

High-Resolution Ultrasonic Spectroscopy: Analysis of Microemulsions

This article introduces the application of high-resolution ultrasonic spectroscopy for the analysis of emulsions and suspensions. The authors outline the principles of the technique and illustrate its application for analysis of the crystallization of lysozyme and the formation of a microemulsion.

Cormac Smyth, Breda O'Driscoll, Jayne Lawrence, Sinead Hickey, Therese O'Reagan and Vitaly Buckin

High-resolution ultrasonic spectroscopy (HR-US) is a novel approach for the analysis of materials that uses the important ability of ultrasound to probe systems non-invasively and report their constitution, microstructure, and intermolecular interactions. The analytical power of ultrasound is well known through its application in medicine, for example, in the screening of unborn babies, where in the ability of the waves to pass through opaque media without causing damage is particularly important.

However, the use of HR-US in analyzing a variety of other materials and solutions has only recently evolved to become an essential laboratory technique. This process is extremely sensitive to the molecular organization, is non-destructive, requires no markers, and can be used on non-transparent and concentrated samples.

Suspensions and Emulsions

Stable dispersions can be divided into two main groups: suspensions and emulsions. A suspension is a dispersion of fine particles of a solid within a liquid, the particles being the dispersed phase, whereas the suspending medium is the continuous phase. Apart from the mixing of an insoluble solid in a liquid, a suspension also can result from the aggregation of dissolved solids; crystallization is an example of such a process.

An emulsion is a dispersion of one liquid in another immiscible liquid, usually in the presence of stabilizer molecules. Emulsions can be either oil droplets dispersed in water or water droplets dispersed in oil, wherein the droplet diameters are in the micron range. Fat in milk is a familiar example of an oil-in-water emulsion. The droplets usually are formed by high-shear mechanical processes and stabilized against coalescence by electrostatic and/or steric barriers around the droplets provided by the stabilizers. Often, this



IMAGE COURTESY DIGITAL STOCK

colloidal dispersion is complex because the dispersed phase is partially solidified or because the continuous phase contains crystalline material (such as in ice cream).

Emulsions and suspensions have two things in common: first, they are thermodynamically unstable and second, because they mostly are optically opaque systems, they are difficult to study using optical analysis methods. Examples of pharmaceutical emulsions include lotions, liniments, creams, and vitamin drops. Except for the medicinal ingredients, the base vehicles in creams, ointments, and lotions are quite similar in formulation to cosmetic preparations.

It has been found that properly formulated pharmaceutical emulsions and dispersions provide more accurate control of the dosage. They also will furnish a method of combining many immiscible ingredients into a single, stable product, which improves the ease of application. The proper

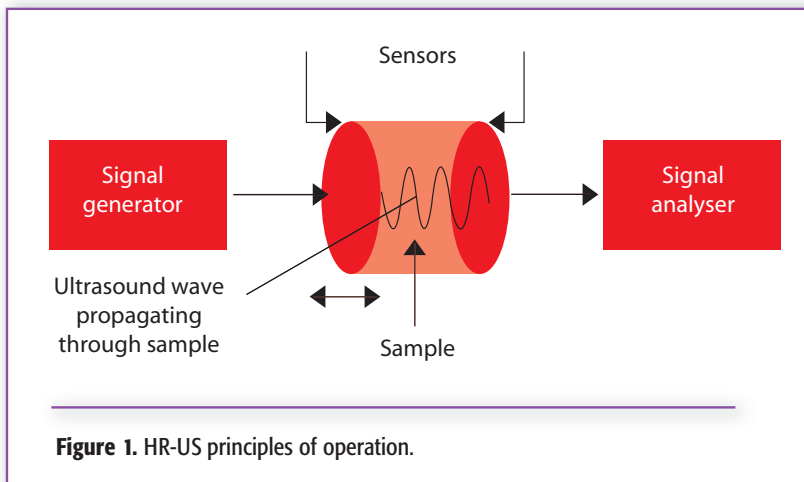


Figure 1. HR-US principles of operation.

formulation and control of particle size and stability bring about the controlled release of the active ingredients. Emulsions are difficult to characterize and their behavior is challenging to understand; however, their application is universal and they are widespread in nature.

Microemulsions are a special class of emulsions. In contrast to “normal” emulsions, they are thermodynamically stable. The droplet size in microemulsions is small (in the range of 10–100 nm) and they are transparent. Microemulsions have attracted consid-

erable interest during the years as potential drug delivery systems because of their unique properties, which include stability, ease of preparation, and their ability to form spontaneously (1).

Analysis of microstructure and intermolecular interactions in emulsions and suspensions is a challenging task. Many traditional analytical techniques require dilution to adjust the transparency of emulsion to an acceptable range. This often affects the weak balance of intermolecular forces in emulsion and its structure. (Microemulsions suffer from similar dilution problems

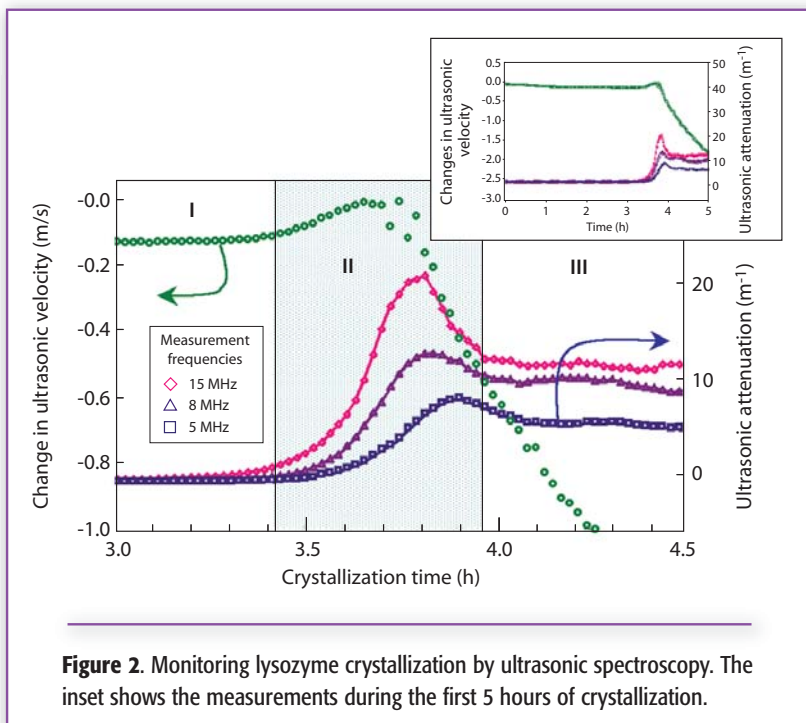


Figure 2. Monitoring lysozyme crystallization by ultrasonic spectroscopy. The inset shows the measurements during the first 5 hours of crystallization.

to emulsions, although in the case of a microemulsion formulated with a short-chain cosurfactant, dilution often leads to the total destruction of the system. Hence it is imperative that most microemulsions are measured in their concentrated state.) Another limitation is the effect of the measuring device on the structure of emulsions, which is common for rheological (viscometry

and dynamic rheology) and other measurements.

Crystallization of Proteins

Proteins are a class of macromolecules universally found in living creatures. Mutation of a protein or a combination of proteins working together can cause diseases such as bovine spongiform encephalopathy (BSE). Subsequently,

having a good understanding of proteins can help fight disease. To appreciate the function of a protein and how it performs this specific function, scientists need to understand its structure.

The method used to study the molecular structure of proteins is X-ray diffraction, in which the protein must be in a pure crystal form. However, because of the complex nature of proteins, and their sensitivity to their environmental conditions, it is difficult to produce good quality crystals. By running thousands of protein crystal screens that use different variables such as pH, salt concentration, and temperature, scientists can narrow down the conditions that are needed to produce high quality crystals. However, this can be a long and arduous process taking months or years. The use of a real-time analytical method that can follow the crystallization reaction can speed up this process.

A Possible Approach

HR-US is a novel technique for non-destructive material analysis based upon precision measurements of parameters of high-frequency sound waves propagating through analyzed samples (Figure 1). These waves propagate through most materials, including opaque samples, and allow direct probing of intermolecular forces. HR ultrasonic spectrometers provide an unprecedented range of new analytical capabilities for research, product development, quality and process control in the biotech, pharmaceutical, food, chemical and petrochemical, and polymer industries. This rapid, inexpensive and non-destructive technique previously has established itself as a powerful tool for

- analysis of chemical reactions;
- conformational transitions in polymers and biopolymers;
- aggregation and gelation phenomena;
- particle sizing;
- phase transitions;
- stability of emulsions and suspensions;
- formation of micelles and critical micellization concentration

- measurements;
- ligand binding; and
- composition analysis.

High-resolution ultrasonic spectrometers can be used for characterization of emulsions and suspensions, and the evaluation of their stability and structure (particle size measurements). The instruments measure two parameters, ultrasonic attenuation and velocity,

elasticity of the medium determine ultrasonic velocity. A major advantage of the ultrasonic technique is the ability to make measurements directly, in the original emulsion, without having to make dilutions. The following examples illustrate the application of the technology for analysis of the crystallization process and the formation of a microemulsion.

THE ANALYTICAL POWER OF ULTRASOUND IS WELL KNOWN THROUGH ITS APPLICATION IN MEDICINE, WHEREIN THE ABILITY OF THE WAVES TO PASS THROUGH OPAQUE MEDIA WITHOUT CAUSING DAMAGE IS PARTICULARLY IMPORTANT.

which are physically independent, allowing the probing of different levels of the organization of the samples to be characterized.

Attenuation is determined mainly by the scattering of ultrasonic waves in non-homogeneous samples (emulsions and dispersions) and fast chemical relaxation (in homogeneous mixtures). The density and the

Crystallization of Lysozyme

Lysozyme protects the human body from bacterial infection by attacking bacterial cell walls, causing them to rupture. By varying the experimental conditions, different amounts and sizes of protein crystals can be produced. The amount of crystals as well as an assessment of their sizes and interactions is an important part of routine analysis in

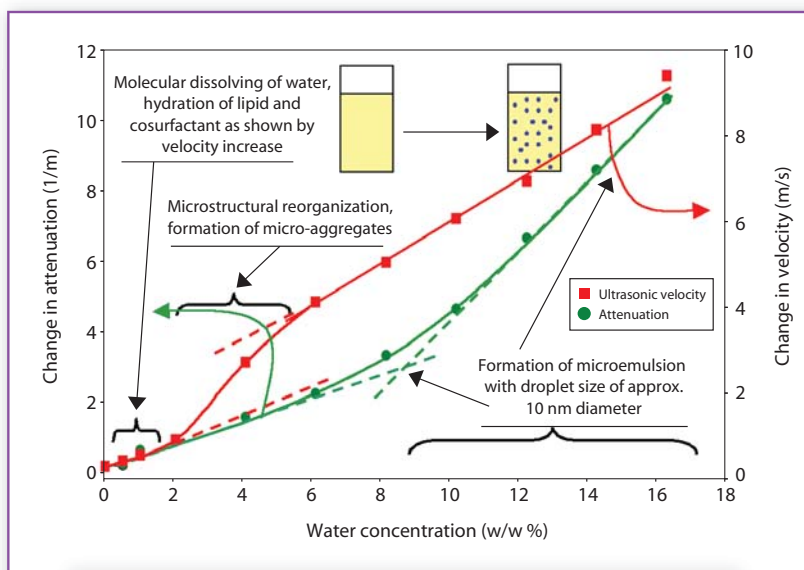


Figure 3. Ultrasonic analysis of microstructural rearrangements and formation of microemulsion.

the pharmaceutical industry. HR-US spectrometers allow measurement as the crystal grows with time and temperature and assessment of the amount of crystals in a sample. Monitoring of crystal formation, in particular the kinetics of the reaction, is essential for the optimization of process control in the batch crystallization of pharmaceutical compounds.

Crystallization of lysozyme measured by HR-US is shown in Figure 2. In this measurement, 1 mL of a solution of lysozyme (40 mg/mL) in 0.1 M sodium acetate (pH 4.8) was placed into the ultrasonic cell. A precipitating agent was added to the sample and the kinetics of crystallization was followed. The spectrometer detects three stages in the crystallization process. During

the first 3.4 h of the reaction, no significant change is detected. At the end of stage I the ultrasonic velocity and attenuation start to increase because of the formation of crystals.

This increase continues through stage II as the concentration and size of the crystals grow. The increase in ultrasonic velocity is caused by the increase in rigidity (decrease in compressibility) of the sample as a result of crystal formation. The rise in ultrasonic attenuation can be attributed to the scattering of the ultrasonic wave on the solid crystals that are formed. The scattering contribution is dependent upon the ratio of crystal size and frequency (for example, the attenuation at the higher frequencies is more sensitive to the formation of small particles). Therefore, multi-frequency ultrasonic attenuation measurements allow analysis of the crystal structure and size. During stage II the ultrasonic velocity starts to decrease; in stage III the ultrasonic attenuation nearly levels off, indicating the end of crystal formation in the micron range size. However, the velocity continues to decrease, as a consequence of the sediment of a small percentage of large crystals from the system.

Ultrasonic Analysis of Microemulsions

This example describes the application of the technology for analysis of the formation of a microemulsion in a system consisting of pharmaceutically accepted components (2, 3), namely isopropyl myristate, lecithin (*Epikuron 200*), n-propanol as a cosurfactant in a w/w ratio 6:1:1 and water (4). The measurements were performed at 20 °C using an HR-US spectrometer equipped with a standard 1 mL cell in the frequency range of 2–20 MHz.

Figure 3 shows the typical dependence of ultrasonic velocity and attenuation on the concentration of water in the system (at 5 MHz). Molecular dissolving of water in the mixture at low concentrations (up to 2 wt%), which is accompanied by hydration of lipid and cosurfactant, is shown by the steady increase in ultrasonic

velocity. Following this, a microstructural reorganization takes place, indicated by an additional increase in ultrasonic velocity in the range of 2–6% water concentration. Formation of the microemulsion can be seen from approximately 8 wt% with ultrasonic attenuation quickly increasing, which is caused by the scattering of the ultrasonic wave on the particles. It should be noted that when compared with light scattering, good agreement above the break point was found whereas below the break point, the possible structuring of the system is not observable with the light scattering. This shows the potential of the technique in providing information previously unattainable by existing methods of analysis.

This article originally appeared in *Pharmaceutical Technology Europe* (June 2004).

References

1. M.J. Lawrence and G.D. Rees, "Microemulsion-Based Media as Novel Drug Delivery Systems," *Adv. Drug Deliv. Rev.* **45**(1), 89–121 (2000).
2. R. Aboofazeli and M.J. Lawrence, "Investigations into the Formation and Characterization of Phospholipid Microemulsions. I. Pseudo-Ternary Phase Diagrams of Systems Containing Water-Lecithin-Alcohol-Isopropyl Myristate," *Int. J. Pharm.* **93**(1–3), 161–175 (1993).
3. www.aapspharmsci.org/view.asp?art=ps020319
4. K. Shinoda et al., "Lecithin-Based Microemulsions: Phase Behavior and Microstructure," *J. Phys. Chem.* **95**(19), 989–993 (1991). ■



Cormac Smyth is a product manager and **Breda O'Driscoll** is managing director at Ultrasonic Scientific Ltd. (Dublin, Ireland). E-mail: info@ultrasonic-scientific.com.

Jayne Lawrence is with the department of pharmacy at Kings College (London, U.K.).

Sinead Hickey, Therese O'Reagan and **Vitaly Buckin** are with the department of chemistry at University College (Dublin, Ireland).

