

DEVELOPMENT OF MICROFLUIDIC DEVICES INTEGRATING METAL ELECTRODES FOR ON-LINE PRECONCENTRATION AND A PROTON-SENSITIVE ISFET SENSOR

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ABSTRACT

This paper reports a development of microfluidic devices integrating an ion selective-field effect transistor (ISFET) sensor with a tantalum pentoxide as a sensing surface to detect protons. To attain high sensitivity in flow injection analysis, a novel on-line preconcentration of ionic analytes was demonstrated by integrating a pair of metal electrodes on a microchannel. In our proposed method, ionic analytes supplied by a continuous flow were trapped at the electrode due to the reversal electrophoretic migration of the analytes. The fabricated device was applied to the analysis of ammonium ions produced by enzymatic hydrolysis reaction of urea with urease.

KEYWORDS: PDMS microchip, ISFET, Enzymatic reaction, On-line sample preconcentration

INTRODUCTION

In the postgenomic era, considerable efforts have been devoted to elucidate biological functions *in vivo*. Recently, proteomics, *i.e.*, the analysis of all the function of celluler proteins, has become a powerful tool for gaining insight into system biology. Among various functions of proteins, the enzymatic activity is one of the important information, so that a rapid and sensitive measurement technique should be introduced in proteomics. To achieve a sensitive and continuous determination of the activity for a small amount of enzymes, we fabricated microfluidic devices integrating an ion selective-field effect transistor (ISFET) sensor with a tantalum pentoxide as a sensing surface to detect protons with a high sensitivity (Figure 1). The pH response of the ISFET sensor was as high as 820 mV/pH in both conventional batch and flow measurements.

To obtain further high-sensitivity in flow injection analysis, a novel and simple

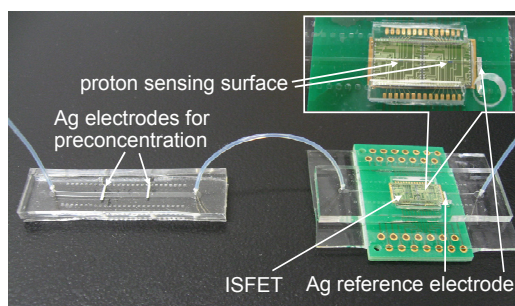


Figure 1. Photograph of the fabricated microfluidic device. The ISFET sensor was embedded in a microchannel fabricated on a PDMS substrate. Ag thin film was installed at the downstream of the ISFET detector as a reference electrode. For on-line sample preconcentration, two Ag electrodes were embedded at the upstream of the ISFET.

method for on-line preconcentration of ionic analytes was developed. In continuous flow analysis on microfluidic devices, solid-phase extraction or electrokinetic filtering on monolithic columns [1] and nanostructures [2] has been mainly employed as a preconcentrator. Apparently, it is difficult to fabricate such structures in the microchannel. In our proposed method, on the contrary, on-line preconcentration of ionic analytes under a continuous flow condition could be realized only by integrating a pair of metal electrodes on a microchannel. The concept of our preconcentration device is shown in Figure 2. In the preconcentration method, ionic analytes supplied by a continuous flow were trapped at the electrode due to the electrophoretic migration of the analytes with a reversal direction against the pumping flow. After the preconcentration, the trapped zone was eluted by switching off the voltage, and then the released analytes were detected by the ISFET sensor. Coupling of such preconcentrator with the ISFET sensor is promising for highly sensitive detection of ionic compounds.

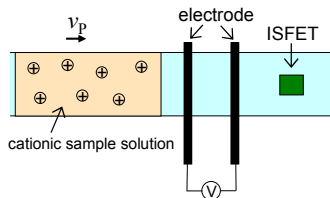
EXPERIMENTAL

Poly(dimethyl siloxane) (PDMS) microfluidic devices were fabricated by a conventional softlithography technique. For on-line preconcentration, a pair of Ag thin films was embedded onto the microchannel. To reduce the flow velocity around the preconcentration electrodes, a wide channel with 1 mm width was fabricated. For ISFET detection, the ISFET sensor was embedded in the PDMS substrate and the Ag/AgCl reference electrode was installed on the microchannel.

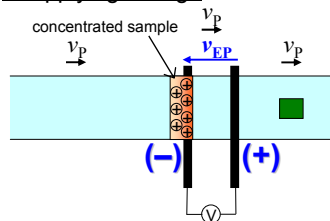
RESULTS AND DISCUSSION

To verify the on-line sample preconcentration mechanism on the device, fluorescent rhodamine 6G (R6G) was concentrated under a continuous flow condition (Figure 3). When no voltage was applied to the electrodes, the constant fluorescent intensity was observed at the outlet side of the cathode. During the application of the voltage of 200 V, the intensity was gradually decreased with time, and then the fluorescence was not detected. After switching off the voltage, a sharp peak appeared due to releasing the trapped R6G. Under this condition, the electrophoretic migration velocity of R6G was estimated to be 0.36 mm/s, which was higher than the

1. Large volume sample injection



2. Applying voltage



3. Detection

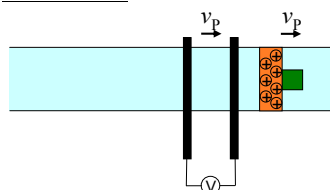


Figure 2. Concept of on-line preconcentration of ionic analytes under the continuous flow condition on a microfluidic device integrating a pair of electrodes. v_p , pumping flow velocity; v_{EP} , electrophoretic migration velocity.

pumping flow rate of 0.13 mm/s. Thus, R6G could not overtake the cathode as shown in Figure 2, *i.e.*, the analytes continuously supplied by the pumping flow could be concentrated on the electrode. When urease was introduced in the continuous flow of urea, the pH change induced by ammonium ions produced in the enzymatic hydrolysis reaction was successfully detected at the ISFET sensor embedded in the downstream of the channel. The limit of detection of urease was 0.1 pmol. When the voltage was applied for 50 s, an enhanced and sharp peak, which corresponded to the trapped and concentrated ammonium ions, was successfully obtained (Figure 4). Therefore, the fabricated device allowed preconcentration and detection of inorganic ions produced by the enzymatic reaction under the continuous flow condition.

CONCLUSIONS

A microfluidic device integrating the electrode preconcentrator and the ISFET sensor was developed. On the fabricated device, cationic analytes were successfully concentrated around the cathode under the continuous flow condition and the concentrated sample was detected by the ISFET. This device is expected to realize highly sensitive detection of ionic products formed by various enzymatic reactions.

ACKNOWLEDGEMENTS

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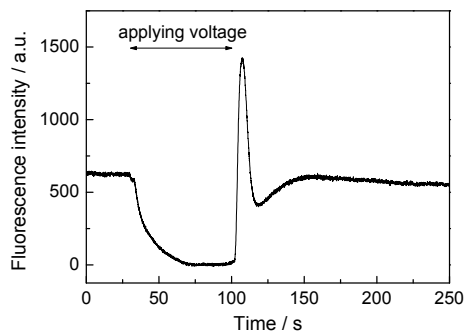


Figure 3. On-line preconcentration of rhodamine 6G continuously supplied by the pressure flow on the fabricated device. Electrode gap, 10 mm; electrode width, 0.5 mm; applied voltage, 200 V; flow rate, 0.13 mm/s; distance of detection point from the cathode, 0.4 mm.

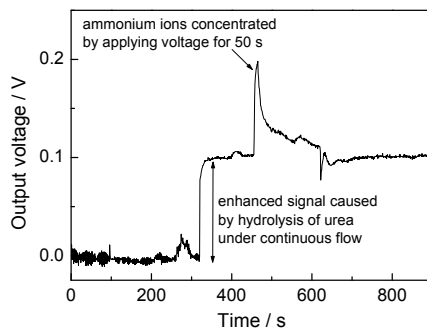


Figure 4. On-line preconcentration of ammonium ions produced by the hydrolysis reaction of urea enzymatically catalyzed by urease under the continuous flow on the fabricated device. Conditions are as in Figure 3.