

# A Capillary Electrophoresis Chip for Rhodamine B and Inorganic Ion detection Utilizing Contactless Capacitance Detection

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## ABSTRACT

A microfluidic channel and a contactless capacitance detector were integrated on a glass chip by a semiconductor manufacturing process for separating and analyzing Rhodamine B and inorganic ion. The electrodes of the capacitance detector were formed successively by sputtering, electroless and electrodepositions. Samples containing fluorescent agent (Rhodamine B) and inorganic ions ( $K^+$ ,  $Na^+$ , and  $Li^+$ ) were injected and separated in the microfluidic channel by the electrical voltage and detected by contactless capacitance measurement proposed in this work. The results showed that all the measured capacitance signals were very sharp and the reproducibility was quite well.

**Keywords:** capillary electrophoresis, contactless capacitance detection, microchip, microfluidic electrophoresis

## 1 INTRODUCTION

Capillary electrophoresis (CE) is an important means for separation and analysis due to its high separation efficiency, quick analysis speed, small sample quantity and wide application field [1-4]. Among the detection methods for CE there chiefly are directly/indirectly optical detections and electrochemical detections [5]. The former contains ultraviolet or visible light absorbance, fluorescence, laser induced fluorescence and so on. The latter contains potentiometry [6], amperometry [7,8], and conductometry [9-11]. The conductometry has contact detection [12-15] and contactless detection modes [16-20]. In the contact conductivity detection the electrodes are formed in the fluidic channel so the fluid filled would be interfered, some unwanted reactions would occur and the manufacture of the detector would be not easy. Therefore, the contactless detection has been more and more studied and developed recently. Up to now there are two different device designs for the contactless conductivity detection for CE. The electrodes with ring shape are formed around the outer surface of the glass capillary tube in one design [21, 22] and the electrodes with film shape are formed near the microfluidic channel in the other design [23, 24]. The latter is more and more important because it can be further miniaturized and its manufacturing process is quite simple.

For the contactless conductivity detection for CE, there are glass (or others), liquid solution and air existing between the two electrodes. When the two electrodes are applied an alternating voltage, i.e. an alternating electrical field, the responses of these medias, i.e. (glass, liquid solution and air) to the alternating electrical field can be represented by a complex dielectric constant  $\epsilon_r$ : [25]

$$\epsilon_r = \epsilon_r' - j\epsilon_r'' \quad (1)$$

where  $\epsilon_r'$  is the real part and  $\epsilon_r''$  is the imaginary part, both being frequency dependent. The impedance  $Z$  of these media is related to the complex dielectric constant by the following formula:

$$Z = d / (j2\pi f A \epsilon_o \epsilon_r) = d / [j2\pi f A \epsilon_o (\epsilon_r' - j\epsilon_r'')] \\ = 1 / [(j2\pi f C) + 1/R] \quad (2)$$

Where  $f$  is the frequency of the alternating electrical field,  $d$  and  $A$  are the distance and area of the two electrodes, respectively,  $\epsilon_o$  is the permittivity of vacuum,  $C$  is the capacitance and equals to  $A \epsilon_o \epsilon_r' / d$ ,  $R$  is the resistance and equals to  $d / (2\pi f \epsilon_o \epsilon_r'' A)$ .

Because the measured signal of the contactless conductivity detection for CE is related with the impedance  $Z$ , its value is greatly affected by the frequency of the alternating electrical field. This is one of problems encountered in the contactless conductivity detection for CE. If the measured signal of the contactless detection for CE is obtained from not the conductivity but the capacitance, this problem will be less serious.

The capacitance of dielectric medium,  $C = A \epsilon_o \epsilon_r' / d$ , is determined by  $A$  (the area of electrodes),  $d$  (the distance between two electrodes) and  $\epsilon_r'$  (the real part of the dielectric constant). The frequency dependence of  $\epsilon_r'$  comes from the polarization of dielectric media, containing interfacial (or space charge), orientational (or dipolar) ionic and electronic modes. The  $\epsilon_r'$  almost keeps unchanged at any frequency but near that  $f = 1/2\pi\tau$ , where  $\tau$  is the relaxation time of electric medium for any polarization mechanism. There is an abrupt change of  $\epsilon_r'$  near microwave frequencies ( $f = 10^6$  Hz) for the orientational

polarization, infrared frequencies ( $f \sim 10^{12}$  Hz) for the ionic polarization, and ultraviolet frequencies ( $f \sim 10^{16}$  Hz) for the electronic polarization. And for the interfacial (space charge) polarization the  $\epsilon_r'$  would vary for  $f < 10^6$  Hz. For glass the polarization mechanism contains ionic and electronic modes. And the orientational, ionic and electronic polarization mechanisms take place in most liquids and air [25].

On the basis of the above consideration, the capacitance can be used for the contactless detection for CE. In the process of contactless capacitance detection for CE, before the samples (charged atoms or molecules) are injected into the capillary or the microchannel, and if the frequency of the alternating voltage applied to the electrodes of the contactless capacitance detection for CE doesn't exceed the microwave range ( $f \sim 10^6$  Hz), the measured capacitance values can be expected to be small and kept steady. And after the samples are injected into the capillary or the microchannel, the measured capacitance values can also be expected to vary greatly due to the space charge polarization of the charged atoms or molecules in buffer solution. Therefore, using capacitance as the measured signal in the contactless detection for CE is one of the aims of this work.

The capacitance  $C (A \epsilon_0 \epsilon_r' / d)$  is also affected by  $A$  (the area of the electrodes) and  $d$  (the distance between the electrodes). The other aim of this work is to make a microfluidic channel and two electrodes on a glass chip in such a manner that the area of the two electrodes can be greatly increased and the distance between the two electrodes can be much decreased for promoting the capacitance detection efficiency.

## 2 EXPERIMENTAL

The measurement system in this work consists of a microchip, a high voltage power supply, an LCR meter and a personal computer. The microchip consists of two sodium glass plates. The top glass plate is used to guide the solution and to protect the microfluidic channel and the two electrodes formed in the bottom glass plate. The microfluidic channel and electrode recesses in the bottom glass plate was manufactured by the semiconductor manufacturing process as the followings:

1. Mask making: A plastic film was used for mask making. The mask pattern was first designed by computer assisted design (Auto CAD) and then printed on the plastic film by a laser printer with high resolution.
2. Photo-resist coating: AZ4620 positive photo-resist was coated on the bottom glass plate by a spin coater to be used as a mask for the glass etching. Because AZ4620 positive photo-resist cannot well adhere to the sodium glass, a film of hexamethyldisilazane (HMDS) was beforehand coated on the bottom glass plate to promote the adherence of photo-resist.
3. Ultraviolet (UV) exposure: The mask was put on

the photo-resist and irradiated by UV. The pattern on the mask was then transferred to the photo-resist.

4. Photo-resist developing: Developing solution was used to dissolve the exposed part of photo-resist and the unexposed part was retained.
5. Etching: The glass uncovered by the photo-resist was etched by buffered oxide etching solution (BOE) and the microfluidic channel and the electrode recesses were then formed.

The electrodes in the bottom glass plate were made by the following processes:

1. The sputtering deposition of Ti: Because Ti can improve the adherence of Cu to glass, a film of Ti was beforehand deposited on the glass plate by sputtering deposition with DC150W power at  $7.0 \times 10^{-6}$  torr.
2. The electroless plating of Cu: After sputtering deposition of Ti, the bottom glass plate was first cleaned in deionized water with ultrasonic vibration and was successively sensitized in  $\text{SnCl}_2 + \text{HCl}$  solution, activated in  $\text{PdCl}_2 + \text{HCl}$  solution, and then deposited a film of Cu in electroless Cu plating bath. This electroless Cu film was for the convenience of the electroplating of Cu because the Ti film was not thick enough for the electroplating of Cu.
3. The electroplating of Cu: A thick film of Cu was deposited on the electroless Cu film in the electroplating bath with 30 mA of current density.
4. The sputtering deposition of Au: To protect the electrodes, thin film of Au was deposited on the electroplating Cu film by sputtering deposition with DC 150W power at  $7.0 \times 10^{-6}$  torr.
5. Lithography and etching: Excepting the mask pattern and the etching solution, the processes of mask making, photo-resist coating, UV exposure, photo-resist developing and etching for the manufacture of the electrodes were all the same as for the manufacture of the microfluidic channel. The etching solutions contained transcene USA for Au etching, nitric acid (10%  $\text{HNO}_3$ ) for Cu etching and hydrofluoric acid (5% HF) for Ti etching.

The top and the bottom glass plates were connected by fusion bonding in a vacuum furnace at 7.6 torr and 650 °C.

The measurement system in this work was shown as Fig. 1. The four solution reservoirs of the microfluidic channel were connected with the positive and the negative electrodes of the high voltage power supply, respectively. The two electrodes in the microchip were connected with the electrodes of the LCR meter for capacitance measurement. The measured capacitance variation with time was input to a computer for storage and analysis. Before the capacitance was measured, the microfluidic channel was first filled with a buffer solution and a sample was then injected and separated in the microfluidic channel by a high voltage. The dimension of microchannel and electrodes were measured by scanning electron microscope and  $\alpha$ -step profilometer.

## 3 RESULTS AND DISCUSSIONS

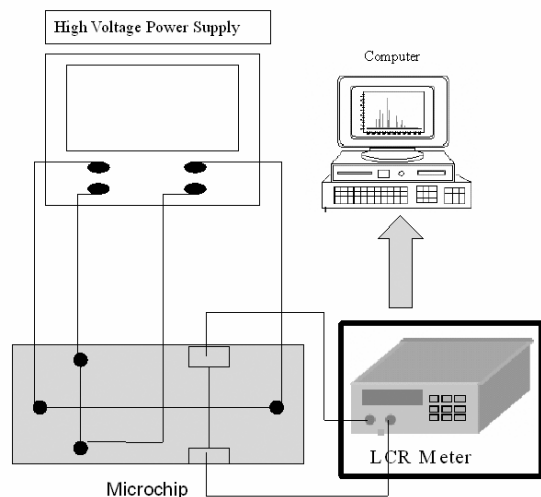


Figure 1: The measurement system consists of a microchip, a high voltage power supply, an LCR meter and a personal computer.

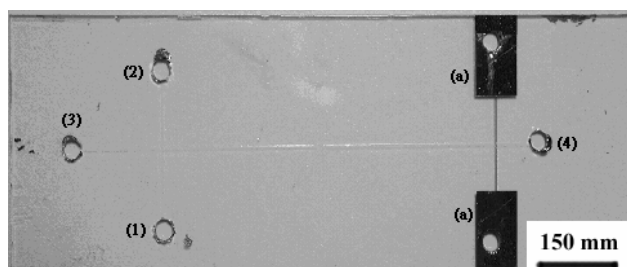


Figure 2: Integrated on-chip contactless two-electrode capacitance detection for microfluidic channel electrophoresis, (a): the two electrodes, (1)-(4): the solution reservoirs, and the microfluidic channels between (1) and (2), and (3) and (4).

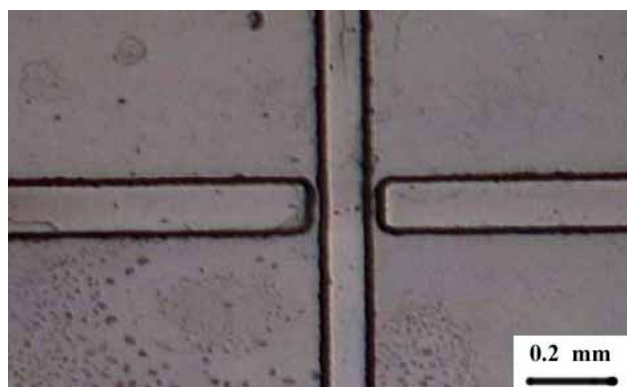


Figure 3: The enlargement of the microfluidic channel and the electrode recesses, the width of the microfluidic channel and the electrode recesses being about 80  $\mu\text{m}$  and 110  $\mu\text{m}$ , respectively the spacing between the electrode and the microfluidic channel being about 20  $\mu\text{m}$ .

The bottom glass plate after fusion bonding with the top glass plate is shown in Fig. 2. There are six through holes in the glass plate, where (a) are the two electrodes, (1) ~ (4) are the solution reservoirs and there are microfluidic channels among them. Fig. 3 is the enlargement of the microfluidic channel and the electrode recesses, where the width of the microfluidic channel and the electrode recesses are about 80  $\mu\text{m}$  and 110  $\mu\text{m}$ , respectively and the spacing between the electrode recess and the microfluidic channel is about 20  $\mu\text{m}$ . The depth of the microfluidic channel and the electrode recess are about 16  $\mu\text{m}$  were determined by step measurement.

A fluorescence agent (Rhodamine B) with  $3 \times 10^{-4}$  M concentration was taken as the sample for measurement. Before measurement a buffer solution (MES/His pH 6.1) with 10 mM concentration was filled in the microfluidic channel. When the fluorescence agent injected into the reservoir 1, a high voltage of 800 volts was applied to the reservoir 1 and the reservoir 3 was grounded. Then a high voltage of 1000 volts was applied to the reservoir 2 and the reservoir 4 and 1 were grounded for the separation of the sample. Fig. 4 shows the result of capacitance measurement. The capacitance response was very sharp. Furthermore, six measurements were successively carried out in this work. It is clearly seen that the reproducibility is very well. From the results described above, the fluorescence agent (Rhodamine B) in the buffer solution (MES/His, pH6.1) can be well detected by the device and the method proposed in this work and also has well capacitance response and reproducibility.

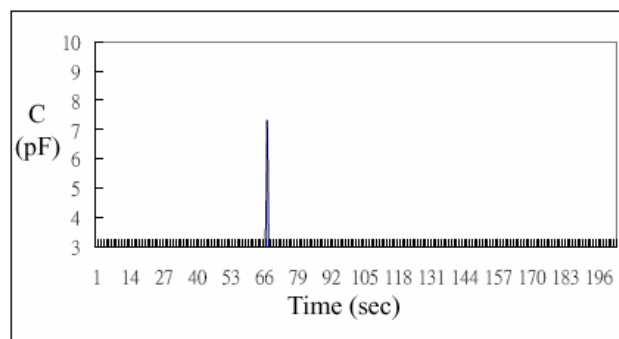


Figure 4: The measured capacitance variation with time for the fluorescence agent (Rhodamine B).

For detecting inorganic ions a mixed solution (one part 1 ml/L KCl, one part 1 ml/L NaCl, and one part 1 ml/L NaCl) was used as samples for separation and measurement by the contactless capacitance detection. The result was shown in Fig. 5. It is clearly seen that all the response signals are quite sharp, the drift velocity of the inorganic ions has the following order :  $\text{K}^+ > \text{Na}^+ > \text{Li}^+$  and the  $\text{K}^+$ ,  $\text{Na}^+$ , and  $\text{Li}^+$  in the mixed solution can be well separated. The drift velocity of inorganic ions in buffer solution can be determined from the channel length and the drift time shown in Fig. 5. The ratio of drift velocity for  $\text{K}^+ : \text{Na}^+ : \text{Li}^+$  is about 2.1: 1.45: 1 in the buffer solution (MES/ His pH

6.1) with 10mM concentration under the drive of 255 volts/cm electrical field.

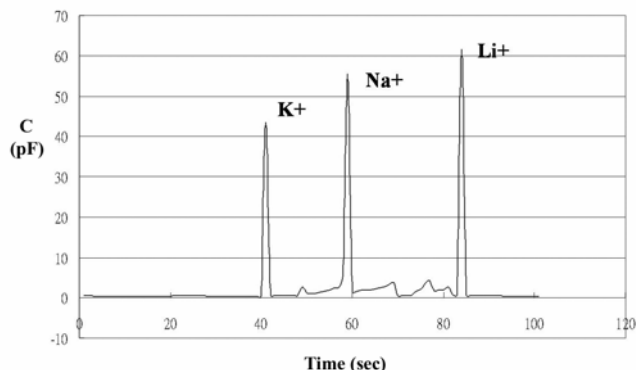


Figure 5: The successive measured capacitance variation with time for the mixed solution (one part 1 ml/L KCl, one part 1 ml/L NaCl, and one part 1 ml/L NaCl).

#### 4 CONCLUSIONS

This work used the semiconductor manufacturing process and various deposition techniques successfully to manufacture an integrated on-chip contactless capacitance detector for microfluid electrophoresis. This device has a structure with two layers. The top layer is a sodium glass plate which was adhered to the bottom layer (a sodium glass plate) by fusion bonding for protecting the microfluidic channel and the electrodes formed in the bottom glass plate. The microfluidic channel is about 80  $\mu\text{m}$  in wideness and about 16  $\mu\text{m}$  in depth. The electrodes are about 110  $\mu\text{m}$  in wideness, about 16  $\mu\text{m}$  in depth (thickness) and about 20  $\mu\text{m}$  in spacing from the microfluidic channel. The electrodes were formed successively by sputtering, electroless, and electrodepositions and on their surface, an Au film was deposited by sputtering deposition for anti-oxidization..

A fluorescent agent (Rhodamine B) and inorganic ions ( $\text{K}^+$ ,  $\text{Na}^+$ , and  $\text{Li}^+$ ) were injected and separated by the electrical voltage and detected by the contactless capacitance detector proposed by this work. The results showed that all the measured capacitance signals were very sharp and the reproducibility was quite well. The drift velocity of the inorganic ions has the following order:  $\text{K}^+ > \text{Na}^+ > \text{Li}^+$  and the  $\text{K}^+$ ,  $\text{Na}^+$ , and  $\text{Li}^+$  can be well separated in the buffer solution (MES/ His pH 6.1) with 10mM concentration under the drive of 255 volts/cm electrical field.

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