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Review

A journey through PROTEOSONICS

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ABSTRACT

Ultrasonic energy is gaining momentum in Proteomics. It helps to shorten many proteomics workflows in an easy and efficient manner. Ultrasonic energy is nowadays used for protein extraction, solubilisation and cell disruption, to speed protein identification, protein quantification, peptide profiling, metal–protein complexes characterisation and imaging mass spectrometry. The present review gives a perspective of the latest achievements in ultrasonic-based sample treatment for proteomics as well as provides the basic concepts and the tools of the trade to efficiently implement this tool in proteomics labs.

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Abbreviations: UE, ultrasonic energy; UF, ultrasound frequency; UI, ultrasound intensity; UA, ultrasound amplitude; TCA, trichloroacetic acid; ECM, extracellular matrix; MS, mass spectrometry; IMS, imaging mass spectrometry; DPD, decision peptide-driven; CH, cup horn; SR, sonoreactor; UB, ultrasonic bath; UP, ultrasonic probe

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1. Introduction

Ultrasonic energy (UE) as a tool in sample preparation is nowadays gaining momentum in proteomics. Some of the firsts attempts to use UE in proteomics were dedicated to protein extraction from complex matrices and to increase the kinetics of enzymatic reactions [1–5]. Since then UE has been reported as a tool to speed/improve several steps of sample handling in proteomics. At present, the use of UE in proteomics spans diverse topics, ranging from high throughput protein identification to quantification and biomarker discovery in biological fluids or tissue samples. UE finds its place to simplify and to shorten the daily work of proteomics researchers [3–5].

The present review is focused in two main aspects. Firstly, it is intended to make easier to the proteomics community to deal with UE. Therefore, the basic concepts about how to handle UE are explained in an easy and straightforward way. This is done on a step-by-step method, including the explanation of the differences among the devices for UE delivery at present available on the market. Secondly, the most important UE applications done to date in proteomics, to the best of our knowledge, are critically described in detail.

2. Ultrasonic energy: the tools of the trade

It is generally agreed that the word "ultrasound" refers to the sound with a frequency ranging from 20 KHz to 10 MHz, out of the 20 Hz–20 KHz, audible range of a healthy young person [6]. The ultrasonic frequency range is itself divided into two main zones, depending on the effects of the ultrasonic waves when passing a liquid medium (see Fig. 1A). High frequency ultrasound, comprised between 2 MHz and 10 MHz, also known as medical ultrasound, is widely used for medical purposes because the physical and chemical properties of the liquid media where the ultrasound is applied do not change. Low frequency ultrasound, is comprised between 20 KHz and 100 KHz, and causes many physical and chemical changes in the liquid media where they are used [7]. These noticeably changes are produced as consequence of a physical phenomena caused by low frequency ultrasound known as *cavitation* (Fig. 1B). Cavitation is the production of microbubles in a liquid, when a large negative pressure is applied [6]. Cavitation occurs when waves cross the liquid fast enough that the liquid molecules cannot follow the cycles of compression and decompression of the wavelength with the same speed. At certain point the forces that maintain liquid molecules together are broken and cavities are created. The formed cavities are known as *cavitation bubbles*. As more energy is delivered to the cavities in the form of ultrasound waves, the cavitation bubbles grow in size through the process called *rectified diffusion* [8]. There are two types of cavitation bubbles characterised by the different effects they promote. *Stable* cavitation is characterised by cycles of compression and decompression, as the wavelength passes through the liquid media but the cavitation bubble never implodes. In *transient cavitation*, the cavitation bubbles grow reaching an unstable size followed by a violent collapse. In these circumstances, cavitation bubbles acts as micro-reactors whereas, according to the hot-spot theory [9,10] temperatures and pressures near 5000 °C and 1000 atm, respectively, are reached. Additional effects are that the mass transfer processes in heterogeneous systems is increased and that the formation of micro-jets of liquid during the implosion at c. a. 400 Km h^{-1} causes erosion and disruption of solid surfaces [6,7]. Also, the described conditions facilitate the formation of highly reactive radical species (RRS) that can be used to enhance chemical reactions. The sonication of water results in the production of small quantities of $OH^{\bullet}$ and $H^{\bullet}$ radicals that undergo several subsequent reactions including the formation of H_2O_2, H_2,and O_2.

The correct application of the UE depends on different variables. In brief, these are ultrasound frequency, UF; ultrasound intensity, UI; ultrasound amplitude, UA; time of application; temperature, external pressure, type of liquid media, and type of gas present in the liquid media. These variables and their effects in the context of ultrasonic-based sample preparation have been discussed in previous publications [11–13] but will be shortly described below.

Common ultrasonic devices are sold delivering a wide range of electrical energy, which is referred as the "power" of the ultrasonicator. It is easy to find sellers classifying ultrasonic apparatus in function of the watts they deliver. The electrical energy is transformed into mechanical (vibration) energy. For instance, this can be visualised as a motion travelling through the ultrasonic tip, causing it to move up and down. The distance of the movement of vibration is called its amplitude. The amplitude of the vibration can be controlled up to a maximum depending on the power of the ultrasonicator. Ultrasonic amplitude and ultrasonic intensity have a direct relationship. The intensity of an ultrasonic wave is proportional to the square of the amplitude. Therefore, the highest is the amplitude the highest is the intensity.

For the same type of sample, if the output power is set to low values, low amplitude and low intensity are achieved. The lower amplitude and intensity the lower the effectiveness achieved with the ultrasonicator. The reverse is also true.

Current proteomics workflows using ultrasonic energy as a tool in sample treatment relay in short times of exposure, generally less than 2 min, and in the use of high intensity devices with capabilities of delivering frequencies between 20 KHz and 40 KHz. Ultrasound amplitudes are generally set up to 50%. As will be seen in further sections, the ultrasonic probe (or multiprobe), the cup-horn and the sonoreactor, are the devices most commonly used nowadays to deliver ultrasonic energy in proteomics.

Temperature can be a problem as many proteomics protocols make use of chaotropes agents, which may covalently modify proteins. As an example, urea is routinely used as denaturing agent in proteomics. However, heat accelerates urea hydrolysis, which leads to the production of isocyanate. This chemical, in turn, promotes the carbamylation of proteins at the N-termini of lysine side chains [14]. However, for the majority of the proteomics applications the time of exposure, less than 2 min, and ultrasonic amplitude, below 50%, are not sufficient to promote an increase in the bulk temperature above the threshold to induce modifications on proteins. If temperature becomes a problem for the reason mentioned above, or some other reason, modern probes can be used in the "pulse" mode. In this working mode, the amplifier switches the power on and off repeatedly, avoiding excessive warming of the bulk sample. External cooling can also be applied.

The ultrasonic energy in proteomics has been always used with success, to the best of our knowledge, under atmospheric pressure. Therefore, external pressure is a variable not to be taken into account. Regarding the liquid media, ultrasound has been successfully applied

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