SYNCHROTRON RADIATION FOR ON-CHIP MID-IR DETECTION AT THE DIFFRACTION LIMIT

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Abstract

Microchips for rapid mixing of two aqueous streams have been produced using CaF_2 wafers and SU-8 microstructuring technology and tested at the IR beamline of Bessy II in Berlin, Germany. Advantage of the synchrotron radiation over thermal globar sources for measuring at sample spots close to the diffraction limit of the employed mid-IR radiation have been documented. The capability of the experimental set-up to initiate and monitor chemical reactions label-free in pL volumina was verified. Simple chemical reactions such as titration of acetic acid and conformational change of the protein β2-microglobulin have been recorded.

Keywords: Mid-IR spectroscopy, synchrotron radiation, micromixing, protein folding

1. Introduction

Dedicated chips for (bio)chemical reaction monitoring using mid-infrared synchrotron radiation are introduced. These chips made of calcium fluoride and SU-8 polymer are designed to take advantage of the highly collimated beam of synchrotron radiation, which when coupled to an IR microscope allows high quality measurements even at spot sizes close to the diffraction limit of the light used. In case of mid-IR radiation the smallest sample spots that can be realized are in the order of 5-10 micrometer. In this context it is important to mention that the throughput advantage of an IR synchrotron light source over a conventional thermal (globar) source is only significant when measurements with spot sizes smaller than 50 micrometers in diameter need to be achieved. To take full advantage of the possible small spot size for a number of different (bio)chemical experiments, dedicated chip based systems are needed. In case of time resolved studies a time resolution of approximately 500 microseconds can be achieved. In case of separation systems like on-chip capillary electrophoresis synchrotron based IR dedication would also be of distinct advantage as here a small detection volume is critical to avoid overlapping elution profiles.

Lab-on-a-chip technology in combination with IR synchrotron radiation sources may thus be regarded as an enabling technology for a number of different experiments such as bio-ligand interaction studies or protein folding.

2. Design, fabrication and working principles of the applied micro-mixers

A 4 µm thick SU-8 layer is deposited on both (bottom- and top-) wafers. After softbake and exposure the wafers are post-exposure baked at 90°C. The 2 µm thick metal layer, which forms the separation membrane, is deposited by evaporation on top of the SU-8 layer on the bottom wafer. Silver is used for this layer, because evaporated Ag-layers do not show any internal stress and they can be deposited using relatively low power. The Ag-layer is patterned in a conventional way using

positive photoresist (AZ 1512 HS) and wet etching. The Ag-etchant, which is used, does not attack the SU-8. Subsequently the SU-8 structure (under the Ag-membrane) is developed.
The treatment of the top-wafer is different and depends on the used bonding method. In case of a simple method the SU-8 structure is developed and the holes for inlets and outlet are drilled. Subsequently the two wafers (top- and bottom-wafer) are bonded in an evacuated chamber upon application of pressure (2000N) and temperature (ramp of 3 °C / min. to 150 °C followed by one hour at this temperature).

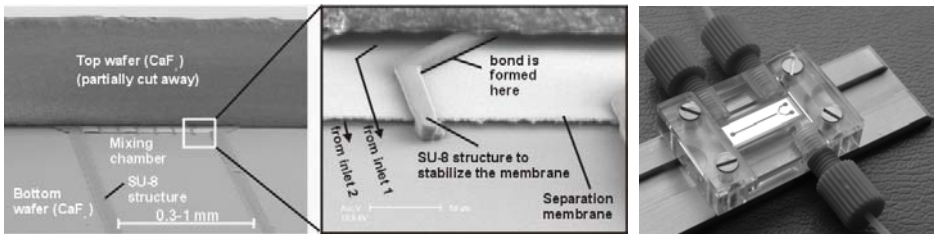

Figure 1. Left and middle: SEM images of the produced chips. Right: Picture of the microchip mounted in the dedicated support for placement under an FTIR microscope.

3. Spectral baseline noise achievable when using a MIR synchrotron source vs. a globar

The dependence of the achievable spectral baseline noise as a function of the probed sample area has been investigated in a systematic study. For this purpose a microchip with a channel cross section of 10*300 μm and filled with water has been used. The aperture of the microscope was set from 10 to 70 μm in increments of 5-10 μm. In case of synchrotron radiation the baseline noise decreased when increasing the sampling spot from 10-20 μm. However, a further increase of the sampling area did not result in an improved spectral baseline. On the contrary when using a globar source a continued improvement in baseline noise was achieved upon increasing the sampling area. At a spot size of 20 μm the baselines noise was approximately 10 times lower using a synchrotron as compared to using a globar source. However, at spot sizes of 50 μm no significant differences in the baseline noise have been observed. These results clearly show that synchrotron radiation is of significant advantage only when measurements at small sample spots need to be carried out. This is the case in chemical reaction monitoring using the continuous flow approach or in separation systems where band broadening in the detection area needs to be avoided.

3. Chemical reaction monitoring in micro-chips by mid-IR synchrotron radiation

The proper functioning of the device was tested using the titration of acetic acid as a simple model system. In figure 2 the spectra are shown which have been recorded at distinct positions along the outlet channel of the mixer. In doing so spectra before and after reaction could be recorded. In a similar way spectra of β2-microglobulin, a single chain polypeptide (99 residues Mw: 11800), at two different pH values have been recorded. β2-microglobulin is of relevance in dialysis-related amyloidosis, a protein conformational disease. There is now broad consensus that partial unfolding of β2-m is required for formation of fibrils. As FTIR spectroscopy is sensitive to the secondary structure of proteins it may be expected that pH induced protein folding may be followed in solution and in-situ by time resolved FTIR studies.

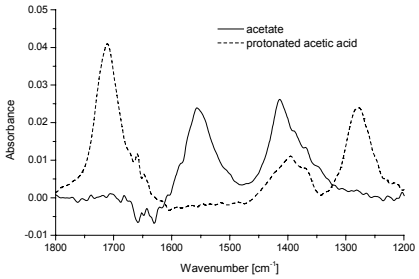

Figure 2. Mid-IR spectra recorded from a sample volume of 9 pL (30*30*10μm). One clearly can see the differences in the mid-IR spectra of acetic acid and acetate.

The spectra of acetic acid and acetate have been recorded in aqueous solution directly. For the study of protein conformation as in case of β2-microglobulin heavy water and deuterated reagents (DCl) had to be chosen. This is because the information rich amid I band of proteins, from which their secondary structure may be told, overlap with the bending vibration of normal water. In the studies performed with the micromixers so far β2-microglobulin solutions of 1g/L have been used. The spectra at two distinct pH values, which have been recorded from a spot volume of 20*40*10 μm are given in Figure 3a. Despite the fact that these spectra are noisy the expected shift of the amid I band to higher wavenumbers upon decreasing pH may be discerned. The observed band-shift reflects the folding (increase in α-helical structure) of the protein. Reference spectra recorded from larger volumes using the attenuated total reflection technique (figure 3b) confirm this observation.

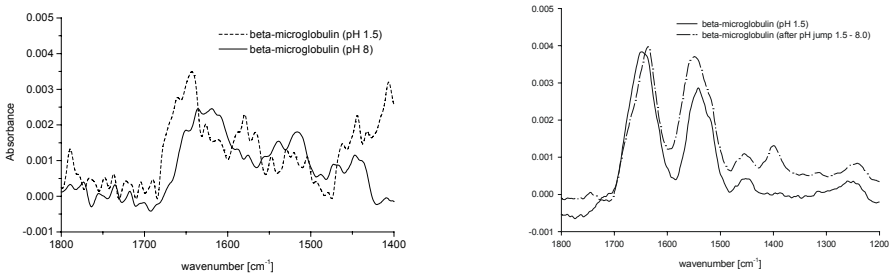

Figure 3. a) Mid-IR spectra recorded from 8 pg (conc.: 1g/L, 20*40*10 μm) of β2-microglobulin at different pH values in the micro-chip using a synchrotron radiation source. b) Reference spectra

4. Conclusions

The successful coupling of MIR synchrotron radiation with micro-chip technology has been shown by recording MIR spectra from a few pL in aqueous solutions only. However, to take full advantage of the sampling capability of synchrotron MIR microscopy the spectral noise needs to be reduced. Efforts are under way which indicate that this will be possible in the near future.

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