SPECTROELECTROCHEMICAL DETECTION OF *p*-BENZOQUINONE AND HYDROQUINONE IN AN ELECTROCHEMICAL MICROREATOR WITH AN INTEGRATED ATR-IR IRE

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ABSTRACT

Cyclic voltammetry (CV) is an established method to study redox reactions, but unfortunately does not offer detailed insights in the (intermediate) products formed. To allow mechanistic studies of electrocatalytic reactions, we have combined a silicon internal reflection element (IRE) for attenuated total reflection infrared (ATR-IR) spectroscopy and an electrochemical microreactor into a single chip. This chip contains boron doped diamond (BDD) electrodes on an insulating layer located 5 µm above the IRE to enable the detection of short lived reaction products. With this 1.3 µL volume spectroelectrochemical chip, the reversible redox pair *p*-benzoquinone and hydroquinone is manipulated and analyzed successfully.

KEYWORDS: ATR, IR, spectroscopy, internal reflection element, IRE, spectroelectrochemistry, electrocatalysis, boron doped diamond, BDD, cyclic voltammetry, CV, microfluidics, quinone

INTRODUCTION

Our research group has previously developed multiple microfluidic chips hyphenated to electrospray ionization mass spectrometry (ESI-MS) [1,2], providing a delayed analysis of electrochemical oxidation reactions of pharmaceutically relevant compounds and peptides. Examples of investigated pharmaceutical compounds are 1-hydroxypyrene, clozapine and amodiaquine. Additionally, the electrooxidative cleavage of peptide-bonds was discovered and investigated on the examples of the tripeptides LWL (L = leucine, W = tryptophan) and LYL (Y = tyrosine) as well as bovine insulin [3,4]. Especially for the elucidation of the aforementioned peptide-bond cleavage reactions, the study of electrochemical reaction mechanisms is a promising research topic. To enable mechanistic studies, a silicon internal reflection element (IRE) was added to a simple electrochemical microreactor to incorporate attenuated total reflection infrared (ATR-IR) spectroscopy as an *in situ* measurement method.

The schematic design of the chip is shown in Fig. 1, the frit channels are exaggerated for clarity. It features three layers: (1) a silicon IRE to guide IR radiation from about 900 cm^{-1} to 4000 cm^{-1} (11 µm - 25 µm), (2) an SU-8 layer, acting as fluidic and spacing layer and (3) a BDD-on-insulator layer. Two facets for coupling the IR-radiation in and out of the chip at the beginning and the end of the channels are etched into the IRE at an angle of 54.7° using KOH. The sample solution is treated and analyzed in three 26 mm long channels. The two outer channels are 2 mm wide, the center one is 3 mm wide. Three BDD-on-insulator electrodes are located in the ceilings of these channels. The center electrode is used as a working electrode, while the other electrodes are used as counter and reference electrodes respectively in a three-electrode setup. The channels are interconnected via small frit channels to enhance electrical conductivity (inset of Fig. 1) as has been previously described by Odijk et al. [5].

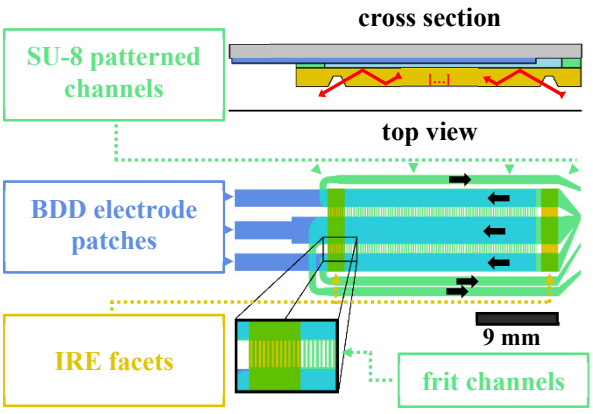

Figure 1: Schematic drawings of the microreactor, cross section (not up to scale) and top view (frit channels exaggerated for clarity). The flow direction is indicated by black arrows.

EXPERIMENTAL

Wires are glued to the contact pads of the chip with a conductive Ag-epoxy-glue. The microfluidic chip is placed in a commercially available sideconnect chipholder from Micronit and connected to a syringe via ETFE tubing to flush or fill the chip (see Fig. 2). For measurements, the chip is filled and the tubing disconnected and then the chipholder is placed and rotated upside down in a custom-built aligner. The aligner with the chip is then put into a Bruker Fourier-transform infrared (FTIR) spectrometer and the wires are connected to a VersaSTAT 3 potentiostat (see Fig. 3). Using the micropositioners and two mirrors of the aligner, the coupling of the IR radiation into the IRE can be optimized. The light source is on the right-hand side of the picture, the detector on the left-hand side.

A reversible redox system consisting of *p*-benzoquinone and hydroquinone in aqueous solution was investigated. The chip was filled with different solutions containing varying concentrations of either *p*-benzoquinone or hydroquinone for the different analytical methods. Spectroscopic measurements were done using concentrations of 20, 40 and 60 mM *p*-benzoquinone in 10% ethanol in water. For electrochemical measurements, an aqueous solution of 75 mM hydroquinone with 0.1 M KNO_3 as a supporting electrolyte was used. Spectroelectrochemical measurements were carried out with aqueous solutions with a concentration of 50 mM *p*-benzoquinone. During these, the compound was reduced to hydroquinone and reoxidized again *in situ* by applying reductive or oxidative electrical potentials of -0.5 V and +0.3 V respectively for 120 s each. IR spectra were recorded initially and after each application of an electrical potential. 128 scans were averaged to obtain suitable spectra. A previously recorded reference spectrum of water inside the chip was subtracted from the measured spectra.

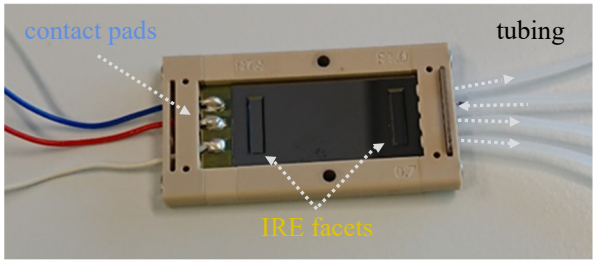

Figure 2: The microfluidic chip in a commercial chipholder. Fluidic connections are made at the right-hand side, the gray arrows indicate the flow direction. Wires for electrical connection are glued to the contact pads at the left-hand side. The two rectangular depressions in the chip surface are the facets of the IRE.

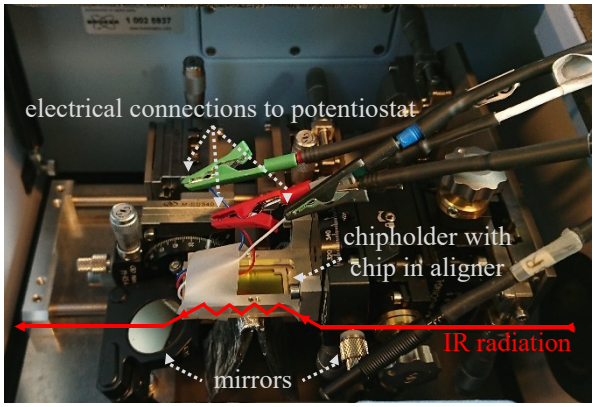

Figure 3: Experimental setup with a custom-made aligner inside a Bruker FTIR spectrometer with connection to a VersaStat 3 potentiostat. The micropositioners allow precise alignment of the mirrors and the chipholder to optimize coupling the IR radiation into the IRE.

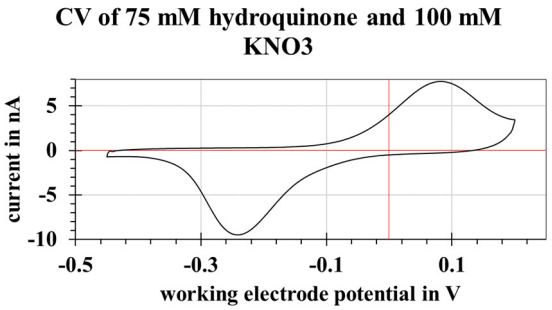

Figure 4: CV of 75 mM hydroquinone in water recorded using the chip, demonstrating the reversibility of the redox pair. The peak separation is a result of mass transport limitations.

RESULTS AND DISCUSSION

The reversible electrochemical behavior of the redox pair inside the chip was observed via cyclic voltammetry (CV) under no flow-conditions (see Fig. 4). The initial hydroquinone was oxidized to *p*-benzoquinone at a potential of +0.09 V and reduced at a potential of -0.24 V. These values are however not precise, because the chip is lacking a robust reference electrode. The exchanged charge between the oxidation and reduction is approximately equal. There is however a second oxidation process that was observed in experiments with a wider potential range and takes place at slightly higher potentials than the first oxidation. This explains why there is a residual current at the far right end of the CV. The relatively large peak separation is caused by mass transport limitations inside of the chip.

In a series of experiments with three different concentrations, this setup was found to still detect 20 mM *p*-benzoquinone easily. The limit of detection is thus estimated to be below 20 mM. To investigate the combination of the two methods, a concentration of 50 mM of *p*-benzoquinone was used. Because IR measurements of a suitable quality would have been too slow to record in real-time, they were instead done before and after each application of an electrical potential. For the interpretation of the spectra, the C=O peak of *p*-benzoquinone at 1655 cm^{-1} was monitored. Upon reduction of the compound to hydroquinone, the C=O bond was converted into a C-OH bond, diminishing the monitored signal. The signal increased again however, when the hydroquinone was reoxidized to *p*-benzoquinone by applying an oxidative potential. The decrease and following increases of the C=O signals is demonstrated in the spectra excerpts given in Fig. 5. Upon application of a reductive potential of -0.45 V for 120 s, the area of the peak was reduced by 45.2% and the two oxidation steps increased it by 11.6% and 5.5%, in total to 71.9%, relative to the original value respectively as can be seen more clearly in Fig. 6. While the signal area could not be fully restored by the oxidation steps, the spectroelectrochemical functionality of the device is clearly proven by these results.

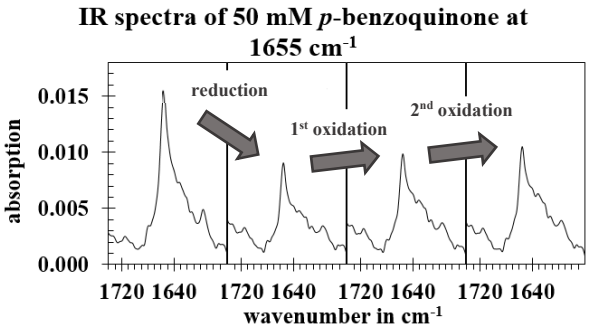

Figure 5: IR spectra excerpts of 50 mM p-benzoquinone in water. The peak at 1655 cm-1 corresponds to the C=O groups of p-benzoquinone.

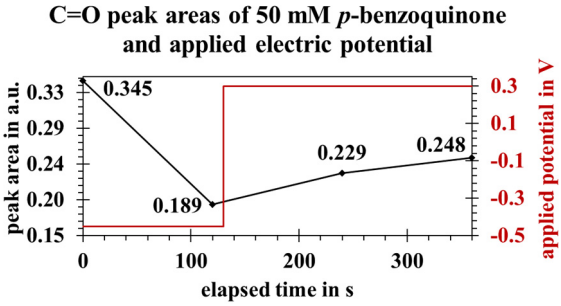

Figure 6: C=O peak areas of p-benzoquinone as a function of time, applied potential in red. The area decreases after 120 s of reductive potential and increases again when an oxidative potential is applied.

CONCLUSION

In summary, we successfully demonstrate the electrically tunable ATR-IR absorption of a reversible redox pair in a novel microreactor. It features BDD-on-insulator as electrode material in very shallow channels of just 5 µm height, a small internal volume of 1.3 µL and an integrated silicon IRE for the ATR-IR spectroscopy. Present research focuses on an improved chipdesign and selection of materials as well as the incorporation of new instrumentation. Studies of reaction intermediates formed especially in the field of proteomics and electrocatalytic oxidation of xenobiotics will be made possible by achieving spectroscopic measurement times in the scale of µs to ms and a limit of detection in the scale of nM to µM.

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