AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

High Resolution Ultrasonic Spectroscopy

Kalyankar Tukaram*[1],Doiphode Nageshwar[1],Shailesh Wader[1].

1.School of Pharmacy,S.R.T.M.University Nanded 431 606 Maharashtra,India.

ABSTRACT

High-resolution ultrasonic spectroscopy (HR-US) is an analytical technique based on the principle of measurement of velocity and attenuation of ultrasonic compression waves propagating through the analyzed sample. HR-US is advantageous over the other spectroscopic technique.This review directs toward the wide applications and recent advances in high-resolution ultrasonic spectroscopy. Ultrasonic velocity and ultrasonic attenuation are the key parameter in the material analysis, microscopic particle size determination in suspension, emulsion and in the analysis of other biochemical products. HR-US spectroscopy in combination with liquid chromatography system has great importance in the analysis of colloidal systems. Recently the HR-US system is used as a detector in titration technique to analyze the sample on the basis of their intermolecular interaction.

Keywords: Ultrasonic Spectroscopy, Optical transparency, ultrasonic attenuation and ultrasonic velocity.

*Corresponding Author Email: dr.kalyankartm@gmail.com

Received 11 January 2013, Accepted19 January 2013

Please cite this article in press as Tukaram K. *et al.*, High Resolution Ultrasonic Spectroscopy. American Journal of PharmTech Research 2013.

INTRODUCTION

The high resolution ultrasonic spectroscopy is widely usedin material analysisareas.The measurement of velocity and attenuation of 'sound' waves at high, ultrasonic frequencies propagating through any material is the basis of this spectroscopic technique. As ultrasonic waves have the capacity to transmit through opaque medium, there will be no requirement of opticaltransparency. Thus it results in making the technique irrelevant for non-transparent as well as transparent samples. In all cases, this method is widely used for analysis of all types of micro and macro properties materials.

HR- US is a powerful technique that enables outstanding resolution (down to 10 -5 %) and fast, high-resolution analysis of small sample volumes. This technique avoids contamination, resupression and biodegradation of the sample. But this method does not use for analysis of aggressive (and sticky/problematic) samples. In addition, HR-US eliminates evaporation of the samples, allows extensive data analysis, and reduces laboratory effort as a result of fasting, digitally controlled analysis.

The analytical power of ultrasound is well known through its application in medicine, for example, in the screening of unborn babies, wherein 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 been recently evolved to become an essential laboratory technique. This process is extremely sensitive to the molecular organization, non-destructive in nature, requires no markers, and can be used on non-transparent and concentrated samples.[1]

Principle:

The basic principle involved in high resolution ultrasonic spectroscopy is the measurement of ultrasonic attenuation and ultrasonicvelocity. Energy losses in ultrasonic waves are the way to determine the Ultrasonic attenuation which is simplyabsorption and scattering contributions. Longitudinal loss modulus and viscosity of the medium are the term used to describe the attenuation. It have ability of analysis of mechanics offast chemical reactions, including the size and shape of particles, coagulation of material, gelatin, microstructure of materials recrystallization and other processes and properties. Density and the elastic response of the sample are used to determine the Ultrasonic velocity,Compressibility. Ultrasonic spectroscopy can be used to analyze particle sizes between 10nm to 1000mm and is suitable for application to concentrated systems (often up to 50%wt). [1,2]

Advantages:

Ultrasonic spectroscopy has many advantages over other existing particle-sizing technologies.

1. Non-destructive in nature

2.Noninvasive in nature

3. Capable of rapid measurements

4.Used to characterize systems which are concentrated and optically opaque

5.No sample preparation. Such as dilution, filtration

6. There are no moving parts.

7. Minimum calibration.

8. The ability to broad range of sample from diluted solution to semi solid materials

9. The analysis of bulk material is done without dilution.

10.Fast measurement for flow through analysis.[1,2]

Disadvantages:

This technique is not suitable for analyzing dilute suspensions i.e. withparticle concentrations <1%wt.[3]

Instrumentation:

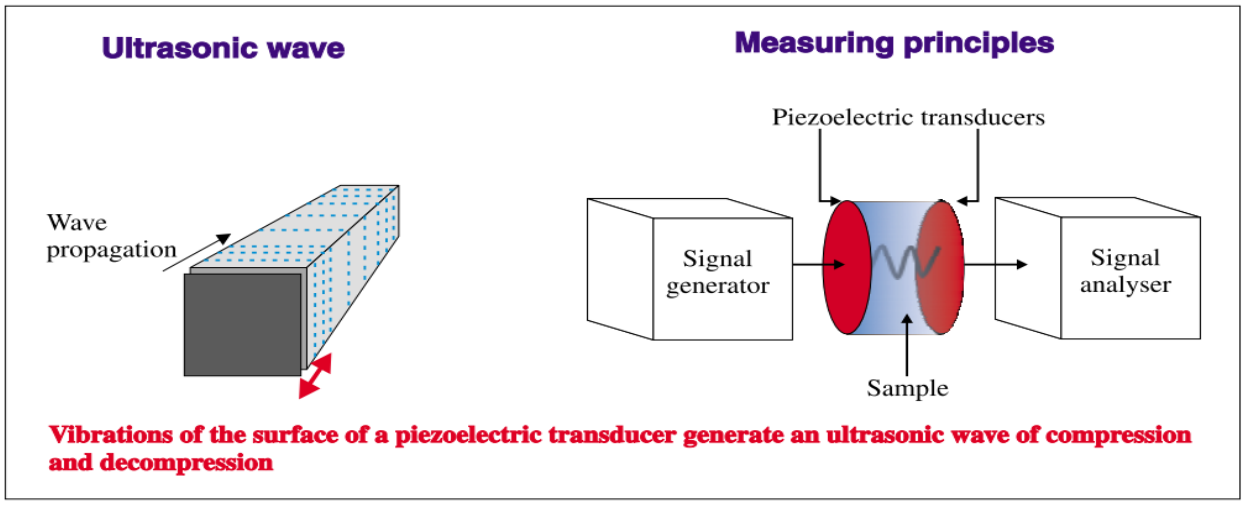

Figure I. Schematic diagram for high-resolution ultra-sound spectrometer.[5]

A schematic instrumentation for high-resolution spectroscopy is illustrated in Figure I. Ultrasonic spectroscopy measures the change in velocity and attenuation of the ultrasonic wave formed by the interaction of sound with the components of the sample. Most frequently, sound attenuation is measured, since this is simple and not temperature sensitive. Attenuation and velocity are sensitive to changes in intermolecular interactions and molecular organization. Most commonly, the measurement is made in a cuvette with a volume of 1 ml typical application of

attenuation measurements include the kinetics of fast reactions and particle sizing in emulsions and suspensions. 2–6 in contrast, velocity changes are a measure of the micro-elasticity of the analytes[4,5].

A sample cell is formed between two piezoelectric transducers. One of the transducers is the driver and the other is the receiver, which measures the intensity and velocity of the signal that has traversed to the sample cell. In the cell, the sample can absorb sound and also change its velocity. A typical ultrasonic spectrometer consists of a signal transmitter, a signal analyzer and a measurement cell.

High-resolution ultrasonic spectroscopy (HR-US) is an analytical technique based upon precision measurements of parameters (velocity and attenuation) of ultrasonic compression waves propagating through the analyzed sample. According to the theory of high resolution spectroscopy signals which are transferred are generated from piezotransducerto the waves. Second piezotransducer is doing another function of conversion of ultrasonic waves to the signals which are required for analysis. The pulse technique had various functions in the analysis of materials. Whenthe procedure is started then the certain amount of frequency is transformed to the sample and received oppositely as well as after the reflection from the wall of the container, back to the source of ultrasound. The size of the sample is going to decrease as compared to the path length. The use of modern advances in ultrasonic design, electronics and digital processing allow the attainment of ultrasonic measurements with much greater resolution (bellow to 10 -5 % for ultrasonicvelocity) in a broad range of the sample volumes, down to a single droplet[6,7].

Benefits of the Ultrasonic Analysis:

1. Ultrasonic transparency allowing the analysis of all types of materials. E.g. pharmaceuticals, agriculture, environmental control, medicine, chemistry, physics and biotechnology.
2. Modern ultrasonic cells do not have sharp corners and can accommodate even strong acids or organic solvents such aggressive liquids can be analyzed without evaporation in the of measurement sizes range up to 4ml.
3. As this technique is fully automated and final analytical report is in soft as well as hard copy. The digitalization of result is possible.
4. The recent advances in the Ultrasonic Scientific HR-US allows to control the temperature of the system, stable to words heat, transition of phase, etc..
5. As the sample requirement is small concentration, down to 0.3ppm (0.3µg/ml), the safety and efficacy of the system is high[7, 8].

HR-US 101 AS LC Detector:

HR-US 101 can be fitted with a flow –through cell, which is connected to an LC column as shown in Figure II. The kinetic regime of HR-US 101 allows continuous monitoring of ultrasonic parameters in the eluted liquid[7].

Table 1. Separation of low molecular weight compounds [7].

Sample injected	20 micro liter of mixture (acetonitrile,nitrobenzene,toulene one volume part of each)
Column	C 18 ODS 5 micro liter, 4.6mm-15 cm
Mobile phase	Methanol/Water (70/30 v/v)
Flow rate	0.8 ml/min-Beckman 128 module
Detection	HR –US 101 fitted with 30 micro liter flow through the cell

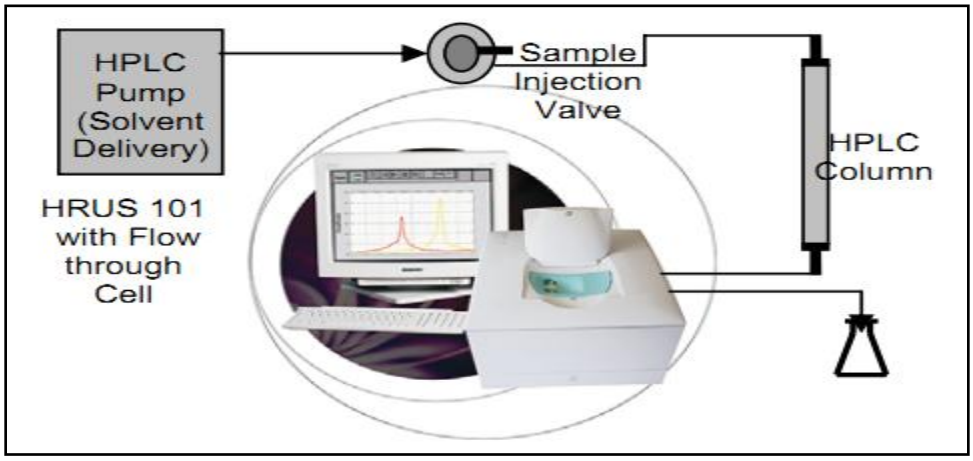

Figure II. HR-US 101 LC detector[7].

APPLICATIONS:

Application in analysis of materials

The measurements of various parameters of ultrasonic waves transferringthrough analyzing samples is the background for analysis of the matter. This provides information on the interaction of ultrasonic waves with the sample's interior, thus allowing analysis of its biological chemical and physical properties. The analytical power of ultrasound is well known for its application in medicine,i.e., the ability of ultrasonic waves to propagatethrough opaque biological tissues is used for visualizing the internal parts of a patient's body and analyzing the bloodstream etc.

Despite this and other successful applications ofultrasound in certainfields of material analysis, thetechnique has not been widely used in research, analytical, and process control laboratories as a routinemethod for material analysis. The major factors thatlimited its applicability were the low resolution ofthe measurements, large sample volumes, and oftencomplicated measuring

procedures. The modern technology employed in the HR-US101 high-resolution spectrometer (Ultrasonic Scientific Ltd., Dublin, Ireland) allows it to overcome theselimitations, and ultrasonic instruments are now commercially available for a wide spectrum of analyticallaboratories in the pharmaceuticals, biotechnological, nanotechnological and physico-chemical science. The device requires a small volume ofsample (typically 1 mL and down to 0.03 mL custommade), and performs analyses with resolution downto 10–5% for ultrasonic velocity. The spectrometer was used in the laboratory of physical Chemistry of Biocolloids at the Department of Chemistry UniversityCollege Dublin to analyze polymer–ligand binding, aggregation in suspensions and emulsion, formation of particle and polymer gels, micellization, andadsorbed on particle surfaces [6,8].

Liquid chromatographic detection.

The HR-US play lot role in liquid chromatography detection and demonstrates that it can potentially be used as a universal LC detector. The technique provides simultaneous measurements of ultrasonic velocity and ultrasonic attenuation at pre-selected frequencies in the flow-through regime, where the ultrasonic cell is directly attached to the liquid chromatography column. The resolution of ultrasonic measurements was 0.2 ppm for ultrasonic velocity and 200 ppm for ultrasonic attenuation. The frequency range of the instrument was 1-20 MHz This novel technique allows the identification of components and measurements of their concentrations in the output of the chromatography column. In the case of colloidal systems the size of particles can be evaluated. The outstanding combination of high resolution in the concentration measurements and high dynamic range allows the application of the ultrasonic technique to a broad variety of systems and concentrations analyzed by liquid chromatography including organic and inorganic low molecular weight components, polymers and others. No optical transparency is required for these measurements.

Parameters measured by HR US in liquid chromatography

a) Ultrasonic velocity

b) Ultrasonic attenuation

Ultrasonic velocity determines the ‘density’ and compressibility of the liquid. While ultrasonic attenuation allows ‘determination of particle size in suspension’ and ‘molecular mass in polymer solution’[7, 8, 9].

Analysis of Microemulsion, suspension and determination of CMC:

Analysis of the 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 to emulsions, although in the case of microemulsions formulated with a short-chain co-surfactant, 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 to emulsions, although in the case of a microemulsions formulated with a short-chain co surfactant, 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[10].

The concentration at which the micelles begin to form is called the CMC. Above the CMC, the concentration of single dispersed surfactant molecules are virtually constant and the rest of the surfactant molecules are in a micelle state. Development of various products involves a vital role of CMC. The experiment is performed by adding aliquots of surfactant into the cuvette and measuring the velocity change. Prior to the CMC, the velocity increase per mass of surfactant does not change, producing the short plateau at low concentration in the left panels. The constant value of the increment of ultrasonic velocity below CMC determines the vacancy of interactions between the surfactant molecules in solution. The sharp decrease in ultrasonic velocity above the CMC is caused by the higher compressibility of the hydrophobic core of the micelles formed. Thus, the inflection point gives the CMC of the surfactant. The right panel shows that the excess attenuation allows the calculation of the micelle size[11].

The analytical power of ultrasound is well known through its application in medicine, where it is used to visualize internal parts of the body. However, the advent of ultrasound in methods to analyze the microscopic details of emulsions and suspensions can be explained by the arrival of breakthroughs in ultrasonic techniques, electronics, new theoretical approaches, and fast computers. Previously, advances were prevented by the technological challenge in generating monochromatic sound beams within a wide frequency range and measurements of parameters of these waves with high resolution. These barriers have now been overcome to permit the development of versatile laboratory instruments, i.e., high resolution ultrasonic spectrometers. High-resolution ultrasonic spectroscopy is a novel technique for material analysis based on precision measurements of the variables of high-frequency sound waves, which propagate

through the sample. Oscillating pressure in an ultrasonic wave causes oscillation of compressions, and, therefore, by its nature, this is a high-frequency rheological wave[10,11].

This provides for one of the great advantages of the technology, including the characterization of concentrated dispersions without the need for dilution. The preparation of serial dilutions is one of the limitations of optical techniques, as optical transparency is required to avoid multiple scattering effects. HR-US plays a vital role in medicine and pharmacy. UltrasonicScientific Ltd. has employed modern technologies to overcome these limitations, thus making commercial ultrasonicinstruments available in practical, laboratory-scale instruments, namely, high-resolution ultrasonic spectrometers[12, 13].An advantage of the technology employed in ultrasonicspectrometers is thein dynamic rheology as well as bulky light sources and other optical parameters of instruments are expensive. Thus representing a robust andmultipurpose instrument, this performs a broad range of analytical functions of fast, non-destructive and non-expensive analysis [15].

Particle size determination.

One of the key elements of emulsion quality and stability is the particle size of the dispersed phase. Batch-to-batch variation in particle size can lead to unpredictable variations in the lifespan and stability (shelf life/heat stability) of the product. Traditionally, measurements of the droplet size in an emulsion are made using optical methods. This means that the sample must be diluted to reach optical transparency and avoid multiple scattering effects. Using the HR-US 102, the size of the water droplets in the original products was measured as 0.9 ml. 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.

This example shows that measurements of particle size using traditional techniques, which require dilution of original and concentrated emulsion, would not provide correct information[14,16].

Ultrasonic Monitoring of various products.

a) Alpha amylase.

Alpha Amylase is an important industrial enzyme that hydrolyzes starch, glycogen, and related polysaccharides by randomly cleaving internal 1,4-gluco-sidic linkages:

$$R_1\text{-}O\text{-}R_2 + H_2O \rightarrow R_1\text{-}OH + R_2\text{-}OH$$

Where R_1 and R_2 are the parts of the oligosaccharide molecule linked by the hydrolyzedO bond. The enzyme is used, for example, as an additive in detergents, for removal of starch sizing from textiles, and proper formation of dextrin in baking. The use of the HR-US 102 spectrometerfor

ultrasonic monitoring of hydrolysis of maltodextrin (1% solution in 0.02 M phosphate buffer, pH 6.9, at 25 °C) catalyzed by amylase, through the measurements of changes in ultrasonic velocity and attenuation. Maltodextrin used in the experiment was a blend of oligosaccharides containing From 11 to 17 dextrose monomers. Five microlitre of a 2-mg/ml amylase solution in the same buffer was added to a 1-mL ultra-sonic cell filled with maltodextrin solution at time 3 min to start the reaction. The hydrolysis of maltodextrin cause an increase in ultrasonic velocity because the hydration level of the products, low molecular weight sugars, is higher than that of the reactants. This increase is proportional to the amount of the substrate transferred to the product and, therefore, the ultrasonic velocity curve was recalculated into the time dependence of the amount of substrate hydrolyzed that is, the kinetic profile of the reaction. The rate of the hydrolysis during the first minutes of the reaction (calculated from the initial slope of the curve) corresponds to 700 mm of glycosidic bonds cut per minute. Reduction in molecular weight of oligosaccharides on the hydrolysis can be monitored also through the changes in ultrasonic attenuation at different frequencies as it is seen in Figure III. These changes in attenuation are related to the decrease in high frequency viscosity of the oligosaccharide solution as the average molecular weight of the maltodextrin decreases [18].

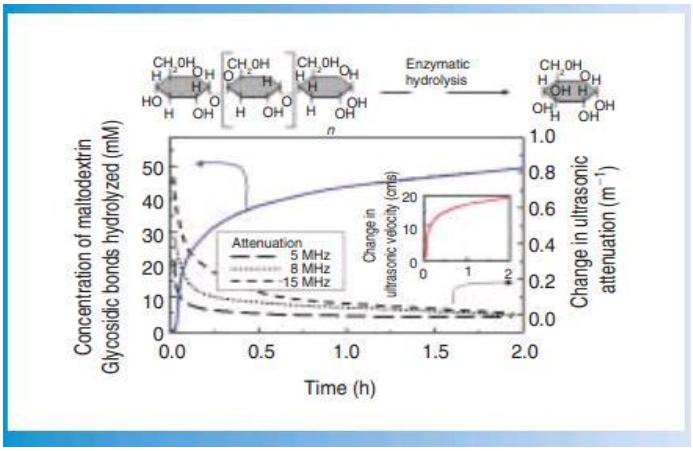

Figure III. Real time monitoring of the hydrolysis of maltodextrin by alpha amylase[18].

b)Hydrolysis of Peroxide by Catalase

Catalase is an enzyme present in almost all animal cells and organs, and in anaerobic microorganisms. Using H_2O_2 as a substrate to produce $H_2O$and O_2($2H_2O_2 \rightarrow H_2O + O_2$), catalase is of commercial interest wherever hydrogen peroxide is used as a germicide. Ithas applications in the food industry, such as pasteurizing milk before cheese-making, as well as microencapsulation. Figure II illustrates the use of the HR-US102 spectrometer for ultrasonic monitoring of enzyme activity of catalase during the hydrolysis of a 19 mm peroxide solution

(0.05 M phosphate buffer, pH 7) by the enzyme at 25 °C. One microlitre of 40 mg/ml catalase solution in the same buffer was added to a1-mL ultrasonic cell filled with the peroxide solution at time 7.5 min to start the reaction[13, 18].

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 50,000 units/mg catalase (one unit is defined as the amount of enzyme activity that will catalyze the transformation of 1 M 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 (only data at 7-MHz frequency are presented). The increase in attenuation is caused by the absorption and scattering of the ultrasonic wave by O_2 gas bubbles, which are formed on decomposition of peroxide[17].

c) Ultrasonic Monitoring of Urea

Urease catalyzes the hydrolysis of urea:

$$(NH_2)_2\,CO + 3H_2O \rightarrow CO_2 + 2NH_4OH$$

In a typical assay the hydrolysis of ureaare measured by coupling ammonia production to a glutamate dehydrogenase reaction and recording the decrease in UV absorbance at 340 nm:

$$2NH_4 + 2\ \text{alpha-ketoglutarate} + 2\ NADH \rightarrow\ 2\text{glutamate} + 2\ NAD + 2H_2O$$

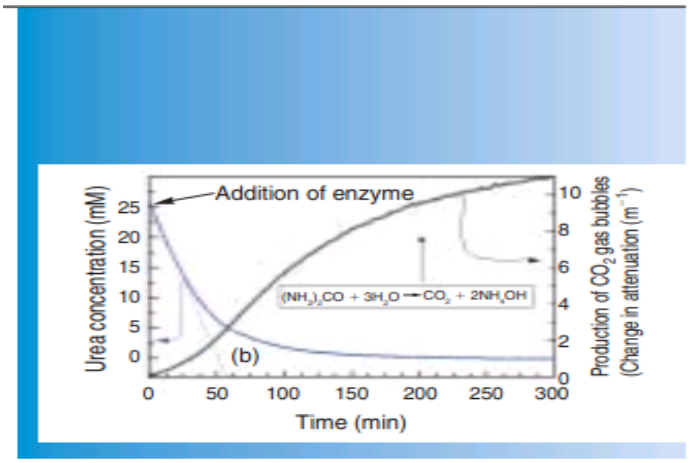

Figure IV. Ultrasonic measurement of the hydrolysis of urea catalyzed by urea's[18].

In comparison with this assay, the ultrasonic method does not require anysecondary reactions or markers and allows the direct detection of the hydrolysis. Figure IV shows the decomposition of urea monitored by the measurements of both ultrasonic parameters: velocity and attenuation

(only data at 7-MHz frequency are presented in Figure (figure 5). Urease from jack beans was dissolved in phosphate saline buffer, pH 7.6, and 40 g of the urease was added to 25 mM urea solution in a 1-mL ultrasonic cell at 25 °C. The change in velocity was recalculated into the change in the concentration of urea. The increase in attenuation could be attributed to a production of CO_2micro bubbles, as in the previous example however; the difference is a delay in attenuation growth relative to the change in concentration of urea. This could be explained by a secondary (coupled with the main) reaction, conversion of carbon dioxide into carbonic acids, which can affect the kinetics of CO_2gas bubble production[18].

New capability:

Titration is a common analytical procedure used in modern laboratories. One of the first types of titration was invented by Joseph Louis Gay-Lussac, known as an author of "The Law of Combining Volumes." Now a days the titration stands for qualitative estimation of the sample using another reagent and indicators. To be suitable for a determination, the end of the titration reaction must be observable. This means that the reaction should be monitored by an appropriate detection technique, for example, potentiometry, color indicators, pH, and so forth. Molecular binding is one of the important applications of titration is analysis, In such a case, an exact determination system results in qualitative information about titration method. The selection of an appropriate detector is the important step in titration analysis which can provide quantitative information on the amount of binding of titrant or reacted with the analyte. A range of techniques can be employed in titration analysis. The list of these techniques includes potentiometry, voltametry, electrical conductivity, fluorescence, isothermal titration, calorimetery, UV/Vis absorbance, and others. However, none of these methods can serve as a universal detector for the binding of titrant to analyte. Fluorescence and UV/Vis absorbance require a change in optical activity of titrant as well as analyte in the binding as well as optical transparency of the solution is the well known example. Therefore, extraction of analyte to make a solution with required optical transparency or other procedures such as attaching optical markers to titrant molecules, often require a complex sample preparation procedure. High-resolution ultrasonic spectroscopy (HR-US) is an analytical technique based upon precision measurements of parameters (velocity and attenuation) of ultrasonic compression wave propagating through the analyzed sample. It can be used as universal detector for titration due to this technique it allows direct probing of intermolecular forces. Any change in molecular structure upon the binding affects intermolecular interactions in the sample and therefore can be detected with ultrasonic measurements. The measured ultrasonic titration profile can be recalculated into the binding isotherm. This

technology is extremely sensitive, requires no markers, and can be used in non-transparent samples such as cell cultures or dispersions (for example, blood or milk). Another advantage of the HR-US titration technique is its ability to analyze molecules in their original state without immobilizing procedures or transferring into another environment.

The basic principle behind the ultrasonic visibility is the resolution of various devices which are used to analysis of material, as like in telescope which is used to charities the stars. Novel principles of ultrasonic detection use the various graded HR-US instruments which increase the resolving capacity of the instruments. Current applications of this technique include analysis of chemical reactions, size of material, gelatin and aggregation phenomenon, transition in polymers and biopolymers, composition analysis and many others. Recently, capabilities of the HR-US technique has been expanded to titration analysis. HR-US titration spectrometers allow analysis of most chemical reactions and molecular bindings[19].

Recent trends:

The current position of high-resolution ultrasonic spectroscopy in the field of material analysis can be compared with that of ultrasound techniques when they were first introduced for medical applications. When the technology reached a required level, it generated an 'explosion' in medical diagnostics through its ability to visualize internal parts of a patient's body, analyze blood streams and other features. Now it is difficult to imagine a modern hospital without a set of ultrasonic instruments.

Similarly, there will be a broad penetration of ultrasonic spectroscopy intoanalytical laboratories in various fields of material analysis and production lines of various industries. Novel high-resolution ultrasonic spectroscopy has the ability to perform a wide range of analyses, which other methods cannot do, as well as to increase the effectiveness and reduce the cost of a number of analytical tasks currently performed by traditional techniques [19].

CONCLUSION:

High resolution ultrasonic spectroscopy is a very advanced modern technique used in the analysis of material. This technique is a combination of various ancient and recent techniques. This technique provides universal detection capabilities for molecular binding, as any binding affects intermolecular forces in the sample, and therefore, can be determined with ultrasonic measurements. Because the measurements do not require any optical transparency, molecular markers or other properties of the solution and solutes, the complex sample preparation procedures in many cases become obsolete.HR-US spectroscopy is a powerful technique for

characterization of emulsions and dispersions. Overall, high-resolution ultrasonic spectroscopy is a new tool for analysis of chemical and micro-structural characteristics of emulsions and suspensions.

REFERENCES:

1. Cormac S, O'Driscoll B, Jayne L, Sinead H, High-Resolution Ultrasonic Spectroscopy: Analysis of Micro emulsions Spectroscopy 2005;20(2).
2. Buckin V, O'Driscoll B, Ultrasonic waves and material analysis: recent advances and futuretrends. Lab plusInternet;2002; 16(3): 17–21.
3. David J.M. Ultrasonic measurement in particle size analysis. In:Robert A. Meyers (Eds.),Encyclopedia of Analytical Chemistry, John Wiley & Sons Ltd, New York, 2006: 1-8.
4. Kudryashov E, Smyth C, Duffy G, Buckin V. Ultrasonichigh resolution longitudinal and shear wave measurements in food colloids: monitoring of gelation processes and detection of pathogens.Progr Colloid Polym Scim 2000; 287–294.
5. Buckin V, Kudryashov E. Supersonics-high resolution ultrasonic spectroscopy. Biochem 2001: 25–29.
6. Smyth C, Buckin V, Making waves. Dairy Industries International; 2002:15–16.
7. Buckin V, Kudryashov E,O'DriscollB, High resolution ultrasonic spectroscopy for material analysis, 2002.
8. Buckin V, Kudryashov E, O'Driscoll B, Spectroscopy perspectives: high resolution ultrasonic spectroscopy for material analysis.American Laboratory (Spectroscopy Perspectives Supplement) 2002:28:30-31.
9. Kudryashov E, MorrisseyS,O'DriscollB, the high resolution spectroscopy: anew tool for PAT analysis.International Labmate; 2003:56-84,
10. Xiaoran G, Jing H, Sen Li, Wenjuan X,Pharmacokinetics and tissue distribution ofa paclitaxel self-microemulsions in rats, Asian J Pharma Sci 2012; 7 (1): 58-66.
11. Aboofazeli R, Lawrence M. Investigations into the Formation andCharacterization of PhospholipidsMicroemulsions:Pseudo-Ternary Phase Diagrams of Systems Containing Water-Lecithin-Alcohol-Isopropyl Myristate. Int J Pharm 1993:161–175.
12. Talegaonkar S, Azeem A, Farhan J,Roop KK, Shadab AP, Zeenat IK,Microemulsions: A Novel Approach to Enhanced Drug Delivery:2008.

13. ShinodaK, Aboofazeli R, Patel N, Thomas M, Lawrence MJ. Investigations into the Formation and characterization of phospholipidsmicroemulsions. Int J Pharm 1995;107–116.
14. Lawrence MJ, Gareth DR. Microemulsion-based media as novel drug delivery systems. Advanced Drug Delivery Reviews: 2000; 45: 89–121.
15. Lawrence MJ. Surfactant systems: micro emulsions and vesicles as vehicles for drugdelivery, Eur J Drug Metab Pharmacokinetic 1994; 257-269.
16. Judith C, John W. Guideline on Speciated Particulate Monitoring by novel techniques: 1998;4-50.
17. Kozo S, Araki M, Sadaghiani A, Khan A, Lindeman B. Lecithin-BasedMicroemulsions: Phase Behaviour andMicrostructure. J Phys Chem 1991;989–993.
18. Edward H, Matthews C,Campbell L. Acoustic Detection of Technology in the Analysis of Bimolecular Interaction. Innovations in Pharma Tech 2006; 30-34.
19. Resa P, Buckin V, Ultrasonic analysis of kinetic mechanism of hydrolysis of cellobiose by beta-glucosidase. Anal Biochem: 2011; 415 (1): 1-11.