Ultrasonic spectroscopy for material analysis. Recent advances

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The relationships between a material's properties and acoustical characteristics have been studied for a long time and ultrasonic techniques have been used in non-destructive testing and imaging for decades. Ultrasound as an analytical tool has revolutionised diagnostics in medicine, but the application of ultrasound to material's analyses has been held back by problems with ultrasonic design, electronics, sample handling, complicated measuring procedures and resolution. Recent advances in computing power and digital techniques have made it possible to design a versatile laboratory instrument with applications ranging from ceramics to polymer science to cell biology and emulsions. The high-resolution HR-US family of ultrasonic spectrometers recently launched by Ultrasonic Scientific is an example of this.

Most spectroscopists are accustomed to using electromagnetic waves in analysis (UV, vis, IR, NMR etc.). Ultrasonic spectroscopy is simply spectroscopy employing sound waves. In particular, it uses a high-frequency acoustical wave (similar or higher to those used by dolphins for communication and bats for navigation). The wave probes intermolecular forces in materials. Oscillating compression (and decompression) in the ultrasonic wave causes oscillation of molecular arrangements in the sample, which responds by intermolecular attraction or repulsion. The amplitudes of deformations in the ultrasonic waves employed in analytical ultrasound are extremely small, making ultrasonic analysis a non-destructive technique.

Of course an ultrasonic wave, unlike its light counterpart, is able to propagate through opaque samples, in fact through most materials. Another advantage is that it is relatively easy to change the wavelength of an ultrasonic wave: unlike optical techniques where the wave originates in a light source and therefore needs special effort to get a required spectral purity, ultrasonic waves are synthesised electronically. Therefore a typical ultrasonic spectrometer can cover a broad range of wavelengths (10–100 times or greater). It could be described as probing the interior of the analysed sample with a set of fingers, which differ in their length by more than an order of magnitude!

Parameters and principles

The two major parameters measured in high-resolution ultrasonic spectroscopy are the attenuation and the velocity of the waves. Attenuation is determined by the energy losses in compressions and decompressions in ultrasonic waves, which include absorption and scattering contributions. As measurements of attenuation do not require high temperature stability of the sample, they can be performed in large samples. That is why attenuation was the parameter responsible for the majority of past ultrasound applications in research, such as the kinetics of fast chemical reactions and particle sizing in emulsions and suspensions.

Ultrasonic velocity is determined by the density and the elasticity of the medium. This is extremely sensitive to the molecular organisation and intermolecular interactions in the sample and can be exploited in the analysis of a broad range of molecular processes. However, its application requires high resolution of the measurements, which cannot be achieved in large samples because of the difficulty of controlling the temperature.

It is because of the difficulty in resolving both sets of information that scientists tended to belong to research groups working on the measurement of either attenuation or velocity and adjusted their instruments for the best performance in the selected area.

The general principles of high-resolution ultrasonic measurements are shown in the Figure 1. The generated electronic signal is transferred by the piezotransducer into the ultrasonic wave travelling through the sample. Another piezotransducer transfers the received ultrasonic wave into an electronic signal for subsequent analysis.

The most widely used approach for the measurements of ultrasonic characteristics in the past was based on the pulse technique. In this technique an ultrasonic pulse generated at a certain frequency is sent through a sample and received either at the opposite side or, after the reflection from the wall of the container, back to the source of ultrasound. Measurements of the amplitude of the wave in the pulse allow the determination of the ultrasonic attenuation and the propagation time (or related parameters), which characterise the ultrasonic velocity. The resolution of this technique is limited by the path-

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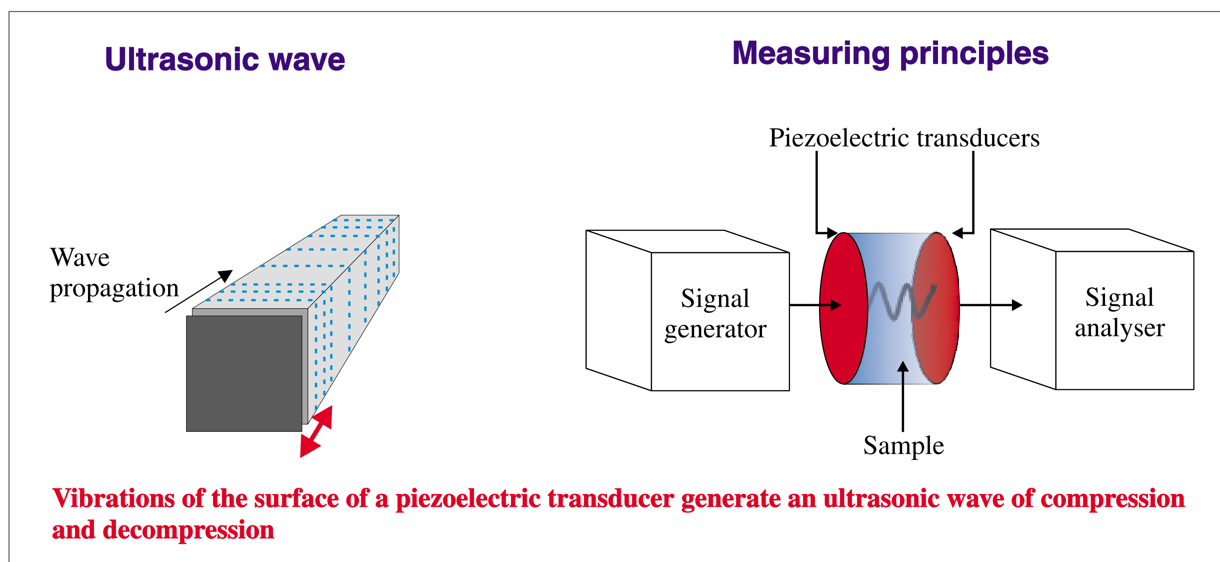

Figure 1. The general principles of high-resolution ultrasonic spectroscopy.

length of the pulse, or by the size of the sample.

The Ultrasonic Scientific HR-US spectrometer employs a novel principle where the pathlength of the ultrasonic wave in the sample exceeds the size of the sample. The use of modern advances in ultrasonic design, electronics and digital processing allow the attainment of ultrasonic measurements with much greater resolution (down to 10^{-5}% for ultrasonic velocity) in a broad range of the sample volumes, down to a single droplet.

Benefits of ultrasonic analysis

Most materials are ultrasonically transparent, allowing the analysis of a broad variety of sample types, chemical reactions and processes. Ultrasonic analysis can now be easily performed in chemistry, physics, biotechnology, pharmaceuticals, food, agriculture, environmental control, medicine, oil, petroleum and gas industries.

Since ultrasonic signals are generated electronically with the use of small piezo transducers, modern high-resolution ultrasonic spectrometers do not have large actuators (as in dynamic rheology) or bulky light sources and other optical parts. This permits the construction of robust and multipurpose instruments, which perform a broad range of analytical functions and are found equally in research, analytical, product development and quality control laboratories, and in process control analysis.

Modern ultrasonic cells do not have any cavities or sharp corners allowing for easy filling, refilling, cleaning and sterilisation. They can accommodate even aggressive liquids such as strong acids or organic solvents without evaporation in a course of measurements. Cell volumes range from 4 mL down to 30 µL. Semi-solid cells are also available for samples such as biological tissues, gels, toothpaste, cheeses, waxes, pastes, creams and so forth.

Users of high-resolution ultrasonic spectrometers can measure concentrations of components, transition temperatures and temperature intervals, characterise the temperature stability and shelf-life of their materials, analyse sizes of particles in suspensions and emulsions, kinetics of sedimentation, kinetics of chemical and physical processes in materials, stoichiometries and affinities in ligand-binding and other parameters of their samples.

Fast measurements allow the analysis of flowing samples and, coupled with the ability to perform measurements on small volumes, make it possible to use high-resolution ultrasonic spectrometers in HPLC and similar applications. Because the ultrasonic velocity and attenuation can be measured simultaneously at different wavelengths as a function of time the instrument can be used for the analysis of the kinetics of chemical reactions and processes, such as the analysis of enzymatic activity. The ability of ultrasound to analyse opaque samples makes it possible to measure the speed of enzymatic reactions in aqueous solutions as well as in blood or tomato juice, samples where traditional spectroscopy fails. It can analyse chemical reactions, transitions and processes as fast as 10^{-5} to 10^{-7} s *without* optical markers, meaning that the reaction or system can be studied in its natural state.

The construction of modern ultrasonic cells allows controlled stirring of the sample, hence permitting measurements under shear and measurements in sedimenting samples. It also allows the measurement of ultrasonic velocity and ultrasonic attenuation in the course of titration, and is useful for the analysis of ligand-binding, and the adsorption of molecules on the surface of particles in colloid systems and the study of complex formation phenomenon.

The design of the HR-US makes possible ultrasonic measurements in the temperature ramp regime for analysis of heat stability, phase transitions, conformational transitions in polymers and other materials. In addition the small sample requirement saves the cost of analysis, which is a key issue in pharmaceutical, biotechnological and biomedical industries and research.

Finally, there is the dynamic range that allows the analysis of solutions of small concentration, down to 0.3 ppm (0.3 µg mL^{-1}). At the other end of the scale the same instrument can be used for concentrated mixtures and non-liquid samples such as biological tissues, hard gels, butter etc. Other techniques such as differential scanning calorimetry would require two different devices for the same set of samples; a high-resolution calorimeter for dilute solutions and another "solid" calorimeter for concentrated samples.

Examples

Here we report a recent application in monitoring the acid-induced gelation of whey proteins.[1,2] Together with caseins, whey proteins are the major protein ingredients of milk. In cheese manufacturing the curdling process sep-

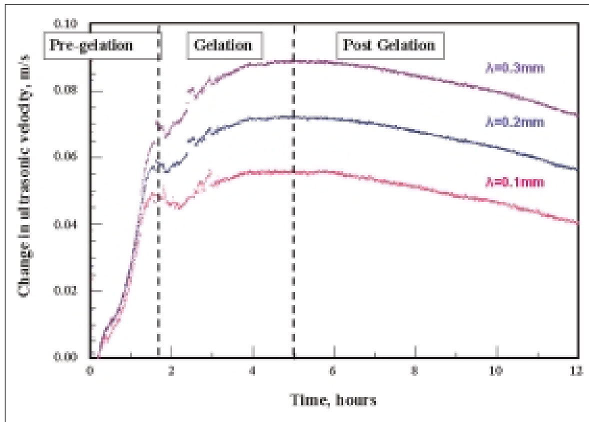

Figure 2. Evolution of ultrasonic velocity in gelation of whey proteins caused by slow acidification. 25°C, 2% protein solution acidified by GDL.

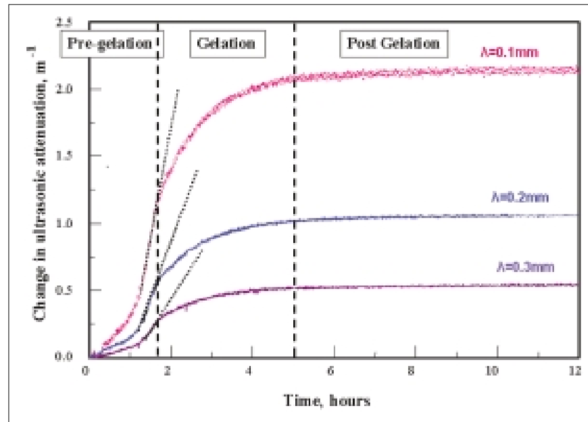

Figure 3. Evolution of ultrasonic attenuation in formation of whey protein gel in aqueous solution caused by slow acidification. 25°C, 2% protein solution acidified by GDL.

arates caseins, which are incorporated into the cheese, from the whey. The dairy industry has been very successful in finding application for the whey fraction and nowadays whey proteins are successfully applied in many foods as a thickening and structuring ingredient. This is possible since whey proteins are capable of forming a space-filling biopolymer network that holds a large amount of water. At a concentration of only a few (weight) percent, the network binds all the water and a gel is formed. It is of great interest to the food industry to know precisely how this network is formed and what the interactions are that hold the network together. This allows more efficient use of the protein ingredients as well as the development of products with improved functionalities such as prolonged shelf life.

The network formation of whey proteins is usually induced by heating, but

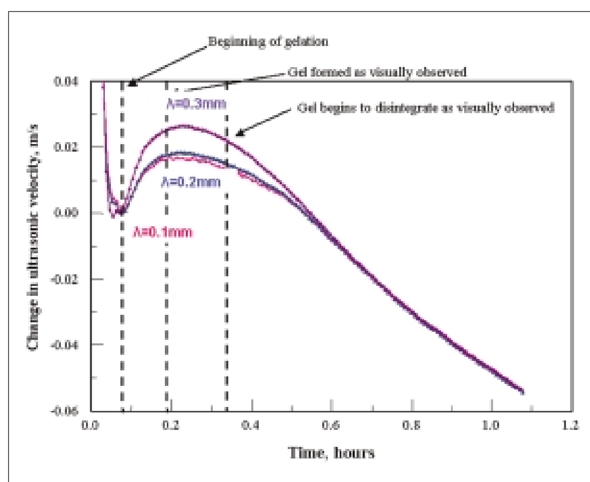

Figure 4. Ultrasonic (velocity) monitoring formation and collapse of gel network in whey protein solution caused by fast acidification. 25°C, 2% protein solution acidified by GDL.

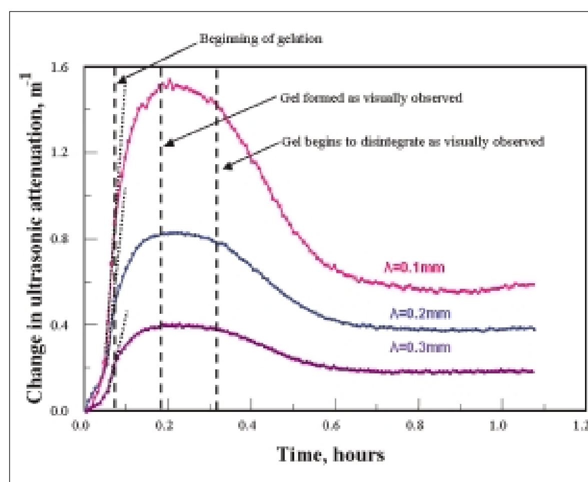

Figure 5. Ultrasonic attenuation profile in the process of formation and collapse of whey protein gel caused by fast acidification. 25°C, 2% protein solution acidified by GDL.

an alternative strategy can be used. Heating induces the denaturation of native proteins. These denatured proteins tend to aggregate and if aggregation proceeds long enough, a gel is formed. However, when heating and solvent conditions are carefully chosen, protein aggregation is not followed by the formation of a space-filling network. In this case a stable solution of aggregates is obtained after cooling. In a second step a complete network can be formed by increasing the interactions between the aggregates. This is typically done by gradual acidification or adding specific salts. Gelation will now occur at room temperature on a time-scale of minutes to days, depending on the chosen conditions and concentrations.

The cold gelation method is attractive because it allows us to study the formation of protein networks in the absence of protein unfolding phenomena. We can also precisely measure and control the properties of the aggregates prior to gelation and thereby obtain direct relations between, for example, their size, shape and charge distribution and the final functional properties of the gels, such as hardness and fracture mechanics. However, the gels are typically opaque to non-transparent which makes it hard to use optical spectroscopy methods. Therefore, and because of the ability to monitor the change in mechanical properties, ultrasonic analysis is an attractive tool to probe the progress of network formation during the cold setting of these types of gels since it is not hampered by the turbidity of the samples.

The kinetics of acid-induced gelation of an aqueous solution of whey protein aggregates were measured. By varying the amount of added GDL (glucono-delta-lactone) we can induce either a fast rate of acidification (typically minutes) or a slow rate of acidification (typically hours). When a large amount of GDL is added (approximately 1%), acidification proceeds rapidly and a gel is formed in minutes. In this case the pH usually decreases below a critical point at which the gels become a liquid again. Adding less GDL (0.15% in our measurements) causes a much slower rate of acidification and gelation then takes hours. Also, the pH settles above the critical value and the system remains in the gel state. Further experimental details have been published elsewhere.[1,2]

Kinetics of aggregation and gelation in protein solution during slow acidification

We measured ultrasonic velocity and attenuation, at frequencies between 4 and 14 MHz, at a temperature of 25°C. The change in ultrasonic parameters with the kinetics of gelation for a slow rate of acidification of a 2% whey protein aggregate solution are given in Figures 2 and 3.

The kinetics of acid-induced cold gelation consists of two stages, which we conveniently refer to as pre-gelation and gelation, both stages are discussed below. We also followed post-gelation processes.

Pre-gelation (slow acidification)

Pre-gelation occurs over the first two hours after acidification of the whey protein solution, and is indicated by increases in the ultrasonic velocity and attenuation over this time period. This increase in ultrasonic velocity shows the hydration changes in the system as a result of protein unfolding and protonation. An increase in particle size is indicated by a sudden increase in ultrasonic attenuation, at 80 min (Figure 3), due to the formation of aggregates, which may include small phases of gel not linked into a complete network structure. At the same time the wavelength (frequency) dependence of ultrasonic velocity appears thus showing a microstructural non-homogeneity of the samples.

Gelation (slow acidification)

At 110 min the onset of gel network formation is indicated by inflections in the ultrasonic attenuation (Figure 3) and ultrasonic velocity (Figure 2) profiles. An important contribution to the increase in ultrasonic attenuation is the viscous losses, caused by friction between the moving liquid and the gel network during compression and decompression of the ultrasonic wave.[3] The ultrasonic velocity increases as a result of the increase in longitudinal storage modulus of the sample caused by the contribution of the gel network.[4] After five hours both ultrasonic parameters reach a plateau as the gel network formation is completed.

A more in-depth analysis of the magnitude of the changes in ultrasonic velocity and attenuation upon the sol–gel transition allows detailed characterisation of microscopic structure of proteins and their aggregates at various stages of the process.

Post-gelation (slow acidification)

The overall gel network remains stable, as shown by the constant value of the ultrasonic attenuation for 12 hours. A decrease in the ultrasonic velocity

with time occurs as a result of ongoing conformational and chemical changes within the gel network. This is in line with previous observations that long-term dynamics occur in these types of systems.[2]

Kinetics of aggregation and gelation in protein solution, fast acidification

The change in ultrasonic parameters with the kinetics of gelation for a fast rate of acidification are given in Figures 4 and 5. The process includes formation of a gel network, followed by temporary gel stability and finally the break up of the gel network.

Gelation (fast acidification)

Figures 4 and 5 show the kinetics of gelation for fast acidification in a 2% whey protein solution. An increase in the ultrasonic velocity with the formation of the gel network (increase in longitudinal storage modulus) is measured. This increase is also seen in the ultrasonic attenuation measurements, as a result of an increase in energy losses from scattering and absorption of the ultrasonic wave on the gel network. The formation of a pseudo-stable gel network is indicated by a plateau in both the ultrasonic velocity and attenuation measurements, after which the network begins to break up. Similar to the case of slow acidification, the gel shows a wavelength (frequency) dependence of ultrasonic velocity, thus again demonstrating a micro structural non-homogeneity of the sample in the gel state.

The collapse of the gel network (fast acidification)

The collapse of the network is shown by a decrease in both viscous losses (ultrasonic attenuation) and the storage modulus (ultrasonic velocity). We see from the ultrasonic attenuation that the gel network has completely collapsed by the 40-minute mark. The ultrasonic velocity continues to decrease at a constant rate after this time, suggesting that chemical or conformational changes are still occurring in the system.

The future

The current position of high-resolution ultrasonic spectroscopy in the field of materials analysis can be compared with the early days of ultrasound application in medicine. As the technology reached a certain level it generated an "explosion" in medical diagnostics through its ability to visualise internal parts of a patient's body, analyse blood streams and other features. Just as it is difficult to imagine a modern hospital without ultrasonic instruments so it will be soon impossible to contemplate an analytical laboratory without one. Modern high-resolution ultrasonic spectroscopy has the ability to perform a wide range of analyses that other methods cannot do, as well as increasing the effectiveness and reducing the cost of a number of analytical tasks currently done by traditional techniques.

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