

SIMULTANEOUS IMPEDANCE AND FLUORESCENCE DETECTION OF PROTEINS IN A CYCLO OLEFIN POLYMER CHIP CONTAINING A COLUMN WITH AN ORDERED PILLAR ARRAY WITH INTEGRATED GOLD MICROELECTRODES

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ABSTRACT

In this work, we report the detection of proteins by means of simultaneous fluorescence and impedance measurements in a cyclo olefin polymer (COP) chip containing an ordered pillar array column, used for reversed-phase liquid chromatography, with integrated microband gold electrodes at the end of the channel.

KEYWORDS: cyclo olefin polymer, gold microelectrodes, pillar array, protein detection, impedance

INTRODUCTION

Reversed-phase liquid chromatography is a very powerful technique for the analysis of peptides and proteins because of a number of factors that include the excellent resolution that can be achieved under a wide range of chromatographic conditions, the experimental ease with which chromatographic selectivity can be manipulated through changes in mobile phase characteristics, the generally high recoveries, and the excellent reproducibility of repetitive separations carried out over a long period of time [1].

Here, we present first experiments using a COP column fabricated via hot embossing to perform simultaneous impedance and fluorescence detection of proteins. The microfabricated COP column has already been demonstrated to be suitable for on-chip pressure-driven reversed phase liquid chromatography [2]. In addition, the microband gold electrodes deposited on a complementary COP sheet were successfully tested through electrochemical experiments in organic media [3].

THEORY

Protein separation is one of the few applications where non-porous columns, like the one fabricated in COP and described in this work, still play a major role. That is because when using porous columns for the separation of large molecules like proteins the inherently small diffusion coefficient has a dramatic impact on dispersion. When the pore size is of the same order as an analyte, the internal surface can become inaccessible or, when accessible, give rise to a small intra-particle diffusion coefficient and hence, a dramatic increase of the C-term, which leads to a dramatic decrease of the efficiency of the porous column.

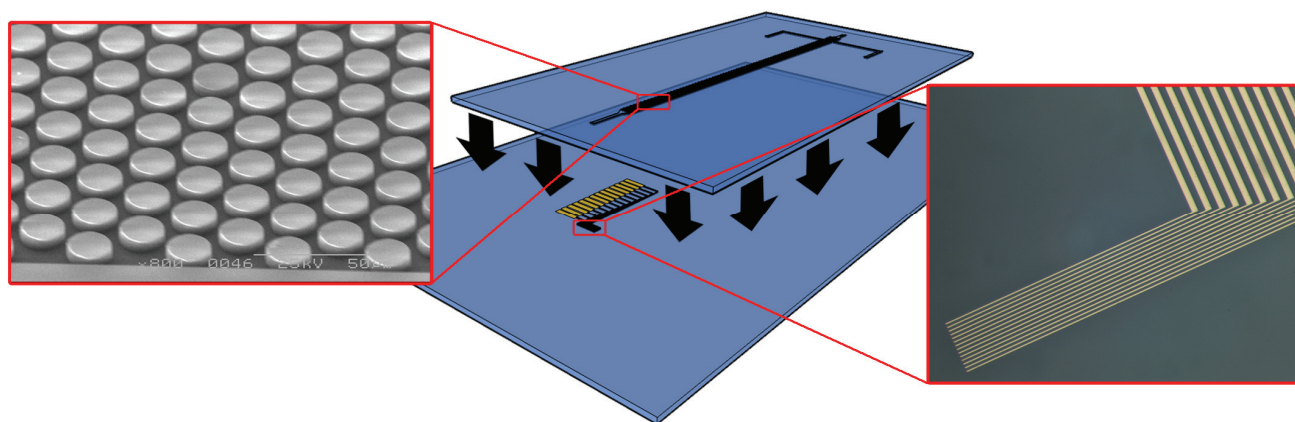


Figure 1: Sketch of the different layers comprising the COP chip. Above, the sheet containing the pillar array column with a detailed SEM image of the pillars (top). Below, the sheet containing the deposited microband gold electrodes with an optical microscope image of the microelectrodes (bottom).

EXPERIMENTAL

The chip presented in this work has been fabricated by pressure assisted thermal bonding between a COP sheet containing a 8.0 μm deep, 318 μm wide and 50 mm long separation column with pillars of 15.3 μm in diameter fabricated *via* hot embossing, and a COP sheet containing the deposited gold microelectrode array (14 μm wide, 16 μm separated electrodes) fabricated by standard lithographic techniques. In Fig. 1, a sketch of the two layers comprising the COP chip is shown, while Fig. 2 shows the end part of the bonded chip where part of the separation column and the microelectrodes are placed.

Firstly, mixtures of different coumarin dyes have been successfully separated (as shown in Fig. 3) and detected using fluorescence in the fabricated COP column demonstrating that reversed-phase separations are possible with this novel column format. Separations are possible due to the hydrophobic nature of the unmodified COP surface as it was firstly demonstrated by Guftafsson *et al.* [4]

Fluorescence and impedance detection of avidin (labeled by means of Alexa Fluor® 488 conjugate), which was dissolved in a phosphate buffered saline solution (PBS) in a concentration of 0.2 mg/ml and injected into the fabricated microchannel using the injection set-up already described in [2], were performed simultaneously. Fluorescence measurements were obtained by a CCD camera while impedance measurements were obtained by connecting two gold band electrodes from the electrode array to an impedance analyzer and monitoring the resistance of the system at 1MHz frequency using an RC parallel mode.

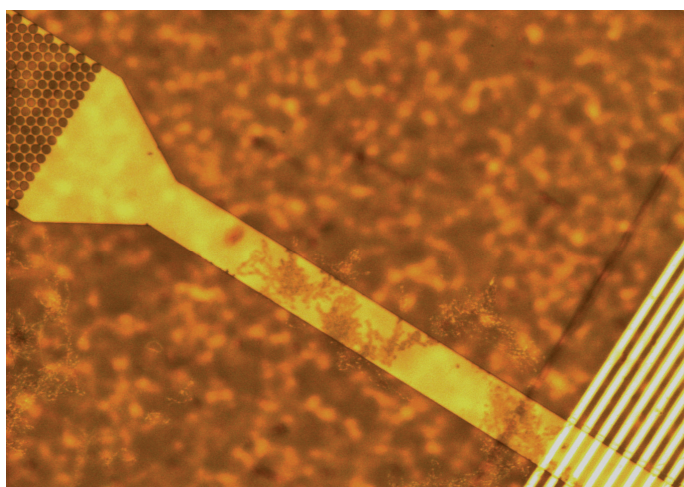


Figure 2: Optical microscope image of the bonded chip showing the end of the pillar array separation column where the microband electrodes are placed.

RESULTS AND DISCUSSION

The results for the fluorescence and the impedance of a detected protein plug have been compared, showing perfect agreement as there is no substantial difference in the peak width, as is shown in Fig. 4. Impedance measurements were demonstrated as well for the detection of Neutravidin dissolved in PBS and, in general, they always showed a good reproducibility. The presence of the protein in the buffer makes the concentration of free ions to increase and hence the resistance of the protein plug is always lower than that of the buffer alone (Fig. 4). The change in the measured resistance due to the transition depends on the concentration of the protein in the buffer. In our particular case, the concentration was 0.2 mg/ml and the resistance changed by approximately 25%; however, due to the very low levels of noise observed (less than 0.5% of the signal), the concentration of the protein could be much lower.

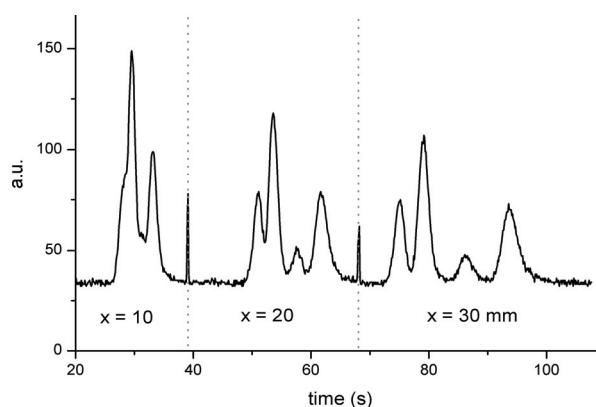


Figure 3: Chromatogram of a reversed phase liquid chromatography separation of a 4-components mixture (coumarins C440, C450, C460 and C480) performed in the presented chip and detected by fluorescence.

These results demonstrate that impedance detection of proteins with integrated electrodes is a powerful and versatile technique that can be applied for any analyte presenting electrical properties that differs from that of the used buffer. In addition, impedance is a label-free technique that only depends on the intrinsic properties of the material. This simplifies any experimental assay as previously unavoidable steps for labeling of the analyte can be eliminated.

Experiments to analyze in more detail, with fluorescence and impedance measurements, an on-chip protein separation are underway. The use of isocratic conditions for reversed-phase protein separation is limited due to the very narrow partition range available for a given protein. Although it has already been proved that it is possible to achieve a separation under these conditions [5], the possibilities of this mode are very limited. For that, gradient elution may be required to perform protein separations. The necessary setup for this separation is being implemented in our lab at this time.

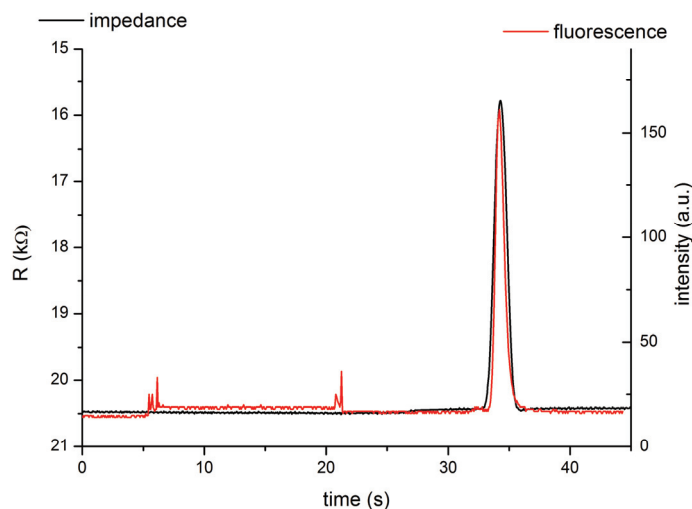


Figure 4: Impedance (black) and fluorescence (red) response of an injected avidin plug detected by an impedance analyzer and a CCD camera, respectively.

CONCLUSION

The presented results demonstrate the feasibility of this chip to be used in biological and chemical analysis where two different detection techniques can be used alternatively or in a complementary way. In particular, the introduction of microfabricated electrodes in a separation column can simplify the detection setup replacing a complex and expensive optical system with a simple electronic label-free detection.

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