

Super sonic

High-resolution ultrasonic spectroscopy

High-resolution ultrasonic spectrometry is a novel analytical technique with enormous potential for the investigation of a wide range of samples and dynamic processes. The non-destructive technique is based on measuring the changes that take place to ultrasonic waves as they pass through materials.

Although these effects have been known for some time, it was impossible to measure them with sufficient accuracy to produce usable data. Ultrasonic Scientific, based in Dublin, Ireland, has managed to find solutions to the problems, and has launched the world's first practical laboratory ultrasonic spectrometer, the HR-US 101 at the Pittcon show earlier this year.

The ultrasonic waves that are used in the spectrometer are the same as those used in hospital diagnosis and the screening of unborn babies. These are applications where the ability of the waves to pass through opaque media without causing damage is important. They can also pass through lab samples, including those that are impervious to the electromagnetic waves that are used in most forms of classical spectroscopy.

Two different parameters are measured by the spectrometer: the velocity and the attenuation of the waves. The velocity depends on two factors — density and elasticity. The second parameter is particularly important, as the elastic response of the sample to compressions and decompressions in ultrasonic waves is extremely sensitive to intermolecular interactions and molecular organization. Therefore, information about the properties of

the sample can be gleaned from the changes that are observed. However, an extremely high resolution is required for the measurements to be useful, and thus it has been impractical to use ultrasound as a laboratory technique.

Attenuation is a measure of the amount of energy that is lost by the wave as it passes through the sample. Important contributors to attenuation are ultrasonic scattering in non-heterogeneous samples, and rapid chemical relaxation. Being able to measure both the velocity and attenuation of the ultrasonic waves as they pass through a sample gives a great deal of information about both the chemical dynamics and the structure of a material.

Standard sample cells for the HR-US 101 have a capacity of 1 ml, and allow the sample inside to be stirred. The dimensions make them

easy to fill, refill, clean and sterilize; they also have screw caps to prevent the evaporation of samples. A range of special cells is also available, including cells with capacities as low as 0.03 ml, cells that allow non-liquid samples to be analysed, and cells that allow an extended frequency range of 0.1–20 MHz to be studied. The spectrometer is sufficiently sensitive to allow low concentration samples (typically about 1 mg/ml) to be analysed.

In some cases, it is even possible to study samples with concentrations in the $\mu\text{g/ml}$ range; concentrated samples do not pose a problem to this system.

Measurements are computer-controlled, and data can be output in graphical and digital formats, being compatible with Microsoft Excel and most standard data-manipulation software. Ultrasonic velocity can be studied at a resolution as fine as 0.00001%, and with an attenuation at 0.2%. Measurements can be made at temperatures between -20°C and 120°C .

The following applications

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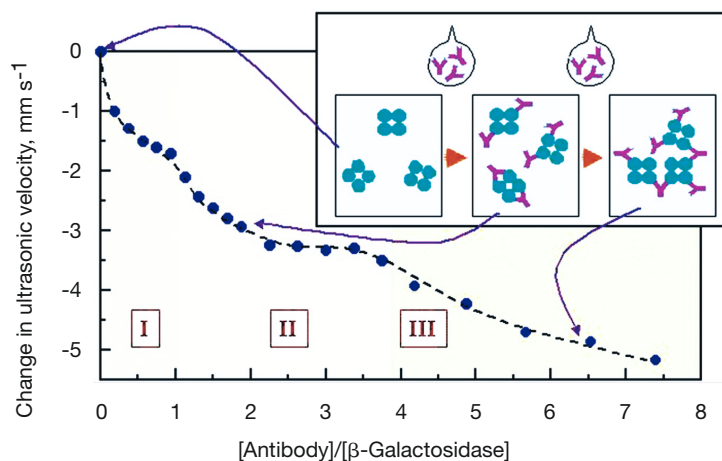


Figure 1. Use of the HR-US 101 to monitor antibody interaction with *E. coli* β -galactosidase.

of high-resolution ultrasonic spectroscopy for analysis of biochemical processes were generated at the Department of Chemistry, University College Dublin, using the HR-US 101.

A good example is in the monitoring of immunochemical reactions, such as the binding of an antibody to β -galactosidase isolated from *Escherichia coli*. This high-molecular-mass enzyme comprises four non-covalently linked subunits, and it hydrolyses lactose into glucose and galactose. The food industry uses this process to remove lactose from dairy products, and it is also used as a molecular sensor in techniques such as ELISA and Western blot immunoassays.

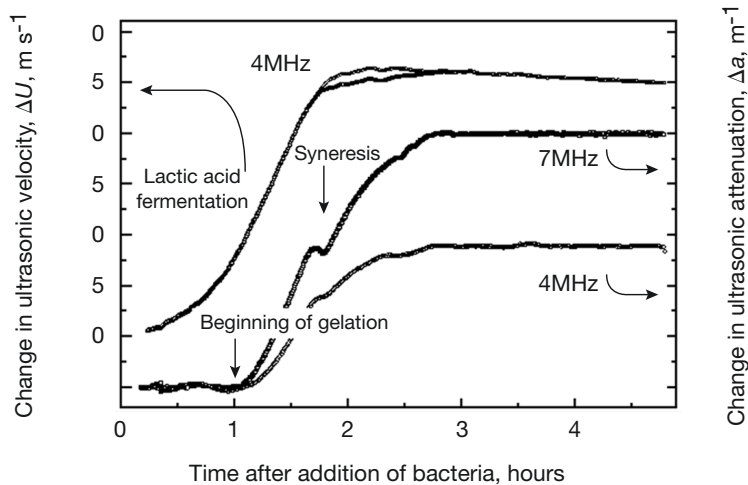
Most immunoassays use attached markers to monitor the antibody–antigen binding reaction, such as optically-active molecules or enzymes that produce coloured components to give a visual signal that a reaction has occurred. High-resolution ultrasonic spectroscopy allows the direct detection of this binding process, thus eliminating the need for markers.

In the example shown in Figure 1, 1- μ l doses of a commercially-available solution of a specific anti- β -galactosidase antibody were added step-wise to an ultrasonic cell containing 1 ml of a very dilute solution of the enzyme, and also into a reference cell containing 1 ml of buffer. The HR-US 101 measured the difference in ultrasonic parameters between the two samples as the antibody was added; the resulting plot is of the interaction between the antigen and the antibody only.

Several different stages of

Figure 2. Progression of milk fermentation by a *Lactobacillus* strain, as monitored with the HR-US 101 system.

Lactic Acid Fermentation



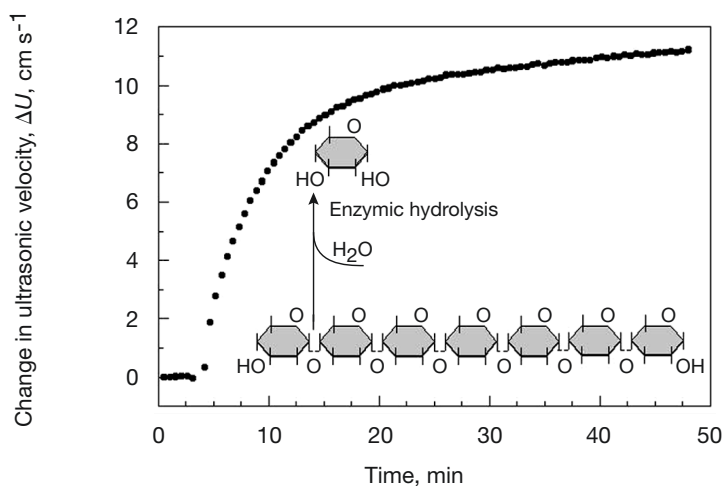
antigen–antibody binding can be observed from the graph, each of which corresponds to a different antigen–antibody complex. At a low antibody–enzyme ratio, in stage I, the antibody binds to all the available binding centres of the tetrameric enzyme, which leads to a decrease in the ultrasonic velocity as the proteins' elasticity increases and water of hydration is released from the binding site. In stage II, as the concentration of antibody rises, the antigen–antibody links redistribute to achieve maximum intramolecular contacts.

In the third stage, further addition of antibody results in the antigen–antibody complexes aggregating. This stage is

characterized by the slow kinetics of the structural organization of these aggregates, which is reflected in the fact that it takes approximately 1 hour for the ultrasonic parameters to reach a steady value. This is in sharp contrast with the first two stages, where the changes occur more at a higher rate, and are essentially dependent on the efficiency of the mixing process. The total decrease in ultrasonic velocity observed at each stage enables structural characterization of the complex to be made; both binding constants and stoichiometry can be calculated from the shape of the curve.

A second example is the

Figure 3. Hydrolysis of maltoheptaose by β -amylase, as monitored with the HR-US 101 system.



monitoring of enzymic activity of a *Lactobacillus* culture in milk. These bacteria are used as starter cultures in the manufacture of fermented food products such as cheese and yoghurt. The bacteria first produce the enzyme lactate dehydrogenase, which turns lactose from the milk into lactic acid. As the pH drops, the negatively charged amino groups on the casein (the main protein in milk) are neutralized, which causes colloidal calcium phosphate to be solubilized and hence destabilizes the inherent structure of the casein micelles within the milk. The destabilized micelles flocculate into clusters, and then link to form a particulate gel.

High-resolution ultrasonic spectroscopy can be used to monitor the pre-gelation and gelation processes as they occur. An ultrasonic cell is filled with milk, and the culture added. As shown in Figure 2, the entire fermentation process is complete within 3 hours. In the pre-gelation phase (during the first hour), the constant level of the ultrasonic attenuation indicates that no structural changes take place in the milk. The rise in ultrasonic velocity represents the production of lactic acid by the culture. However, after approximately 1 hour, the casein micelles begin to aggregate followed by the formation of the gel network, which leads to an increase in ultrasonic attenuation.

After 1.7 hours, the bacterial activity slows down, and the ultrasonic velocity levels off as a result. However, as can be seen from the ultrasonic attenuation measurements, the gel's structure continues to change until 2.5 hours have elapsed. At this

point, the velocity curves are dependent on the frequency, which is characteristic of non-homogeneity in the sample. After this time, the gel becomes homogeneous, as indicated by the velocity curves at different frequencies overlapping again.

A final example of the uses of the HR-US 101 system is in the study of the hydrolysis of maltoheptaose by β -amylase. A 5 μ g sample of enzyme was added to a 3.5 mM aqueous solution of the sugar, and the progress of the resulting reaction was studied by the continuous monitoring of changes in ultrasonic velocity. As the reaction proceeds, ultrasonic velocity increases because the hydration level of the product is higher than that of the starting substrate, as shown in Figure 3. It is simple to recalculate the ultrasonic curve to give the time dependence of the amount of substrate that has been hydrolysed, which provides the kinetic profile of the reaction, and allows the enzyme's activity to be calculated.

These three examples show the potential of ultrasonic

spectrometry in the monitoring of biochemical reactions. Indeed, the HR-US 101 was given the Silver Award for best new product at the Pittcon Editors Awards this year, in recognition of the potential of the technique. Its ability to monitor both structural and biochemical processes in real time in the same machine makes it a simple yet powerful new analytical technique.

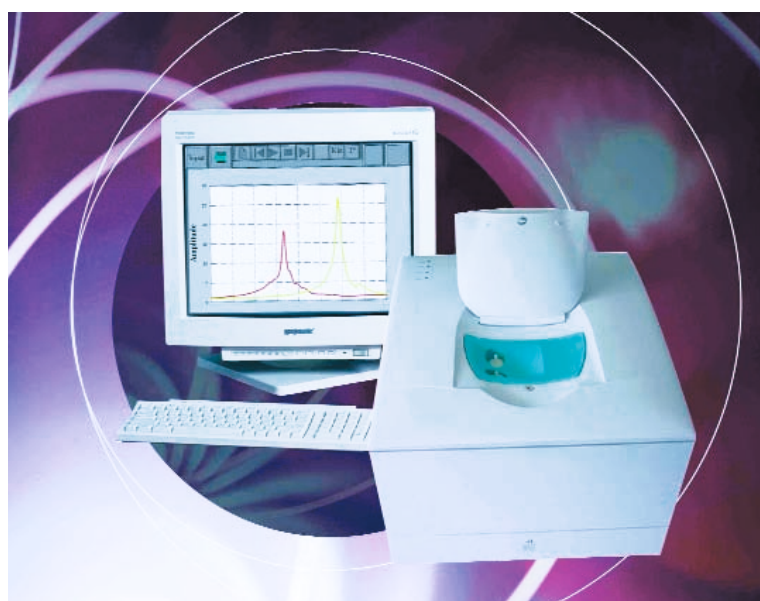
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The HR-US 101 from Ultrasonic Scientific