Ultrasonic particle manipulation exploited for infrared and Raman spectroscopy of suspensions

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Abstract: Ultrasonic separation techniques are based on the so-called acoustic radiation forces exerted on suspended particles within an ultrasonic standing wave in the megahertz frequency range. Among the systems employing the aggregation effects caused by sonication, acoustic cell filters are successfully applied in biotechnology.

In this work, an ultrasonic standing wave field of 2 MHz was used to concentrate particles in the light path of a confocal Raman microscope. Due to the increased spatial concentration of matter in the focus of the laser beam, an increase of the signal-to-noise ratio of the recorded Raman spectra of the particles was achieved.

The utilized biomacl suspensions of yeast cells are important, e.g., for on-line monitoring of bioprocesses. Spectra are presented taken by the Raman microscope pointing at cell aggregates induced by ultrasound. As an indication of feasibility, this data are compared to measurements taken when no means of concentration were at work and to measurements of dried yeast cells, respectively.

A further field of application was identified in the control of crystallisation processes with emphasis to discriminate polymorphs. Therefore, suspensions of theophylline crystals were investigated following a similar protocol as with the yeast cells. Here, the data show significant differences in the spectroscopic feature structure between dried matter and crystals in contact with water. In addition, SNR is increased by several orders of magnitude.

Key words: ultrasonic standing waves, Raman spectroscopy, optical measurement, ultrasonic particle separation

A. Introduction

Within an ultrasonic standing wave, forces are exerted on suspended particles directing them towards so-called pressure nodes, i.e., regions of vanishing sound pressure of the ultrasonic field. This technique is referred to as ultrasonic particle separation.

Ultrasonically Enhanced Settling (UES) devices based on this principle are exploited industrially as cell filters in biotechnology [1]. This is possible due to its non-destructiveness for biological cells - in contrast to the common ultrasonic cleaning devices which utilize the effect of cavitation at frequencies of a few ten kHz. In fact, micro-organisms such as yeast cells, are handled very gently by a megahertz standing wave field [2].

Ultrasonic particle separation as exploited here has been increasingly used for particle manipulation in small cavities recently [3]. The present work describes the results of Raman spectroscopy measurements when a small resonator was used to control the spatial residue of suspended particles relative to the light path of the Raman microscope, i.e. agglomerates of yeast cells and theophylline crystals dispersed in water were deliberately moved in and out of the focus of the instrument.

Raman spectroscopy is an increasingly popular technique in process analytical chemistry because it directly provides molecular specific information on the sample under investigation. In Raman spectroscopy, a sample is irradiated with a focused laser beam and the inelastically scattered light measured. The recorded intensities at different frequency shifts are termed Raman spectrum which provides information on characteristic vibrational transitions in the sample under study. Raman spectra can be recorded from solids, liquids and gases, providing qualitative as well as quantitative information on the chemical composition of the sample.

In case of monitoring reacting suspension, such as fermentations or crystallization processes, Raman spectroscopy holds great promise due to possible non-invasive measurement strategies. Furthermore, based on the Raman spectrum, polymorphs of a given substance during crystallisation processes [4] may be distinguished as well as the physiological status of micro-organisms assessed [5]. Both exemplified applications turn out to be difficult for standard on-line Raman spectroscopy because Raman photons from the solid matter (crystals or micro-organism) need to be distinguished from Raman signals originating from the pure liquid phase. This problem is of special relevance in case of low concentration of suspended particles.

Thus, combination of ultrasound technology for particle manipulation and Raman micro-spectroscopy is an interesting avenue to increase selectivity and sensitivity of on-line Raman measurements. This is because by ultrasonic particle manipulations, particles may be concentrated or removed from the Raman measurement spot, thus allowing to selectively measure the liquid and solid phases, respectively. In addition to
the gained selectivity, an increase in sensitivity may be expected due to the local pre-concentration of the particles by means of ultrasound separation technology.

**B. Experiment**

**B.1. Ultrasonic separator**

The ultrasonic separator consisted of an aluminium spacer between two glass sheets (microscopy slides). The lid was equipped with in- and outlet thus building up a flow cell (see Fig. 1 top). To one sidewall of the spacer a PZT ceramics (1x25x7 mm$^3$) was glued for the excitement of the ultrasonic standing wave. The other sidewall acted as a sound reflector. The device was placed under the Raman microscope with the glass sheets perpendicular to the light path. Thus the acoustic nodal planes where oriented parallel to the incident light beam, allowing to control their locations relative to the light path by changing the excitation frequency (~2 MHz). The PZT was connected to an FPS 4025 frequency power synthesizer (PSI Systems, Austria). A few tens of one Watt were used as true electrical power input.

The described agglomeration of particles by the ultrasonic radiation forces is visible in the two pictures in the bottom row of Fig. 1. The right light micrograph of a nodal plane clearly shows the increased particle concentration when compared to the left hand side picture, where no ultrasonic field was present.

**B.2. Suspension & Raman measurements**

The yeast-in-water suspension was prepared with dried yeast (traditional dried active yeast, Allinson, UK) in distilled water at a concentration of a few 10$^7$ cells/mL.

A fairly over-saturated theophylline solution was used for the observation of the hydration process (solubility of theophylline in water is 8.3 mg/mL). For the experiments on improving the SNR by means of ultrasonic particle agglomeration, a slightly over-saturated theophylline solution (9.3 mg/ mL) was investigated.

Fig. 1. top: flowcell equipped with ultrasound transducer bottom: left freely suspended (left) and US agglomerated (right) theophylline crystals

![Fig2. Raman spectra of yeast in water (grey), yeast cells agglomerated in the nodal plane of the ultrasonic field (black with dots) and for comparison dried yeast cells on quartz (black).](image-url)
The Raman spectra were recorded on a LabRAM HR 800 confocal Raman microscope from Jobin Yvon (Bensheim, Germany) using a 632.8 nm HeNe laserline (14.5 mW), a 600 grid, and a 20x magnification objective with a working distance of 20.5 mm. The confocal hole was set to 500 µm and the slit to 100 µm, recording time was 30*18 s.

C. Results

C.1. Yeast in water

The flow cell was filled with suspensions of yeast cells in water. The grey line in Fig. 2 shows the resulting Raman spectrum, when the optical focus was somewhere in the liquid layer. However, when an ultrasonic standing wave was applied and the Raman measurement was taken at a location within an agglomerate of yeast cells in a nodal plane the black line with dots was the result. Significant features of yeast could be identified around wave numbers 2850 cm⁻¹, 1660 cm⁻¹, and 1437 cm⁻¹, which arise from the symmetric CH2 stretching vibration, the amide I band and the amide III band, respectively. For reasons of comparison, Fig. 2 includes the Raman spectrum of dried yeast on a quartz glass plate (black), where the scatter intensity was found to be almost twice as high as for the measurement of the agglomerate, however, the feature structure was conclusively similar.

C.2. Raman spectra of theophylline

The Raman spectra of theophylline crystals were measured in a saturated solution within the sediment to define the achievable resolution (Fig. 3, grey). In comparison to data acquired from water free theophylline (black) crystals the recorded Raman spectra are significantly different. This difference resulted from the formation of theophylline monohydrate, which is another crystalline form of theophylline. Most significant changes can be seen in the absence of the band at 1690 cm⁻¹ and additional bands at 1668 cm⁻¹ and 1710 cm⁻¹ with further small bandshifts across the whole spectrum. The measured scatter intensity of both polymorphs was within the same order of magnitude.

The results of investigations aiming on the influence of the separation of the theophylline crystals by an ultrasonic standing wave are collected in Fig. 4. The aim was to compare the Raman signal of freely suspended and hence moving theophylline crystals (black) with measurements of agglomerates brought about by the ultrasonic separation (black with dots). Fig. 4 shows clearly that a significant increase (3 to 6-fold) of scatter intensity was found when the ultrasonic wavefield was applied. Moreover, the data suggest better resolution, e.g., between wavenumbers 1600 cm⁻¹ and 1700 cm⁻¹ when the theophylline crystals were concentrated ultrasonically.

In contrast no significant differences were found for regions of the ultrasonic standing wave where no particles are present (typically the displacement nodes). The Raman spectrum of a crystal-free theophylline solution (grey) was not different from a measurement taken with the optical focus positioned within this depleted region (grey with dots).
C.3. Comparison

Agglomerates of yeast cells showed a slightly lower level of scatter intensity when compared to dried material. Similarly, dry theophylline crystals showed the highest Raman signal. However, when comparing sedimented theophylline crystals – which should be packed tightly enough – with agglomerates brought about by the ultrasound, even a slight increase of scatter intensity was measured when the standing wave was present.

In all measurements the ultrasonic field did not significantly influence the structure of the Raman spectra.

D. Conclusion

The presented results of Raman measurements indicate that the ultrasonic particle separation technique is a suitable means to achieve higher Raman scatter intensities when applied to suspensions. Two substantially different types of particles – yeast cells and theophylline crystals - were used in this study to cover a broad range of applications this combination of optical and acoustical techniques can be applied to.

The beneficial effects on the measurement of Raman spectra of the two components of a suspension was the consequence of the vast increase of the particle concentration in the nodal planes of the ultrasonic standing wave, in comparison to the arbitrary distribution of particles when no ultrasonic field was present. Moreover, it was shown for theophylline crystals that the very low concentration of particles between those planes enables one to specifically take measurements of the host liquid.

E. Literature


