



Automatic electrochemical sequential processing in a microsystem for urea detection

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ARTICLE INFO

Article history:

Received 28 July 2009

Received in revised form 10 October 2009

Accepted 20 October 2009

Available online 30 October 2009

Keywords:

Automatic processing

Electrowetting

Valves

pH regulation

Ammonia

Urea

ABSTRACT

To demonstrate the possibility of automatic serial operation in a microanalysis system, components for valve operation, adjustment of mixing time, pH regulation, and sensing functions were integrated. Electrodes for actuation and sensing were formed on a glass substrate and flow channels and compartments were formed using poly(dimethylsiloxane) (PDMS). When a urea standard solution was filled in half of a mixing area, a mixing valve opened automatically and the solution merged with a urease solution initially filled in the other half. The length of time required for the mixing of components was adjusted by using a delay line, which extended from the electrode used for the mixing valve. After a time delay, an injection valve at the end of the mixing chamber was opened automatically. After the solution was injected into the compartment of the pH regulator using a nonstandard three-electrode system, the solution pH was elevated automatically. Gaseous ammonia produced accompanying the change was detected using an air-gap ammonia sensor. The existence of ammonia was clearly indicated by a change in electrode potential. The dependence of the potential on the logarithm of the concentration was linear, confirming the Nernstian response.

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1. Introduction

Micro-bio/chemical analysis systems have witnessed considerable developments over the last decade, and therefore, increasingly sophisticated devices are expected in the coming decade. Although many issues have been noted with regard to the realization of sophisticated, next-generation analysis systems, automatic sequential operation of components has been emerging as a new trend [1–7]. Biochemical analyses include steps such as separation, mixing of components, adjustment of pH, and sensing. Thus far, however, few microchips are available that can carry out the abovementioned series of steps. Although some interesting devices have been proposed for automatic device operation, especially some that take advantage of the spontaneous volume change in stimulus-responsive gels [8–10], a more likely and realistic approach will be to use the transported solutions themselves for switching.

For related components to be switched on by a transported solution, electrochemical principles are advantageous, because electrodes used for driving can easily be coupled with the transported solution. To realize on-chip microfluidic transport, electrowetting-based valves are advantageous [11–13]. Since an

aqueous solution moves spontaneously in a hydrophilic micro-flow channel by capillary action, solution transport has been controlled by using valves located in some critical portions. Otherwise, an electrowetting-based valve was formed in the form of a gold electrode to separate two compartments. The valve opened when the compartments were filled completely [6]. Furthermore, an electrowetting-based valve was coupled with a pH-sensitive electrode to realize a pH-responsive valve [14]. A pH filter that passed a solution whose pH fell in a limited range was also fabricated. These devices have opened up a new possibility of carrying out switching operations without requiring software control. Based on the concepts used in these devices, automatic switching of more complicated sequential functions could be realized.

In addition, electrochemical devices can be used advantageously to drive other components. We have proposed an electrochemical pH regulator that uses a nonstandard electrochemical three-electrode system [15]. As in the case of the electrowetting-based valves mentioned earlier, these devices could be switched on upon the introduction of a solution in the compartment with the electrode. In this study, we demonstrate the sequential operation of devices that can be used to carry out the steps required in an enzyme analysis. In addition to the functions mentioned earlier, we integrated functions to adjust the length of time required for mixing and to measure the concentration of ammonia and urea. The construction and performance of the device are discussed.

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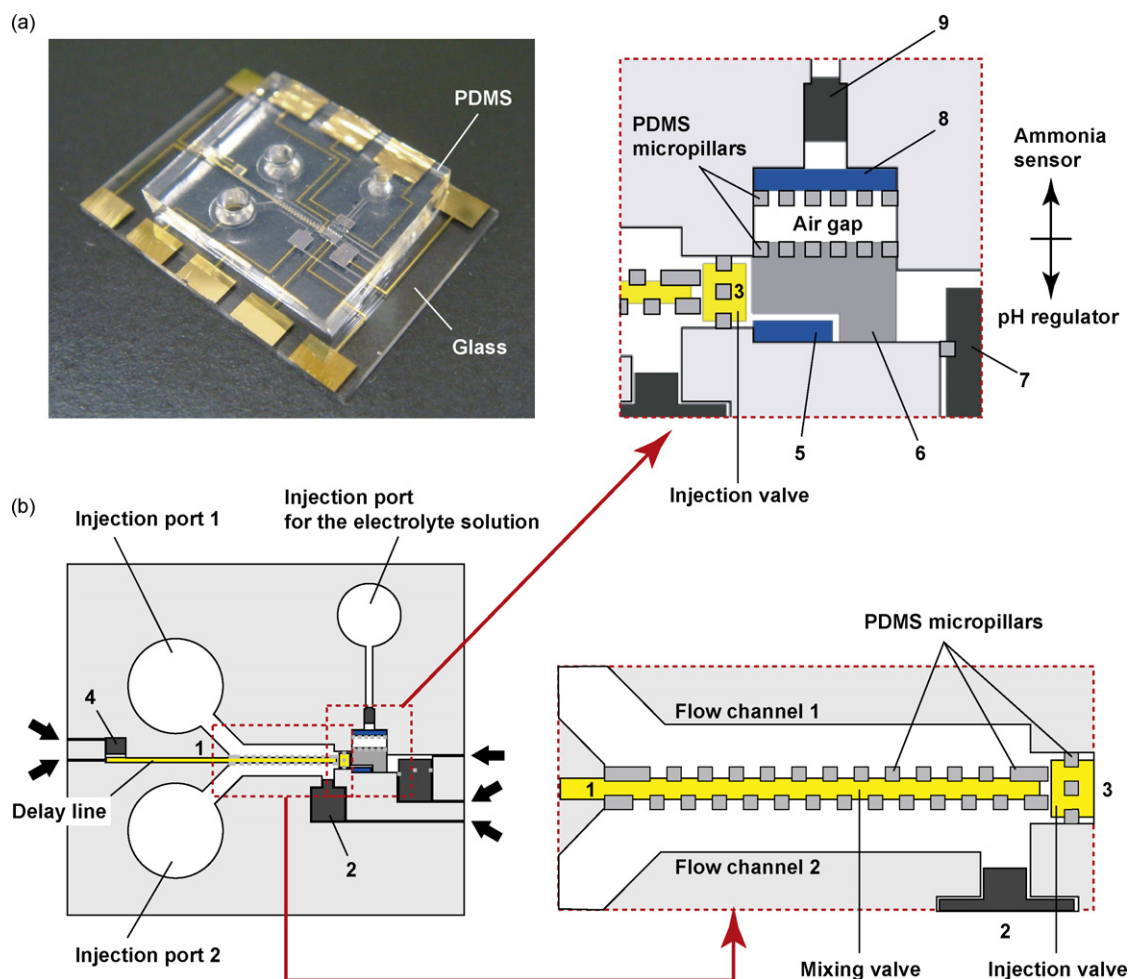


Fig. 1. Device for automatic sequential operation. (a) Completed system. (b) Layout of the integrated components. The figures on the right show magnified views of the corresponding areas in the figure on the left. Leads are omitted to avoid complication. The numbers indicate the electrodes described in Table 1. Black arrows indicate the positions of air vents. Dimensions of the chip are 20 mm × 15 mm.

2. Experimental

2.1. Materials and reagents

Materials used for the fabrication of the device were obtained from the following commercial sources: glass wafers, No. 7740 (diameter: 3 in, thickness: 500 μm), from Corning Japan, Tokyo, Japan; a negative photoresist, OMR-83, from Tokyo Ohka Kogyo, Kawasaki, Japan; a thick-film photoresist, SU-8, from MicroChem, Newton, MA, USA; and a PDMS precursor solution, KE-1300T, and room-temperature vulcanizing (RTV) silicone rubber, CAT1300, from Shin-Etsu Chemical, Tokyo, Japan. A universal pH-indicator dye was purchased from Nihon Finechemical (Tokyo, Japan). Urease (E.C. 3.5.1.5, from Jack Bean, 113 U/mg) and other reagents were purchased from Wako Pure Chemical Industries, Osaka, Japan, unless otherwise noted. All solutions were prepared using distilled deionized water.

2.2. Structure and construction of device

Fig. 1 shows the structure and construction of the device. The components integrated on the chip included the following: an electrowetting-based mixing valve, a delay line to regulate the time required for mixing, an electrowetting-based injection valve that injects the mixed solution into the compartment of a pH regulator, the pH regulator, and an air-gap ammonia sensor. All

the components were operated based on electrochemical principles.

The device was constructed with a glass substrate with electrodes and a PDMS substrate with structures for flow channels and compartments. The necessary structures in the PDMS substrate were additionally formed by replica molding using a template formed with the thick-film photoresist (SU-8). Through-holes were formed at the inlets of the flow channels. Air vents were also formed at appropriate locations to release pressurized air and facilitate solution transport. Electrode materials were selected for their respective purposes (Table 1). The electrodes were formed by a thin-film process including metal deposition by sputtering, formation of photoresist patterns, chemical etching, and lift-off. First,

Table 1
Material and role of the electrodes.

Electrode	Material	Role
1	Au	Mixing valve (W.E.) and delay line
2	Ag/AgCl	R.E. for the mixing valve (R.E.1)
3	Au	Injection valve (W.E.)
4	Ag/AgCl	R.E. for the injection valve (R.E.2)
5	Ir oxide	pH-sensing electrode for the pH regulator (R.E.)
6	Ir	Actuator electrode to cause pH changes (A.E.)
7	Ag/AgCl	W.E. for the pH regulator
8	Ir oxide	pH-sensitive electrode for the ammonia sensor
9	Ag/AgCl	R.E. for the ammonia sensor

W.E., working electrode; R.E., reference electrode; A.E., auxiliary electrode.

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