KINETICS OF INSULIN ADSORPTION FROM REAL TIME OF MEASUREMENTS IN A MICROFLUIDIC CHIP

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ABSTRACT

We propose an original method to monitor insulin adsorption by EOF mobility measurements. Kinetics have been real-time recorded by particle anemometry in a Wheastone microfluidic bridge. Hybrid glass/photosensitive PDMS/glass microfluidic chips were fabricated to perform mixed hydrodynamic and electrophoretic cycles, with different insulin concentrations. From the real-time measurements the constants of adsorption kinetics were determined by fitting the extracted data according the Langmuir model. This platform offers accurate results even at 10^{-5} M insulin concentration.

KEYWORDS

Real-time monitoring, adsorption kinetics, electro-osmotic mobility,

INTRODUCTION

The surface instabilities in microfluidic systems are key issues for the development of highly reliable µTAS. Biomolecule adsorption onto the inner wall of the microfluidic channel often decreases the resolution and reproducibility of analytical experiments based on electrophoresis. Moreover medicinal peptide delivery by microdevices could also suffer from these unexpected concentration variation. To study peptide adsorption during mixed hydrodynamic and electrophoretic sequences we designed a hybrid glass/photosensitive PDMS/glass microchip in a Wheatstone bridge geometry [1] that can precisely measure the electro-osmotic mobility (EOF) modulation resulting from biomarker adsorption.

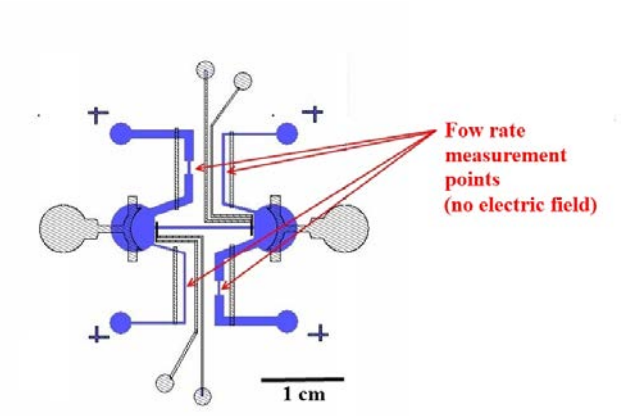

Figure 1. Lithographic masks of the Microfluidic Wheatstone Bridge (µFWB) [1] with H shape channel geometry for the fluidic network (in blue), the grey parts are platinum electrodes and short-circuits. The EOF velocity is measured by real-time particle anemometry in the four lateral channels of the µFWB.

EXPERIMENT

In this context, we present a novel approach to study insulin adsorption by particle anemometry. Neutral polystyrene fluorescent microbeads have been spread into the insulin buffer solutions to measure EOF by cross-correlation image treatment. In short, EOF of the central channel is calculated from the velocity of the beads in the four lateral channels of the microchip. As soon as insulin covers the polarisable interface the EOF decreases because of the Zeta potential modulation. It allows to quantify precisely protein interfacial adsorption on various materials. The material used in this study is a 100 nm thin SiC layer that was integrated onto the central microchannel (Figure 2) similarly as gate electrode in a polarisable fluidic transistor [2].

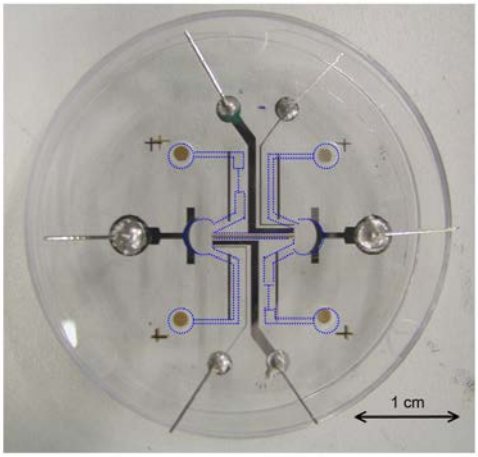

Figure 2. The µFWB chip that integrates the different electrodes, the amorphous 100 nm SiC layer in the central channel (see the small and long brown horizontal rectangle in the center of the image). This hybrid device is fabricated to have a sandwich "glass/photosensitive PDMS /glass" assembly [3-4]. The blue dashed lines show the fluidic network as a guideline for the eyes.

A glass/PDMS/glass technology was used to do sandwich microchips in a clean room. The borate buffer solution used for insulin was prepared with deionized water at a concentration of 40 mM and pH 9 and filtered using a filter of 0.22 microns. Then 1 micron diameter fluorescent polystyrene beads were added to perform particle anemometry measurements. A 50 volt electric field has been applied to create an electrophoretic migration of the insulin solution. The electroosmotic mobility values were obtained from the measured velocities taking into account the calibration factor of the chip and the length of the central channel, the applied voltage and the fluidic resistances of the channels.

RESULTS AND DISCUSSION:

The fast insulin adsorption onto the surface [3] challenges the feasibility of such EOF measurement at low peptide concentration. Indeed the literature reports only few data about fast biomolecular adsorption phenomena in µTAS. This is mainly due to the lack of fast and real time instruments to perform these experiments. The observed adsorption phenomenon arises from the surface interaction and from the low velocity of the fluidic stream at the solid-liquid interfaces. Three different concentrations of insulin have been prepared according to the concentration values used in conventional µTAS electrophoresis experiments: from 10^{-5} to 10^{-3} mol/L. Isotherm profiles of the adsorption have been plotted and fitted according to the Langmuir model [4].

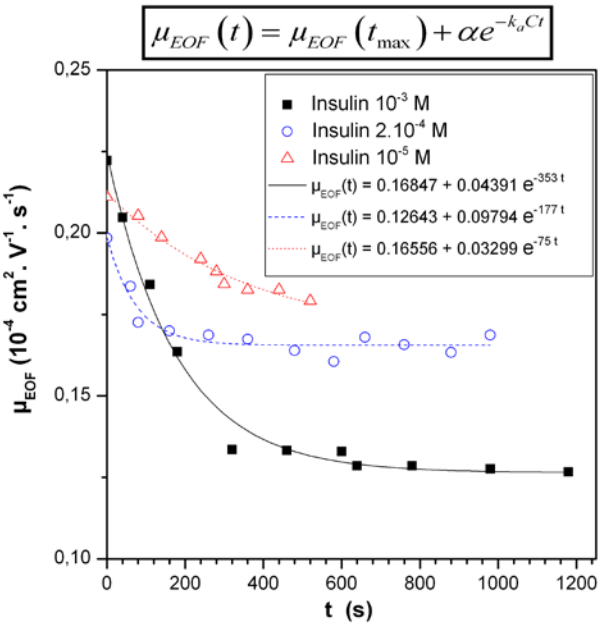

Figure 3. Variation with time of EOF during insulin adsorption experiments with the µFWB chip. At each concentration of insulin, the EOF curve has been fitted according to the Langmuir model [4].

While the adsorption of the 10^{-5} mol/L is rather slow, the higher concentrations induce a fast covering of the surface of the central channel. The kinetics parameters of adsorption extracted from Langmuir fits are reported in Table 1. By comparison with other classical methods for K_aC determination, our Wheatstone bridge chip allows precise extraction of kinetics parameters even at very low concentration (down to 10^{-5} M). The hundreds of seconds necessary to level off the exponential EOF modulation underline the idea that, during a typical time of one analytical experiment, the migration could be largely altered by insulin physisorption.

$C_{insulin}$	μ_{EOF} (t_{max}) ($10^{-4}.cm^2.V^{-1}.s^{-1}$)	α ($10^{-4}.cm^2.V^{-1}.s^{-1}$)	$k_a C$ (s^{-1})	K_a ($m^3.mol^{-1}.s^{-1}$)
10^{-3} M	0.12643	0.09794	177	177
2.10^{-4} M	0.16556	0.03299	75	375
10^{-5} M	0.16847	0.04391	353	35300

Table 1. Extracted data from the Langmuir model fitting.

CONCLUSION

To conclude, we have developed a fast and accurate platform that permits to present a comprehensive study of the kinetic of insulin adsorption at typical concentrations used for analytical purposes. An influence of the insulin concentration over two factors has been observed: the maximum adsorption time and the surface coverage.

In the future, we need to study the repeatability of our measurements to calculate relative standard deviations since these unspecific adsorption phenomena may be not reproducible. Moreover we started to study the adsorption of insulin on the fluorescent beads at low concentrations to know if it may affect our results.

ACKNOWLEDGEMENT

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