Ultrasonic Scientific

DIRECT MONITORING OF BIOCHEMICAL REACTIONS AND DETERMINATION OF ENZYME ACTIVITY USING HIGH-RESOLUTION ULTRASONIC SPECTROSCOPY

No optical markers or optical transparency required

High-Resolution Ultrasonic Spectroscopy is a novel technique for 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 101 and 102 ultrasonic spectrometers from Ultrasonic Scientific Ltd. 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.

Fig. 1 Principles of operation

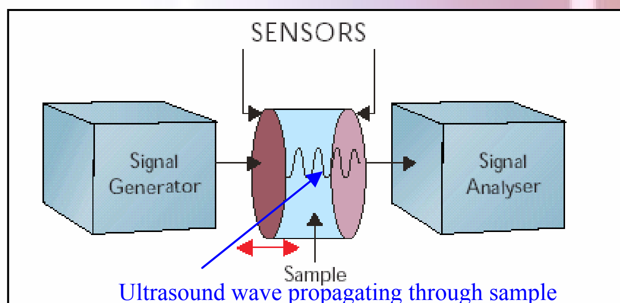
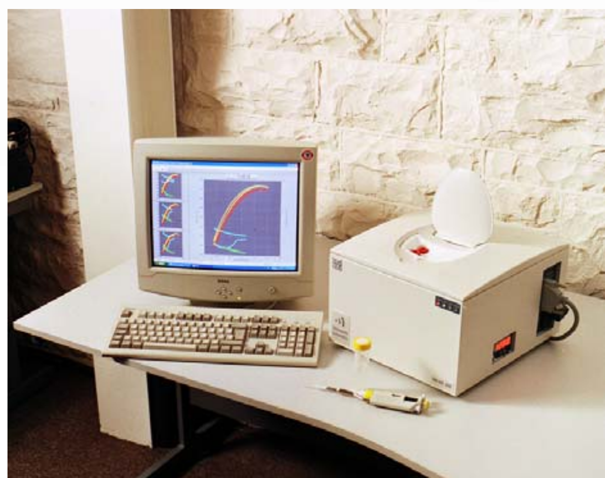


Fig. 2 Dual award-winning HR-US 102 Spectrometer



This publication describes some examples of the application of high-resolution ultrasonic spectroscopy for monitoring of biochemical reactions and determination of enzyme activity. The technique provides the capability to study enzyme kinetics, which is of key importance in the control of biological processes, the development of new and improved products and process optimisation for biotech, pharmaceutical, food and other industries.

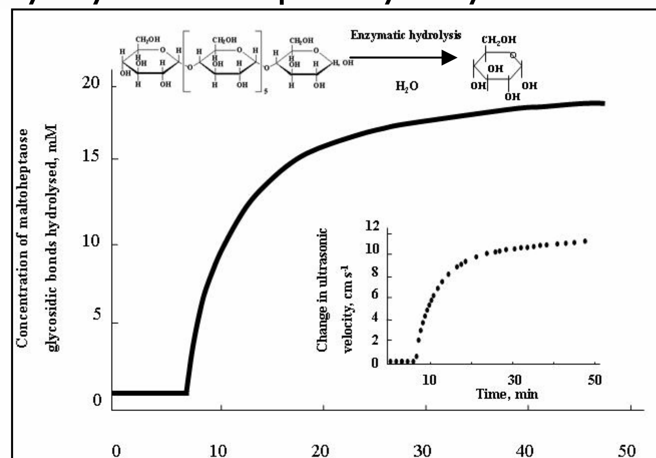
The most common method for detecting enzymatic reactions is based on standard UV / visible and fluorescence spectrophotometric assays. However, this requires optical activity of the reactants or products and in the absence of these, makes the analysis more complicated, as optical markers are needed.

The HR-US family of high-resolution ultrasonic spectrometers allows direct detection of an enzymatic reaction and determination of enzyme activity by monitoring the change in concentration of the substrate and product. This technique is non-destructive, requires no markers and can be used in non-transparent samples, such as tomato juice, milk etc.

Ultrasonic monitoring of α-amylase hydrolysis of maltoheptaose

α-amylase is an important industrial enzyme which hydrolyses starch, glycogen, and related polysaccharides by randomly cleaving internal α-1,4-glucosidic linkages. It is used, for example as an additive in detergents, for removal of starch sizing from textiles and proper formation of dextrin in baking. The figure below illustrates the ultrasonic (HR-US 102) monitoring of enzyme activity of α-amylase at 25°C through the measurements of changes in ultrasonic velocity during the course of a hydrolysis of 3.5 mM maltoheptaose solution (0.02 M phosphate buffer, pH 6.9) by the enzyme. 5μL of a 1 mg/ml amylase solution in the same buffer was added to 1ml ultrasonic cell filled with maltoheptaose solution at time eight minutes.

Fig. 3 Ultrasonic monitoring of enzyme hydrolysis of maltoheptaose by α-amylase

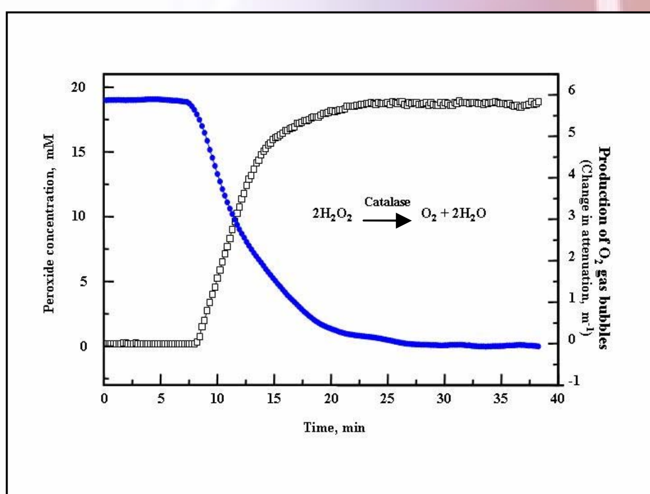


The amylase-maltoheptaose hydrolysis causes an increase in ultrasonic velocity, as the hydration level of the hydrolysis product is higher than that of the maltoheptaose substrate. The ultrasonic velocity curve (insert) was recalculated into the time dependence of the amount of substrate hydrolysed, i.e. the kinetic profile of the reaction. The enzyme activity calculated from this curve is 700 units/mg amylase (1 unit is defined as the amount of enzyme activity which will catalyse the transformation of 1 micromole of the substrate per minute).

Ultrasonic determination of enzyme activity of catalase in a peroxide hydrolysis

Catalase is an enzyme present in almost all animal cells and organs, and in anaerobic micro-organisms. Using H_2O_2 as a substrate to produce H_2O and O_2, catalase is of commercial interest wherever hydrogen peroxide is used as a germicide. It has applications in the food industry, in pasteurizing milk prior to cheese-making, as well as for micro-encapsulation. The figure below illustrates the ultrasonic (HR-US 102) monitoring of enzyme activity of catalase during the hydrolysis at 25°C of a 19 mM peroxide solution (0.05 M phosphate buffer, pH 7) by the enzyme. 1μL of 40 mg/ml catalase solution in the same buffer was added to 1 ml ultrasonic cell filled with the peroxide solution at time 7.5 mins.

Fig. 4 Ultrasonic monitoring of enzyme hydrolysis of peroxide by catalase



The hydrolysis results in both a change in ultrasonic velocity and attenuation. The change in velocity is directly related to the change in chemical composition of the solution, allowing the concentration of peroxide during the enzymatic reaction to be calculated. The figure shows the kinetic profile of the reaction calculated from the velocity curve. The calculated enzyme activity of catalase is 2700 units/mg catalase (1 unit is defined as the amount of enzyme activity which will catalyse the transformation of 1 micromole of the substrate per minute). In addition to the reaction, the kinetic profile of the production of O_2 gas bubbles in the solution can be monitored with attenuation measurements. The increase in attenuation is caused by the absorption and scattering of the ultrasonic wave by O_2 gas bubbles, formed upon decomposition of peroxide.

Conclusion

HR-US 101 and 102 ultrasonic spectrometers provide a universal capability for analysis of kinetics of enzymatic reactions. They do not require any optical activity and transparency of the sample and allow direct monitoring of the changes in the concentrations of the substrates and products. The measurements can be performed at low and high concentrations and volumes 0.03mL and higher.

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