Development and Electrical Analysis of DNA Aqueous Solution on Microchannel Biochip

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Abstract

A microchannel biochip employed by micro-electro-mechanical systems (MEMS) and based on Si substrate was designed and developed in the research. The ionic CH_3COOH or DNA aqueous solutions were filled in the microchannel for the reagents in the experiment and the DC electrical characterization of the samples was carried out and analyzed when applying voltage in the microchannel. Furthermore, the curve of current versus voltage of these reagents was measured precisely by a high-resolution measurement system. In the experiments, it was successfully to distinguish the reagents as dATP, dGTP, dTTP, and dCTP. Moreover, the curves displayed oscillation phenomena with time because the accumulating charges generated a diffusion effect to counteract the electrical drift effect caused by applying voltage. The initial results demonstrated that the designed microchannel biochip is feasible to be used and distinguish different DNA molecules. The method should be beneficial for the advanced studies and applications of biomedical diagnostic technology.

***Keywords*:** Biochip, Microchannel biochip, DNA analysis, Electrical characterization

Introduction

The nucleus of every living cells holds chromosome composed of Deoxyribonucleic Acid (DNA) and proteins [1]. The DNA molecules bear the whole genetic information of the individual species through the genes. For instance, human genome consists of 80000 genes [2] and access to genetic information is ultimately limited by the ability to screen DNA sequence. Therefore, DNA chips have been developed for years to measure the sequence and size of DNA [3]. DNA chips comprise a wide range of microsystem applications that are attracting an increasing level of interest in recent years.

Microsystem technology application in the field of DNA diagnostics began in the 1990s. Microsystems for DNA analysis, also termed DNA chips, give clear signs of a short-term improvement in traditional DNA analytical procedures both in terms of speed and cost [4].

According to the micro-total-analysis-system (μ-TAS) [5] fields, chemists developed the conventional DNA chips and other nonsilicon-processing related engineering, which leads to the appearance of many devices, based on glass, plastic [6] and ceramic substrates. With the beginning of development in DNA chip, silicon substrates were studied and developed, as they should offer many more possibilities than other substrates, such as control circuits and detect circuits. However, glass and plastic substrates are still the leading technology in this sector because of their low price and insulating properties.

DNA biochip is one of the latest techniques for DNA analysis. Electrophoresis enables researchers to discriminate and identify DNA fragments with respect to their relative sizes [7]. Traditional electrophoresis systems operate in the 300 V [8] range on slab-gel preparations. Negatively charged DNA migrates through the gel and becomes separated in terms of its relative size. Separations on the 300 V range are slow, with a varying scope stretching from the 20 minutes of a conventional fragment separation, to the almost 2 hours of a sequence resolving run. In order to achieve higher speeds, this research is developing a new chip to measure the current and voltage of the fragment of DNA in the microchannel on biochip combined with a high-resolution measurement system. Besides, micro-electro-mechanical systems have many advantages, such as: high precision, high performance, low energy consumption, and mass production realization. Therefore, the subject chooses the MEMS technology to develop the DNA microchip.

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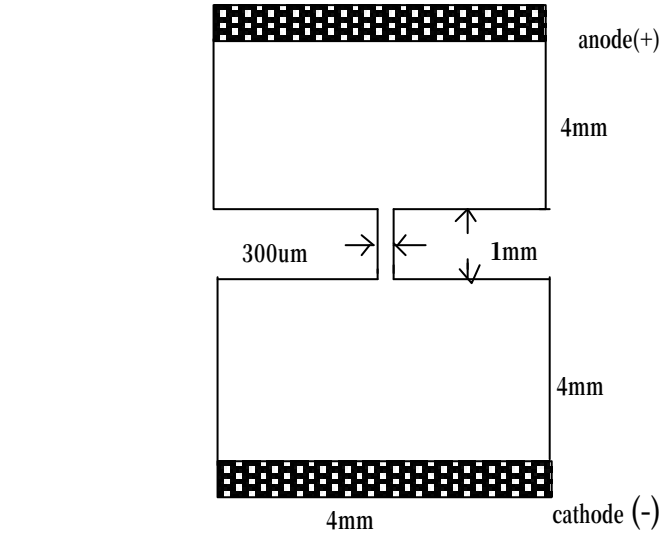
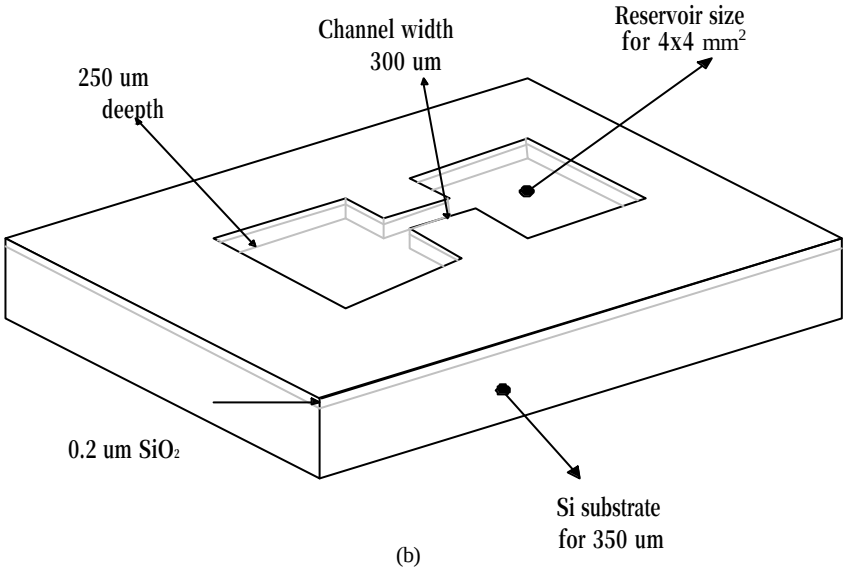


(a)



(b)

Figure 1. (a) Schematic of top view of the microchip; (b) Schematic of the whole microchip

Design and Fabrication Process of Microchip

This study utilizes applied voltage to drive different DNA molecules for generating current in the microchannel because DNA molecules carry negative charges. This method can be used to identify different DNA fragments by measuring the difference of current during the experiment. The diameter of DNA is about 3.4Å for each base-pair and the minimum volume of pipets which we use in the experiment is 1μL. So the schematic of top view of the designed microchip is shown as Figure 1 (a). The research designs a microchannel between two reservoirs [9] in the chip to measure the mass transfer of different DNA reagents in the microchannel. The dimensions of the designed microchip are 300 μm for the width, 1mm for the length, and 250 μm for the depth. As shown in Figure 1 (b), the area of the two reservoirs for loading reagents is 4×4 mm^2 and the depth is 250 μm, too. According to the results of designing several different dimensions of the microchannel for testing, we find 300 μm for the width of microchannel will let us to get the best result. In other words, the current signal in the microchannel with narrower width than 300 μm is too slight to be measured. Besides, the surface of the microchip is coated with SiO_2 by 0.2 μm for thickness to prevent leakage current while applying voltage; two tungsten electrodes [10] are placed on the walls opposite to each other to output DC voltage and prevent chemical reactions from occurring on the electrodes [11]. The subjective can use pipets to load chemical sample like ionized groups or biological sample like DNA molecular, blood etc into the reservoir. The microchannel is the main region of mass and electron transfer [12]. The schematic of the whole

Table1 Samples with specification in the study are listed.

Reagents	Concentration	Volume
ddH2O	100%	500 ml
CH3COOH	99.70%	300 ml
dATP	100 mM	molecular weight=491.2
dTTP	100 mM	molecular weight=482.2
dGTP	100 mM	molecular weight=570.2
dCTP	100 mM	molecular weight=467.2

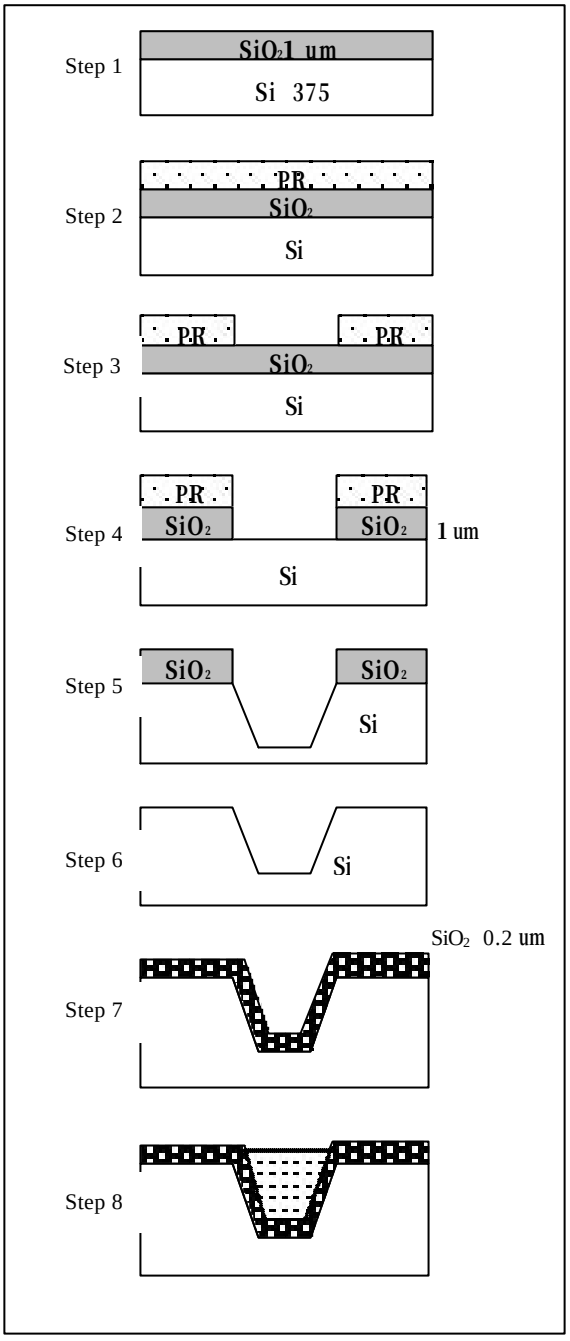


Figure 2. Fabrication flow process of microchip.

microchip is as shown in Figure.1 (b).

The method of fabricating the microchip in the study adopts Si fabrication process of MEMS technology [13]. Among many types of micro-fabrication processes, Bulk micro-machining is utilized in the research. Besides, Si is selected as the substrate, and the method of anisotropic etching is used to etch the depth at 250 μm in the fabrication of microchip.

During the anisotropic etching process of the experiment, SiO_2 is chosen as etching mask. The thermal oxidation SiO_2 is chosen to realize the film deposit of SiO_2. Furthermore, a layer thick of photoresist is coated on SiO_2 by the method of spin-on twice. HF aqueous solution is used as the anisotropic etchant to etch at the designed depth in the experiment. After the etching is performed, photoresist must be removed by aceton. Finally, KOH aqueous solution is selected as the etchant of Si substrate. The microchip is put in KOH aqueous solution for about 48 hours to be etched Si substrate at 250 μm. In addition, because Si is a kind of conductor, it must be separated form DNA molecule with negative charges. Therefore, it is designed to deposit SiO_2 for thickness of 0.2 μm as an insulater on Si substrate. The whole process of fabrication is as shown in Figure 2.

Experiment materials and methods

All materials used in the experiment on the microchip are listed in table 1. Different concentration of CH_3COOH solution and different kinds of DNA solution are chosen as reagents on the microchip in this study. The study adopts the method that it is necessary to make up the reagents by taking the amount of CH_3COOH, and adding ddH_2O (double deionized water). Besides, it's the same way to prepare the DNA reagents for measurement. For example, DNA powder will become soluble in ddH_2O for different concentration. In the experiment, the concentration of different DNA solution is the same (100 mM). It should be noted that the DNA reagents have to be stored up at the temperature of –20 ^{0}C to prevent from any unexpected chemical reaction. While the beginning of using the DNA reagents, the DNA reagents have to be unfreezed at about 1~3 hours.

As shown in Figure 3 the measurement system is composed of a source measure unit (SMU, Keithley, model 236), trigger controller (Keithley, model 2361), black box and personal computer. The chip was put on a heat sink to avoid thermal effect during measurement. The device under test was put in the black box for lowering the noise. After the measurement apparatus was set up, the different reagent was loaded into microchip by a pipet. The tungsten electrodes connect to the source measure unit. The source measure unit can apply a DC voltage to the device to generate a region of electric field in the reservoir and through microchannel [14], and the trigger controller can make SMU to output different voltage. During the process of mass and electron transfer, the source measure unit can measure current flow through the chip. The apparatus offer us to measure current versus time and voltage.

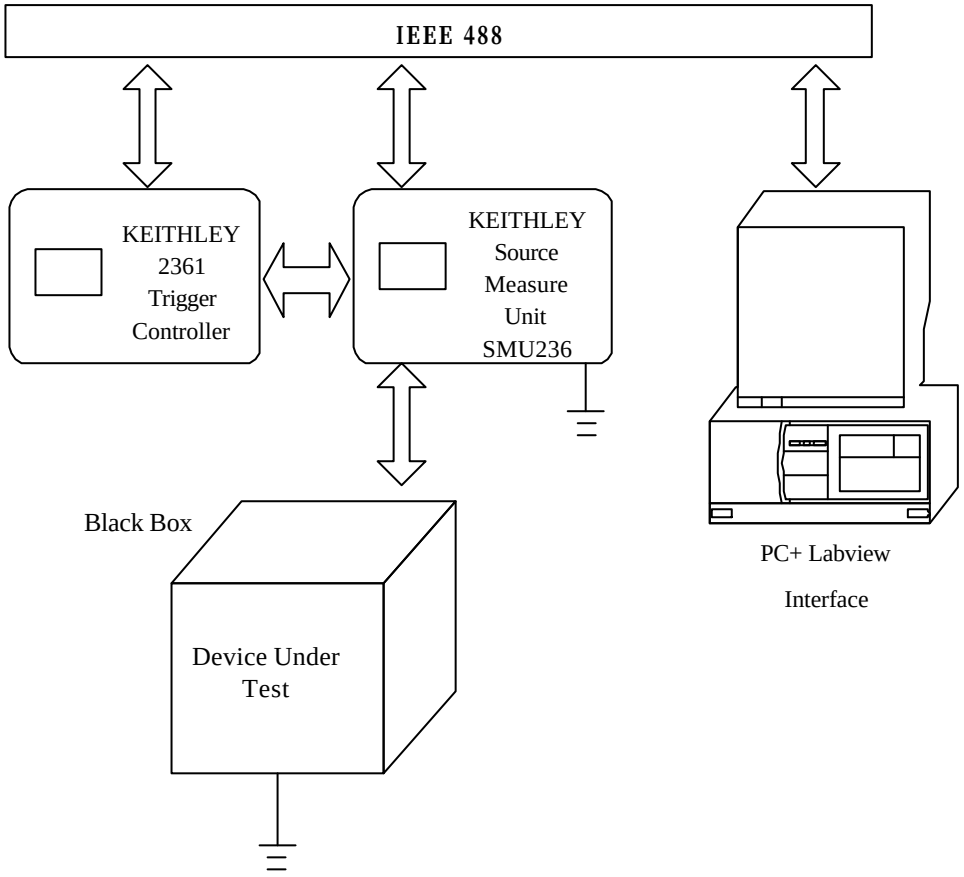


Figure 3. The block diagram of the electric signal measuring system.

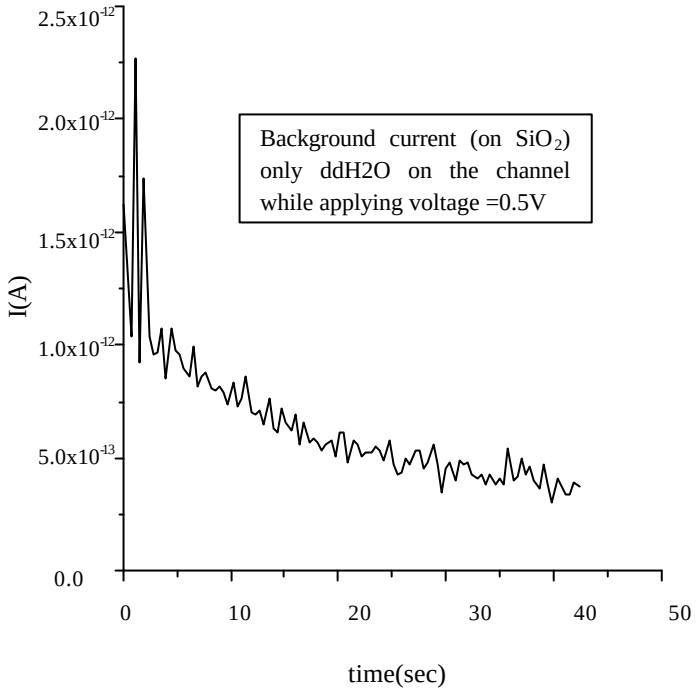
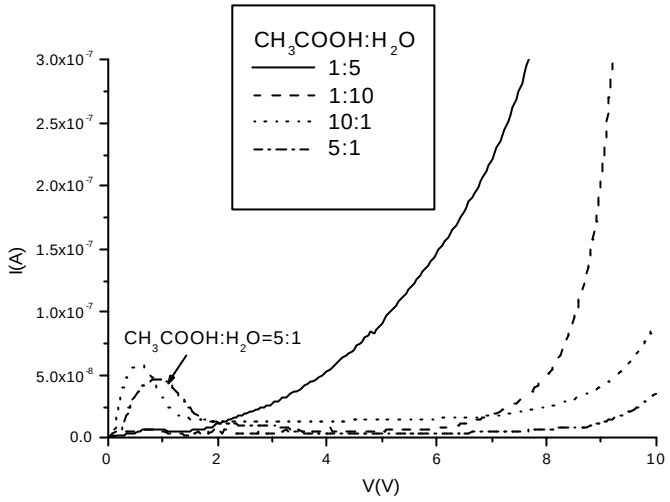


Figure 4. The measurement of current (I) versus time (T) measurement for only in the presence of solvent (ddH2O) on the chip.



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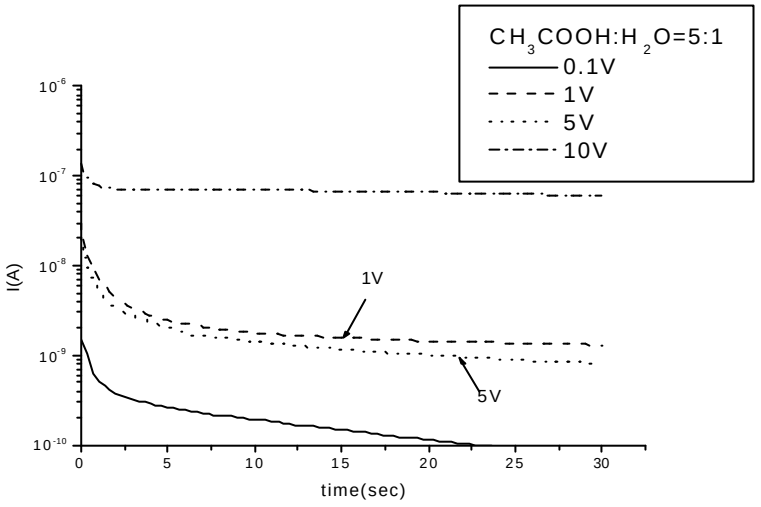


(b)

Figure 5. (a) Current (I) and Voltage (V) measurement of CH_3COOH for different concentration.; (b) Current (I) and time (T) measurement of CH_3COOH for applying different voltage.

Results and discussion

The background current was measured only in the presence of solvent (ddH2O) on the chip. Two electrodes were located on surface of chip. Figure 4 displays the background current about the order of magnitude 10^{-12}A (PA) when applying 0.5V on the chip, and the values of current are about 10^{-3}~10^{-5}A (mA~ μ A) when the reagents are put on the microchannel with applied voltage in the experiments. Comparatively, the value of the background current is much smaller than that while samples put on the microchannel. Therefore, background current can be ignored in this study. The experimental results will be described and discussed as following paragraph.

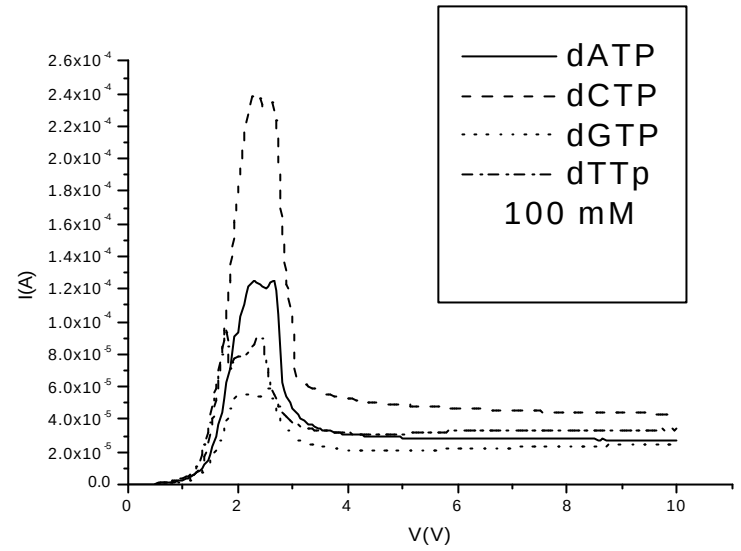
CH_3COOH for reagent

I-V Measurement

Figure 5(a) shows the measured current-voltage (I-V) curves of CH_3COOH:ddH_2O at concentration ratios of (10: 1), (5:1), (1:5), and (1:10).

There are two different kinds of profile including dense CH_3COOH solvent and dilute CH_3COOH solvent in the I-V curves. Taking the reagent of CH_3COOH: H_2O =5: 1 (dense CH_3COOH solution) as example. There is a peak current at the voltage equal to 1V. Briefly speaking, there are four regions in the profile. The current flow involves the transfer of charge to or from an electrode.

The first region is from 0V to 1V. The current instantaneously increases at this region. The instantaneous current produced by the increase of ionization of electrolyte (CH_3COOH). The second region is from 1V to 2V. The current values decrease with increasing of the voltage values. The phenomenon is like the negative resistance. It is believed that it is the absorption of CH_3COO^- and cathode electrode, and the reaction area of electrode is lower and lower to make current



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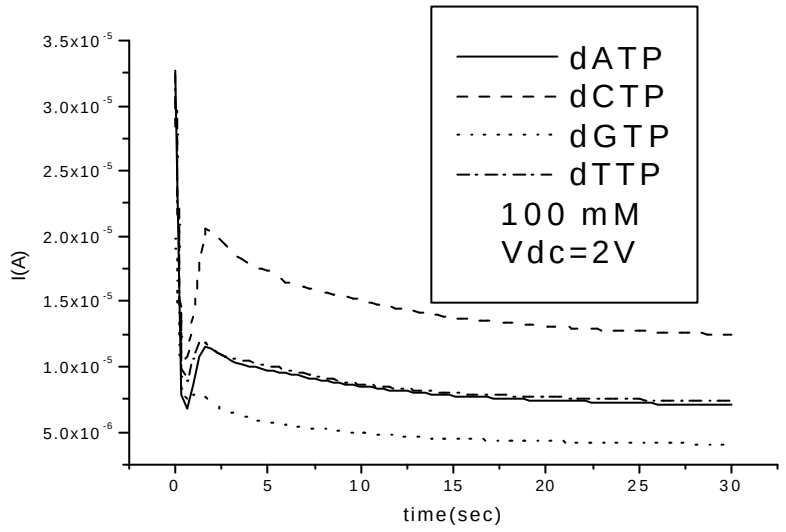

(b)

Figure 6.(a) Measurement of I-V curves of dATP,dGTP,dTTP,dCTP.; (b) Measurement of I-T curves of dATP, dGTP, dTTP, dCTP.

decrease. The third region is from 2V to 8V. The current doesn't change with various voltages except at the case of concentration ratio 1:5. In this case, the current increases monotonically as the bias voltage increases. However, the current maintains a constant value in the region. According to thermal diffusion, the concentration near electrode is higher than the bulk of solution. So ions near the electrode attempt to move away from electrode to the bulk in solution. Therefore, at the third region, it is balance of the driving force of electrical field and thermal diffusion. No matter applying voltage increases, current is still the same. The fourth region is defined as after raising the voltage up to 8V. The current increases slowly when raising the voltage in dense CH3COOH solvent. Relatively, the current increases rapidly as the voltage increasing in the solution with dilute solvent.

I-T Measurement

As shown in Figure 5(b), the current (I) and time (T) measurement for applying four different voltages at CH_3COOH: H_2O=5:1. According to Figure 5 (a), The current (I) on DC bias at 5V is less than that at 1V. Also, From Figure 5(b), the result is that the current at 5V is really less than that at 1V.

DNA for reagent- dATP, dCTP, dGTP, dTTP

In the study, four different one base-pair DNA are taken as reagents [15]. They are dATP, dCTP, dGTP, and dTTP.

I-V Measurement

As the same method described previously, the current (I) and voltage (V) for four different kinds of DNA are measured in a very short time. Figure 6 (a) shows the current-voltage curves for the four different DNA. From the I-V curves, it is found that the DNA has the same characteristics with dense CH_3COOH solvent. The I-V curve can be divided for three regions. The first region is from 0V to 2.5V. The current instantly increases at this region. The instant current produced by the increase of ionization of charged DNA. However, the biasing voltage from 2.5V to 3V in the second region shows the

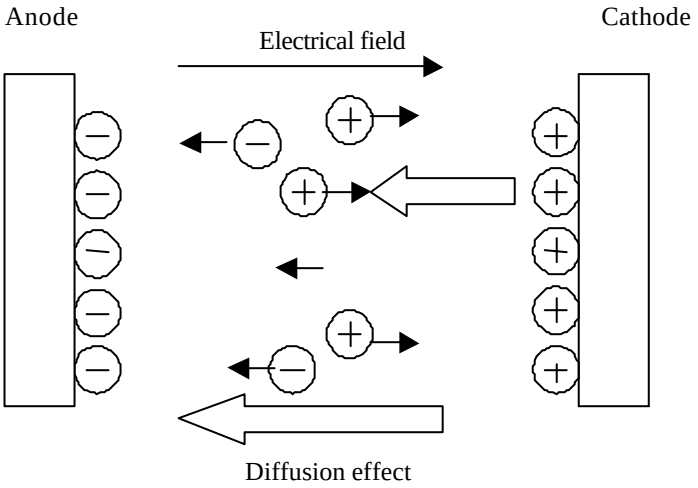

Figure 7. Schematics of diffusion effect counteracting electrical drift effect. Take positive ions for example, the accumulated positive ions near the cathode generate diffusion force on subsequent positive ions coming to the cathode. The diffusion force on positive ions will counteract the electrical driving force generated by applying voltage.

transient behavior like the negative resistance. The value of current decreases very rapidly. That is the absorption of DNA^- and cathode electrode, and the reaction area of electrode decrease. So the current falls down. Finally, the third region is from 3V to 10V. The current isn't proportional to the voltage. The current keeps at a fix value in this region. It should obey thermal diffusion theorem, the concentration near electrode is higher than the bulk of solution. It causes ions near the electrode move away from electrode to the bulk in solution. In the third region, it is balance between the thermal diffusion and electrical field.

I-T Measurement

The current (I) and time (T) measurement (I-T curves) for the four different DNA shows in Figure 6 (b) as the transient response of current. There are two special phenomena in the experiment. First, it is obvious the I-T oscillation in the short time (between 0 sec-2.5 sec). Second, the order of current value is always the same.

Discussion

In the experiment, the concentration of the four kinds of DNA is 100 mM and the applied voltage is 2 V. Obviously, the four kinds of DNA all have the oscillation phenomena of current. The current increases and then decreases as time goes by. The concept of these phenomena is supposed and drawn schematically in Fig.7 to interpret it. It is perhaps because the H^+ ions (symbol circle with plus sign) in aqueous solution move rapidly toward the cathode to generate higher current during the beginning of applying voltage in the microchannel. At the same time, the heavier DNA molecules (symbol circle with minus sign) move slowly toward anode and accumulate near anode. It means that the negative charges carried with DNA molecules generate static electric field to resist the electric field generated by applying voltage and repel the following negative ions moving toward anode. Besides, the concentration of ions near the electrodes is higher so that the concentration gradient also results in the effect of repelling the subsequent ions moving toward the electrodes. In other words, the diffusion effect [16-18] generated by the accumulating charges counteracts the electrical drift effect caused by applying voltage. The further theory deduction and simulation model are underway. It may be the main reasons why the current oscillates.

Conclusions and Future Prospect

A simple Micro-Total-Analysis-System (μ-TAS) by MEMS application has been set up and successfully fabricates the micro electrophoresis chip on silicon substrate. In the study, the new method is emphasized to execute the experiment. In some condition, the chip is really powerful to shorten the measurement time from 20 minutes to less than 30 seconds.

In the research, different sizes of etching depth for the microchannel are studied to get better performance. After successfully fabricating the chip, some kinds of material are used as the reagents. By the experiments, different known concentration of CH_3COOH solution and the 4 different one base-pair DNA can be distinguished. As long as the concentration of DNA is the same, they can be identified by the difference of their molecular weight. In the work, it is successfully to distinguishe the reagents as dATP, dGTP, dTTP, and dCTP. As the shown results, the curves display oscillation phenomena with time. From the experiments, some proper mathematical and chemical models are being studied to explain these phenomena. The models will be beneficial for the advanced studies and applications of biomedical diagnostic technology.

On the other hand, there are some factors to affect the experiment results.

1. The environmental noise: It's necessary to isolate the chip with outside noise. Thus, we measure data in the black box.

2. The purity of our DNA: Only using high purity DNA could get the accurate data. The precise data can't be measured if it exists impurities.
3. Control of time: Because the reagent will evaporate in short time, measurements have to be proceeded in very short time.

The study of the microchip just began to be developed. At initial, there are not lots of DNA reagents to be measured. If it' s able to make up a set of database of DNA, maybe the faster experiment can be done. It is also important to improve the knowledge of biology and chemistry for studying the chemical diffusion model. Finally, the biochip will be combined with the control circuit. And by designing the other forms of chip, the various DNA chip with microchannel will be produced in the future.

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