An Acoustic Trap combined with Attenuated Total Reflection Infrared Spectroscopy for Enzyme Kinetic Studies

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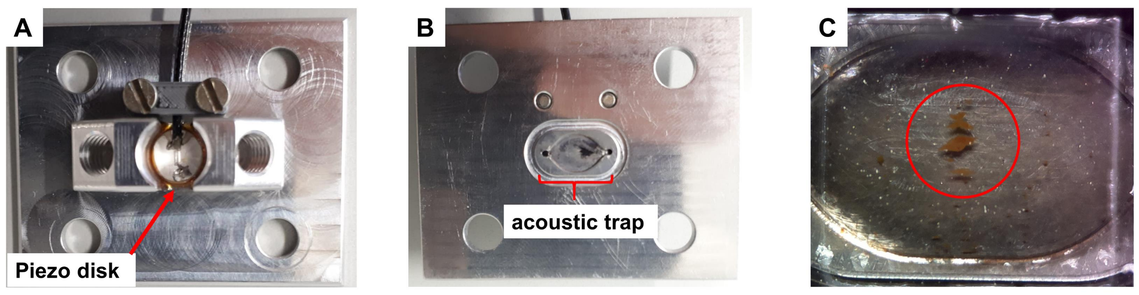
Introduction

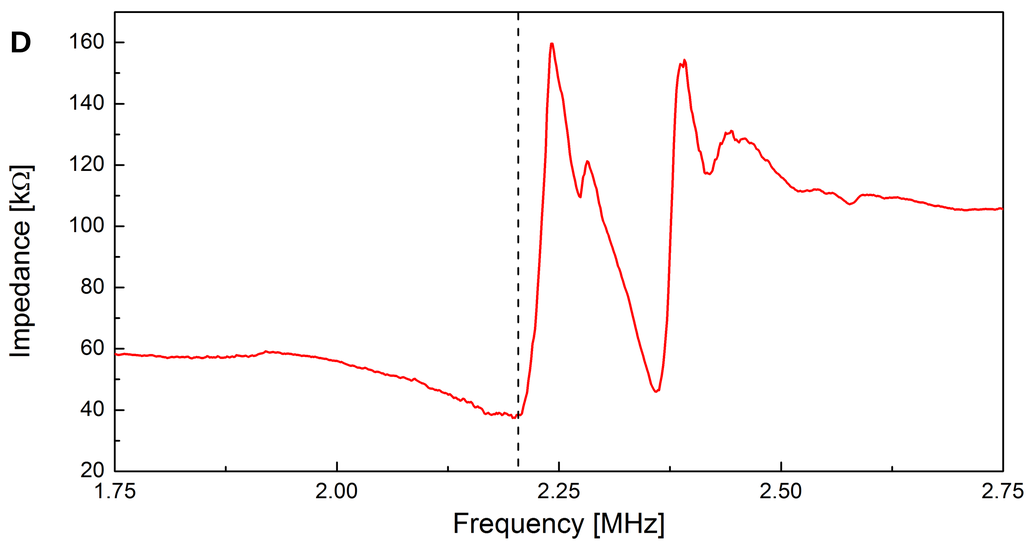
In this work, we present the combination of an acoustic trap [1] and attenuated total reflection (ATR) Fourier-transform infrared (FTIR) spectroscopy [2], to monitor the activity of immobilized enzymes. By mounting an acoustofluidic cell on top of a custom-built ATR setup, we were able to trap beads labeled with the enzyme alkaline phosphatase (AP) without the need of mechanical retention elements [3]. To showcase potential applications of the presented setup for enzyme kinetic studies, we monitored the conversion of *p*-nitrophenylphosphate into *p*-nitrophenol and phosphate ion via beads carrying the immobilized enzyme. Multiple experiments were performed by retaining the labeled beads via ultrasound (US) particle manipulation and resulted in excellent experimental reproducibility. Beads could be discarded in a straightforward manner by switching off the ultrasound. This proves the potential of the acoustic trap combined with ATR-FTIR spectroscopy for monitoring reactions catalyzed by trapped particles.

Acoustic trap



Pictures of the top view of the assembled acoustic trap (A), the bottom side (B) and trapped beads, encircled in red (C).

The acoustic trap is made of aluminum. The liquid compartment has a volume of approximately 20 µL and a height of 500 µm. Above the sample compartment a 8 mm piezo disc (lead zirconium titanate, Type PRYY 0059; PI Ceramics, Lederhose, Germany) with wraparound silver electrodes was directly glued to the aluminum body using a two-component epoxy resin (Polytec PT, Karlsbad, Germany). The assembled acoustic trap was mounted on top of a custom build ATR fixture, holding a multibounce zinc sulfide ATR element (17 x 10 x 1 mm^3 ,45°). Due to the transparency of the ATR element real-time visual observation of acoustic trapping via a Pi camera mounted under the ATR fixture was possible.



Impedance spectroscopy was used to analyze the frequency response of the acoustic trap. An impedance minimum corresponding to the resonance of the acoustic trap mounted on top of the ATR fixture was found at 2.20 MHz. Experiments were performed using a ISX 3 (Scio Spec, Bennewitz, Germany) impedance analyzer. The measurement setup was set to a minimum frequency of 1 MHz and a maximum frequency of 4 MHz in 2000 steps at a precision of 5 resulting in a total measurement time of 12.6 s. The acoustic trap was operated using a sonic amp (USEPAT, Wien, Austria) US driver set to the identified impedance minimum of 2.20 MHz and a gain of 65%. Prior to sample handling the US was turned on for 30 min to allow the acoustic trap to thermally stabilize.

Conclusions & Outlook

We introduced the combination of an acoustic trap with ATR-FTIR spectroscopy, for monitoring reactions catalyzed by trapped particles. ATR-FTIR spectroscopy is a perfect match for the acoustic trap as the ATR element acts not only as a reflector for particle manipulation via the standing ultrasound wave but also as the sensing element. The presented results pave the way for enzyme kinetic studies exploring non-chromogenic natural enzyme-substrates via ATR-IR spectroscopy. Future studies will focus on retaining bacteria within the acoustic trap and perform ultrasound enhanced assays for monitoring microbial drinking water quality.

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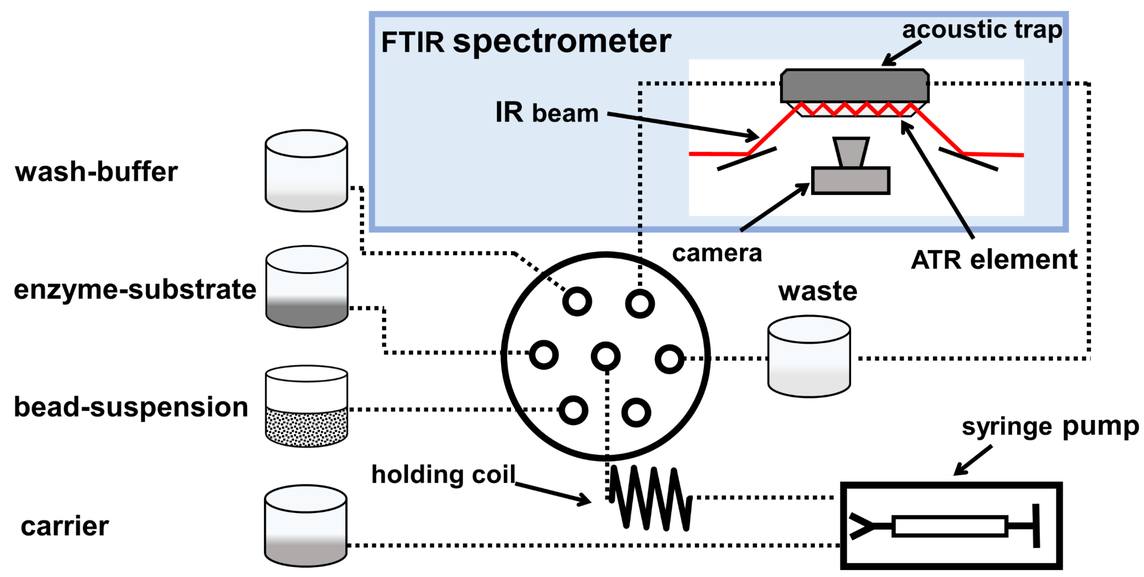
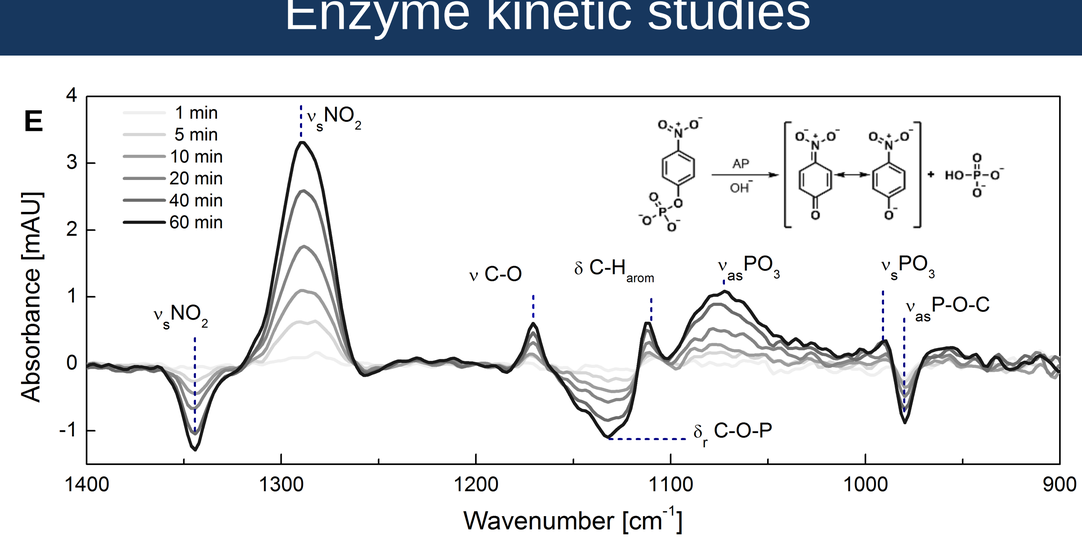
Experimental setup



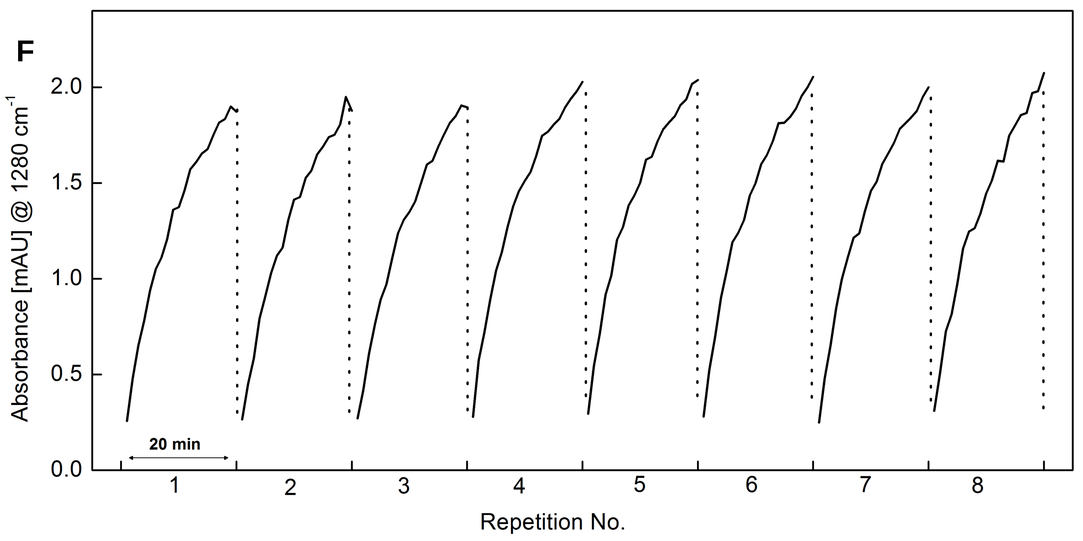
ATR measurements were performed by guiding the IR beam of a FTIR spectrometer (Vertex70v, Bruker Optics) through a custom-made ATR setup with the acoustic trap on top.

Automated liquid handling is performed via a sequential injection analysis manifold consisting of a selection valve and a syringe pump.

Enzyme kinetic studies



Ultrasonic radiation forces were used to retain beads within the acoustic trap, above the ATR element, while enzyme substrate was pumped into the cell. IR spectra monitoring the enzymatic conversion of *p*-nitrophenylphosphate into *p*-nitrophenol and phosphate ion catalyzed by the beads were recorded over the course of 60 min (E). The first of these consecutively recorded spectra was used as the background. The spectra show increasing product-related bands at 1280, 1170, 1075, and 990 cm^{-1}, whereas the substrate-related bands at 1345, 1130, and 980 cm^{-1} decrease over time.
Spectra were recorded with a spectral resolution of 4 cm^{-1}, and a total of 128 scans were averaged per spectrum. All spectra were acquired at room temperature. The aperture was set to 4 mm, restricting the IR beam to the width of the acoustic trap.



In addition, reproducibility experiments were carried out by using the band height at 1280 cm^{-1}, originating from the nitro-group of the product, as analytical signal. Fresh beads were trapped eight consecutively times, supplied with enzyme substrate and the subsequent reaction was monitored for 20 min, by recording a sequence of 20 IR spectra via ATR-IR spectroscopy. After each injection, the beads were discarded and the acoustofluidic cell was thoroughly rinsed with wash solution. The acoustic trap demonstrated high reproducible bead trapping capabilities (F).

[1] Tenje, M., Xia, H., Evander, M., Hammarström, B., Tojo, A., Belák, S., Laurell, T. and Leblanc, N. Analytica chimica acta **853**, 682-688 (2015).

[2] Ramer, G. and Lendl, B., Attenuated total reflection Fourier transform infrared spectroscopy, John Wiley & Sons, Ltd., (2013).

[3] Freitag, S., Baumgartner, B., Tauber, S., Gasser, C., Radel, S., Schwaighofer, A. and Lendl, B. Analytical Chemistry **91**, 7672-7678 (2019).