

HIGH-RESOLUTION ULTRASONIC SPECTROSCOPY FOR DETERMINATION OF EMULSION STABILITY, SEDIMENTATION AND PARTICLE SIZING IN BIOPHARMACUETICAL APPLICATIONS



No optical transparency required

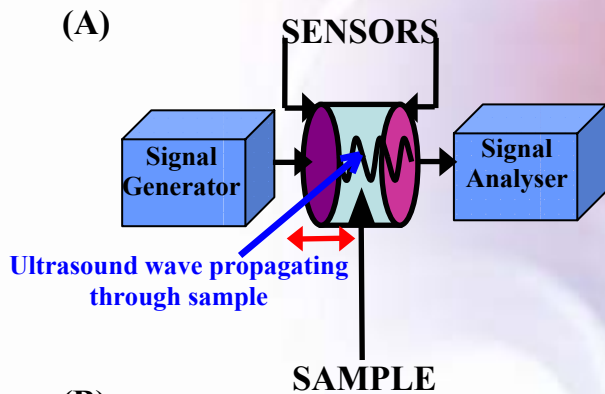
High-Resolution Ultrasonic Spectroscopy is a novel technique for non-destructive material analysis based on precision measurements of parameters of high-frequency sound waves, propagating through analysed samples. These waves propagate through most materials including opaque samples and allow direct probing of intermolecular forces. Award winning HR-US ultrasonic spectrometers from Ultrasonic Scientific provide an unprecedented range of new analytical capabilities for research, product development, quality and process control in biotech, pharmaceutical, food, chemical and petrochemical, polymer and other industries. Applications of this technique include 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 CMC measurements, ligand binding, composition analysis and many others.

major advantage of the ultrasonic technique is the ability to make measurements directly, in the original emulsion, without having to make dilutions. The preparation of serial dilutions is commonly required when using optical techniques to reach the optical transparency and avoid multiple scattering effects..

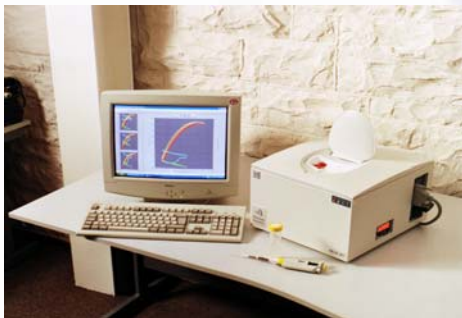
The following examples illustrate the application of the HR-US 102 spectrometer for the analysis of heat-stability and sedimentation of emulsions and suspensions and the determination of particle size in emulsions.

Fig. 1

- (A) Principles of operation
- (B) Dual award-winning HR-US 102 spectrometer



(B)

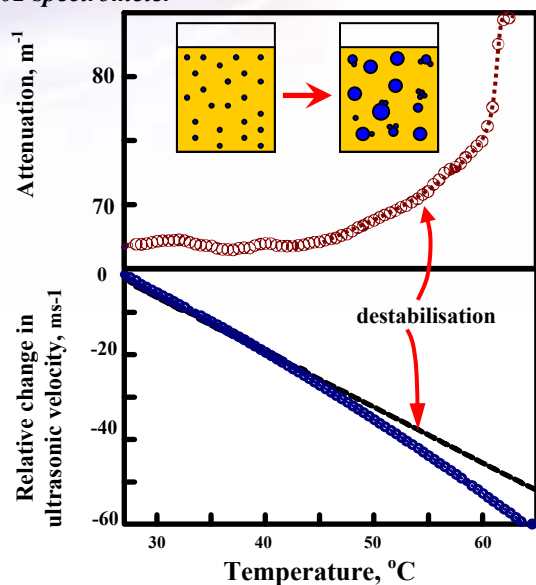


This publication describes the application of HR-US series of high-resolution ultrasonic spectrometers for characterisation of emulsions, evaluation of their stability and structure (particle size measurements). HR-US high-resolution ultrasonic spectrometers measure two parameters, ultrasonic attenuation and velocity. The parameters are physically independent, allowing to probe the different levels of organization of the samples to be characterized. Attenuation is mainly determined by the scattering of ultrasonic waves in non-homogenous samples (emulsions, dispersions) and fast chemical relaxation (in homogenous mixtures). The density and the elasticity of the medium determine ultrasonic velocity. A

Ultrasonic determination of heat stability of emulsions

Stability of emulsion (including the composition and microstructure) is a key element for the determination of the lifetime, temperatures and other parameters for manufacturing, handling, storage and the use of the product. HR-US spectrometers allow simple procedures for the evaluation of the stability of emulsions. Figure 2 shows the analysis the thermal stability of a water/oil emulsion (0.7µm). The analysis was made using temperature ramp mode of HR-US 102, which allows gradual heating (and cooling) of the sample with a desired heating (and cooling) rate. The changes in the attenuation and relative ultrasonic velocity with temperature were measured. The arrows in Figure 2 indicate the temperature corresponding to the destabilisation of the sample (temperature measurements were taken at various frequencies in the range 2-15 MHz and data for 6MHz are given in the Figure). The rise in the attenuation at 45°C provides clear evidence of the changes in the microstructure of the emulsion. The increase in the attenuation can be attributed to the flocculation of dispersed aqueous droplets induced by heating. As seen in Figure 2, the change in ultrasonic velocity deviates from the baseline at the same temperature at which the ultrasonic attenuation begins to rise. At temperatures above 65°C an irreversible changes in the emulsion takes place followed by phase separation.

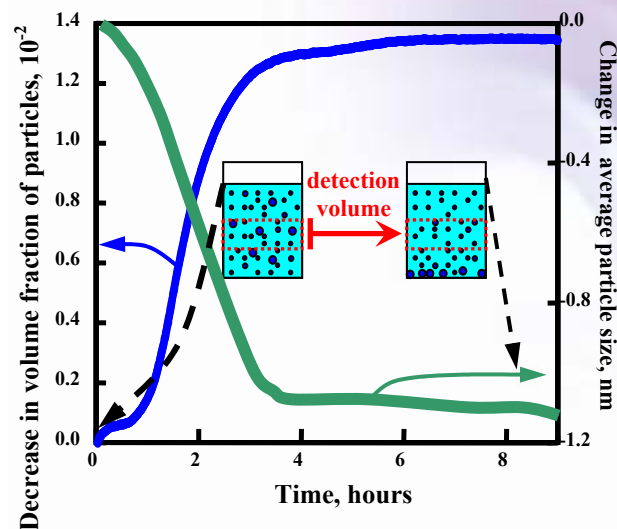
Fig. 2 Characterisation of heat stability of an emulsion using HR-US 102 spectrometer



Ultrasonic monitoring of sedimentation in emulsions and suspensions

Figure 3 illustrates the ultrasonic monitoring of sedimentation in a perfluorocarbon emulsion. Perfluorocarbon liquids are well known for their high capacity to solubilise gases such as oxygen and carbon dioxide and therefore have applications as synthetic blood substitutes. Commercially available blood substitutes are typically supplied as concentrated perfluorocarbon emulsions in water. The functional properties of the emulsion are determined by the stability of perfluorocarbon droplets. Perfectly suspended emulsion generally consists of small particles (approximately 100nm). The droplet size of these emulsions increases with storage time. In particular, the fast precipitation of perfluorocarbon droplets is related to the growth of droplet size because of the higher density of the perfluorocarbons compared with water. This affects the functional properties of the emulsion. HR-US 102 spectrometers enable the direct detection of the changes in the structure of the emulsion, allowing the efficiency or usefulness of emulsion to be assessed. 1 ml of a commercial emulsion of perfluorocarbons in water (10% v/v) was agitated and loaded into the ultrasonic cell. The precipitation of large particles resulted in a shift of particle distribution across the cell, which was monitored by measuring ultrasonic parameters in the middle of the cell (that is, the area of the ultrasonic beam). The measurements were performed simultaneously at various frequencies in the frequency range (2-15 MHz). The ultrasonic velocities were used to calculate the emulsion concentration in the beam (volume fraction) and the ultrasonic attenuation was used to calculate the average particle size of perfluorocarbon droplets. The results shown in Figure 3 enable the ageing of the emulsion to be directly evaluated.

Fig. 3 Ultrasonic monitoring of the sedimentation in a perfluorocarbon emulsion



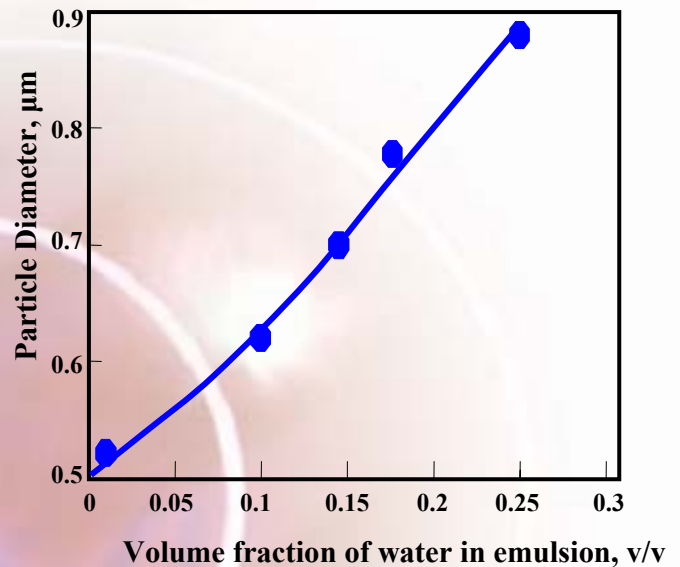
Determination of particle size in dilute and concentrated emulsions

One of the key elements of emulsion stability is the particle size of the dispersed phase. Batch-to-batch variation in particle size can lead to unpredictable variations in the life span and stability (shelf life/heat stability) of the product. Traditionally the measurement of the droplet size in an emulsion is made using optical methods. This means that the sample must be diluted to reach optical transparency and avoid multiple scattering effects. Figure 4 shows the application of the HR-US 102 spectrometer to measure the particle

size in a concentrated (undiluted) water-in-oil emulsion and its change in the course of dilution. Using the HR-US 102, the size of the water droplets in the original products was measured as 0.9 μ m. As the product is diluted stepwise to a lower concentration where optical measurements can be performed (1% v/v water-in-oil), the droplet size decreases in accordance with the ultrasonic measurements. Effectively, the particles are drying out in the course of dilution.

This example demonstrates the capacity of the HR-US 102 high-resolution ultrasonic spectrometer to measure size of particles in optically opaque products in their original form and the change in particle in size in different batches without sample dilution.

Fig. 4 Ultrasonic measurements for determination of particle size of emulsions



Conclusion

These results demonstrate the power HR-US high-resolution spectrometers in characterisation of emulsions. The measurements can be performed in small samples (typically 1 ml, lower and higher volume of sample compartments available), are done under well-controlled temperature conditions (down to 0.01 $^{\circ}$ C).

For more information on our products and their application visit our web site:

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This technology is subject to protection by granted patents and pending patent applications.