectrum. Particle size changes can indeed be determined with ultrasound attenuation data, however, a significant frequency range, from 1 to 100 MHz, is needed. Measurements at low frequencies limit the ability to observe the details of change in small particles [3, 6, 12]. The attenuation of the ultrasonic wave is caused by thermal, visco-inertial and scattering events during its path [3, 12, 13]. Thermal attenuation of the ultrasonic wave is the most important component in dairy colloids as the dispersed phase a low density-contrast with the solvent. Thermal attenuation depends on the thermodynamic properties of the dispersed components, such as heat conductance, thermal expansion and heat capacity. When

the ultrasonic wave propagates, the thermoelastic scattering is result of the temperature oscillations caused by the adiabatic compressibility of the particles. That is to say that compression and decompression of the ultrasonic wave generate a heat flow between the dispersed particle and the solvent causing a temperature gradient at the boundary layer. This effect is then strongly dependent on the total surface area of the dispersed particles. The particle boundary layer will expand and contract acting as a secondary source of ultrasonic waves, and a thermal wave will flow away from the particle and attenuate along its path [3, 12]. As previously mentioned, in oil-in-water emulsions or milk, the contribution of the visco-inertial dissipation to the total attenuation of the system is negligible due to the low density-gradient between the oil and the water (or the casein micelles and the water) [12, 13]. On the other hand, when there is a gradient in density between the dispersed particle and the solvent, visco-inertial effects become significant. Lastly, the total attenuation from scattering by particles becomes important as the size of the particles increases to diameters of $50\ \mu\text{m}$ and above. In this case, the particle size is large enough to redirect the ultrasound energy away from its path [3].

There has been significant experimental research devoted to the changes in ultrasonic attenuation during renneting of milk. In general, an increase in attenuation occurs at the early stages of the aggregation when enough casein micelles have been destabilized by the rennet enzyme [7]. Attenuation of sound seems to be less sensitive to changes in the structure of the gel once the gel has formed [7]. In contrast, in acid-induced gels, most of the changes in attenuation can be attributed to the dissolution of calcium phosphate from the micelles. Here again, the effects of gelation per se on the attenuation of the sound wave are quite small [10, 11]. Very little differences in the development

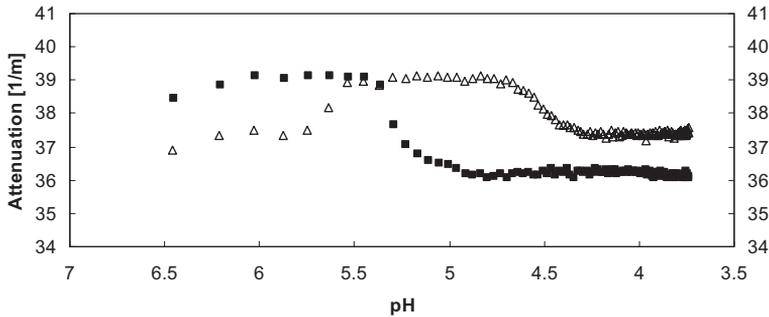


Figure 2. Ultrasonic attenuation, measured at 8.7 MHz, as a function of pH for 10% oil-in-water emulsions containing 0.5% sodium caseinate (■) and same emulsion with 0.5% high methoxyl pectin (degree of methoxylation 67) (△). Acidification of the emulsions was induced by addition of 1.5% GDL at 22 °C.

of ultrasonic attenuation during acidification have been reported for heated and unheated milk, once again confirming that although the rheological parameters are quite different with heating, the attenuation parameter is affected very slightly by the effect of gelation [10, 11].

In oil-in-water emulsions attenuation can be successfully used to follow the structural rearrangements of the oil droplets in solution [6, 14]. An example is shown in Figure 2. Attenuation decreases in flocculated systems [6], for example, when a negatively charged polysaccharide is present in emulsions stabilized by sodium caseinate at high pH. The negatively charged oil droplets covered by sodium caseinate do not interact with high methoxyl pectin at a pH of 6.8, but they start to interact when the surface charge on the oil droplets decreases (pH of around 5.6). The ultrasonic attenuation decreases due to droplet rearrangement [17]. Emulsion aggregation can also be measured by observing changes in attenuation. At pH < 5.4 casein-stabilized oil droplets lose their surface charge and aggregate, showing a dramatic decrease in attenuation. The presence of high methoxyl pectin caused the pH of destabilization to decrease to lower pH (about 4.6).

This is seen as a sudden decrease in the attenuation curve. By measuring changes in ultrasonic attenuation, it is possible to follow the dynamics of destabilization of dairy based emulsions [6, 14, 17].

3. DIFFUSING WAVE SPECTROSCOPY

Dynamic light scattering (DLS) is a technique commonly employed to study food colloids. The intensity fluctuation of the scattered light is measured and from a time-resolved correlation function the diffusion coefficient of the scatterers can be derived. From DLS experiments information on size and size distribution of the particles can be derived [9]. This experimental approach has played an important role in improving our understanding of the mechanisms leading to milk proteins aggregation. DLS can be employed only on extremely diluted systems, as multiple scattering needs to be avoided. This limits substantially our ability to measure changes during aggregation in more realistic conditions, when the concentration of the scatterers is significant and hence the measurements are conducted in the multiple scattering regime. In recent years, diffusing wave spectroscopy (DWS) has been

developed to overcome this limitation. In contrast with DLS, DWS requires turbid or concentrated systems as the photon path through the sample is treated as a random walk phenomenon [20]. DWS is an ideal technique to study the dynamics of change in dairy systems, such as renneting or acidification of milk or destabilization of dairy emulsions [2].

While traditional light scattering experiments can yield information on size, shape or polydispersity of the particles, the interest in DWS moves beyond the determination of size information and onto particle interactions and particle dynamics. Output of the DWS instrument is a time-correlation function with a characteristic decay time of $\tau(l^*/L)^2$ (where, for free diffusing particles, $\tau = (Dk^2)^{-1}$, D is the diffusion coefficient of the particles and k is the wave vector of the light $k = 2\pi n/\lambda$, n is the refractive index of the serum phase, λ is the wavelength of light and L is the thickness of the sample measured). In the case where there is no correlation between particles, values of diffusion coefficient can be treated as in traditional DLS and used to calculate the average diameter of the particles present in the sample. However, the value of l^* has to be determined; l^* is defined as the photon transport mean free path [2, 20] and it is related to the randomization (or decorrelation) of the photon path over many scattering events. This randomization is related to the physical properties of the sample as well as their spatial arrangement. The measurement of l^* not only allows for the calculation of the diffusion coefficient in DWS measurements, but more importantly relates to changes in the interactions between the particles. For this reason, interest in the DWS technique for dairy systems lies in its potential to be used as a tool to measure the dynamics of interactions in situ with minimal sample disturbance [2, 15, 20].

By combining DWS and rheological measurements on acid-induced aggrega-

tion of skim milk (using glucono- δ -lactone, GDL) it is possible to observe changes occurring in the preceding stages of aggregation by following the development of l^* [1, 2, 10]. In both heated and unheated milk, values of $1/l^*$ increase much before the change (because of aggregation) in the size of the casein micelles. The change in $1/l^*$ demonstrates that the casein micelles change their spatial correlation ahead of the actual aggregation which occurs at much lower pH, as shown by rheology. In unheated milk, the interactions appear later and at a lower pH than for heated milk [1, 10].

From the correlation function data obtained with DWS it is also possible to determine values of mean square displacement (MSD), from which we can derive information on the particle movement at very short time scales. This parameter is affected by the interactions between the particles (hydrodynamic, steric, electrostatic, etc.) and their restriction (if any) of movement [20]. The coupling of l^* , MSD and rheological measurements clearly results in defining the mechanisms of aggregation in colloidal systems, allowing the observation of interactions preceding particle size changes or changes in viscoelastic moduli as measured by rheology. It has been clearly demonstrated [1] that during acidification of milk by addition of GDL, gelation occurs earlier in heated milk because of the interactions between soluble whey protein aggregates with casein micelles. The gelation point appears earlier in DWS compared to rheology measurements, because DWS probes interactions at a much smaller length scales than rheology, which can only detect the formation of a gel after a space-filling network is established. For this reason, DWS is much more sensitive to particle-particle interactions than rheology. Observation of acid-induced gelation of casein micelles with DWS has also demonstrated that the changes occurring in milk are quite gradual, reflecting an increasing

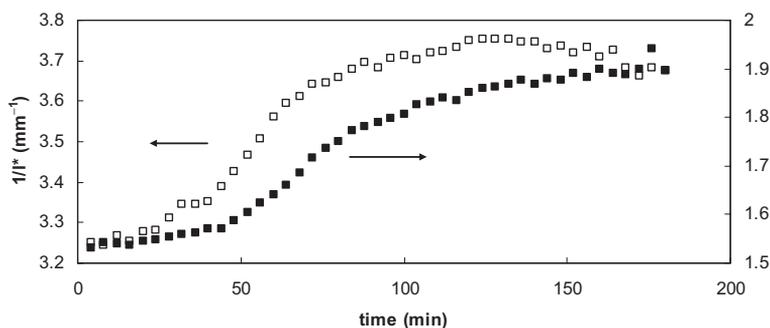


Figure 3. $1/l^*$ during renneting of skim milk (■) or of skim milk with 4% anhydrous milk fat emulsion droplets covered with whey protein isolate (□) note the difference in the scale.

tendency of the particles to interact as the pH decreases. On the contrary, rheological measurements would suggest that gelation occurs in a narrow pH range. It is important to note that during rheological measurements a shear stress (although minimal) is applied to the sample.

Measurements of milk treated with rennet by DWS confirm that the casein micelles start interacting with one another much earlier than the point of gelation measured by rheology [2, 15]. Figure 3 illustrates the development of $1/l^*$ in fresh skim milk after the addition of rennet. The increase in the $1/l^*$ value indicates that the particles begin to interact with each other much earlier than the detected gel point. Most of the research conducted so far on milk gelation with DWS has focused on the aggregation of casein micelles. Research has yet to be reported on the aggregation of skim milk by renneting or acidification in the presence of fat globules. Presently, we are expanding the development of this technique beyond simply skim milk to include systems containing a combination of casein micelles and fat globules. Figure 3 illustrates the difference in the $1/l^*$ development when rennet is added to skim milk containing 4% fat, consisting of droplets of anhydrous milk fat stabilized by whey proteins. Very little is so far understood on the interactions between oil droplets and

casein micelles in the stages preceding aggregation, but studies on model systems will allow to build knowledge which will aid in the interpretation of more complex systems.

Transmission DWS has also been used to study the interactions between charged polysaccharides and dairy protein-stabilized emulsion droplets. It has been demonstrated that DWS is a much more sensitive technique to identify bridging flocculation or structural rearrangements of the oil droplets at their early stages of the interactions in systems containing those stabilizers often used in dairy products [4, 14].

4. CONCLUSION

Although the theoretical framework for the interpretation of the results has still to be fully developed, DWS and US techniques have shown great potential to be used in the study of dairy systems, because of their sensitivity to the changes in the interactions between the particles. These non invasive, non disruptive techniques have already resulted in great advances in our fundamental understanding of the aggregation behavior of dairy systems and have the potential to be implemented in real time monitoring of industrial processes, such as yogurt and cheese making.

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