# NANOFLUIDIC DEVICES FOR PROTEIN CONCENTRATION AND ENZYMATIC REACTION KINETICS

**Xing-Hua Xia* and Chen Wang**

*Key Laboratory of Analytical Chemistry for Life Science, School of Chemistry and Chemical Engineering, Nanjing University, China*

## ABSTRACT

We demonstrated a simple and cost-effective technique for fabricating nanofluidics devices via an UV-irradiation photochemical decomposition reaction. The nanochannel size can be tuned continuously by controlling the irradiation time and light intensity. For demonstration, two kinds of nanofluidics devices respectively for proteins concentration and enzyme kinetics study in spatial confinement have been fabricated. The results promise that the present nanofluidic devices can well be used for chemical and biological analysis system at nanoscale.

**KEYWORDS:** Nanofluidics. UV-ablation. Protein concentration. Enzymatic reaction kinetics. Spatial confinement.

## INTRODUCTION

Nanofluidics has been attracting extensive attention and become a rapid developing research area. Nanofluidics manipulating fluid flow in nanosized structure has at least one of the dimensions falling in the nanosize range of several to one hundred nanometers. Nanofluidics devices further reduces costs, processing time, amount of reagents necessary for assay as compared to the microfluidics and would find wide applications in bioanalysis. The main research activities of nanofluidics is focusing on understanding of fundamentals of nanochannels and extending its application in analytical chemistry and bioanalysis. Up to now, nanofluidics devices have been successfully used for protein concentration, DNA analysis and immunoassays [1-4]. A key issue on the development of this technique is the nanochannel fabrication. Although various methods including bulk machining, surface machining, and mold machining have been developed, they are labor intensive, and require expensive equipments and sophisticated processing procedures. This paper reports a simple and cost-effective UV-ablation technique for creation of size-tunable nanofluidics devices via photochemical decomposition reaction. The resulted nanofluidics have been used for protein concentration and study of enzymatic reaction kinetics.

## EXPERIMENTAL

Materials and reagents. All Phosphate buffer (PBS, pH 7.0, 10 mM) solution was used as the buffer system. Glucose oxidase (GOx) was obtained from Sigma and used as received. Glucose was from Nanjing Chemical Reagent Company. Glucose stock solutions were allowed to mutarotate overnight at room temperature before use. All solutions were kept in a freezer to prevent deterioration. All liquid samples were filtered with a 0.22 μm syringe filter to remove particulates before use. All aqueous solutions were prepared from deionized water (18 MΩ·cm, PURELAB Classic, PALL, USA).

Micro/nanofluidics chips Fabrication. The micro/nanofluidic chip for protein concentration was composed of an upper PDMS cover with microchannels network and a PC substrate with nanochannel. The fabrication procedures can be described as follows. Briefly, a polycarbpnate (PC) substrate possessing nanochannel networks was first formed by UV-lights irradiation through a PET photomask. Then, a PDMS cover with four reservoirs was sealed to the PC plate. This sealing process is reversible. The total length p–q =16 mm o–r was 8 mm (Figure 1A). The fabrication of nanofluidic chip for enzymatic kinetics study was similar as described above. The whole fabrication process includes three steps: (1) nanochannel formation on PC by UV-irradiation, (2) Enzyme (GOx) immobilization on nanochannel, (3) the final formation of PC/PDMS nanofluidics chip. The width was 200 μm. The total length o–r was 8 mm and s (or t)–o was 4 mm (Figure 1B). For microfluidics chip fabrication (18 μm high, 200 μm wide), a PDMS film with microchannels was prepared using a silicon mold. To realize the immobilization of enzymes, a PC substrate was irradiated by UV-lights for 120 min without PET mask. The final PC/PDMS microfluidics chip was fabricated by sealing the PDMS with microchannels with the PC substrate.

Enzyme immobilization. A PC plate of 30×30×0.5 mm was exposed to the UV-lights emitted from a low-pressure mercury lamp in atmosphere with the utilization of a PET photomask at a lamp-to-plate distance of 2 cm. Then, the exposed PC plate was rinsed with water and immersed in a solution containing EDC/NHS (0.1 M of each in pure water) for 30 min. After washing with water and drying with $N_2$, the EDC/NHS-activated PC plate was immediately spotted with 100 μL of GOx solution (100.0 mg/mL) and placed in a sealed Petri dish that was saturated with water vapour.

Characterization. The depth of etched nanochannels was measured by a profilometer (Dektak3, Veeco Inst Inc., USA). A scanning electron micrograph (SEM, Sirion, FEI Company, Holand) and atomic force microscopic (AFM, Picoforce, Veeco Inst Inc., USA) were used to characterize the morphology of the nanochannel fabricated. X-ray Photoelectron Spectroscopy (XPS) (an ESCALab220i-XL electron spectrometer from VG Scientific using 300 W Al Kα radiation), the UV-vis spectra (a UV-3600 UV-vis spectrometer, Shimadzu, Japan) and an inverted fluorescence microscope (Nikon, Ti-U, Japan) equipped with a digital CCD camera were used to characterize the surface components of PC surfaces and the immobilization process of enzyme on nanochannels.

Electrochemical Measurements. A home-made plexiglass holder integrated with a 3-D micromanipulator was fabricated for fixing the microchip and housing the detector and reservoirs, as reported previously [1,2]. Proper sealing of the solution reservoir at the end of microchannel was achieved by using silicone grease and fixing with double-sided adhesive tape. The reservoir served as both the cathodic buffer reservoir for the CE system and the electrochemical detection cell. A three-dimensional micromanipulator (Shanghai Lianyi Instrument Factory of Optical Fiber and Laser, Shanghai, China) was fixed on the plexiglass holder for precise positioning of the working electrode. Alignment of the working electrode to the microchannel end was performed under a microscope (Jiangnan Optical Instrument Factory, Nanjing, China). A home-made saturated Ag/AgCl reference electrode, a platinum counter electrode, and a ground Pt electrode for CE were also placed in the reservoir along with the working electrode. Amperometric detection was carried out in a three-electrode configuration on an Electrochemical Workstation CHI 650 (Shanghai Chenhua Instrument Company, Shanghai, China).

## RESULTS AND DISCUSSION

As illustrated in Figure 1, UV-irradiation through a PET photomask results in continuous decomposition of poly(carbonate) (PC), forming nanochannel and carboxyl groups (as confirmed by XPS analysis) on the surfaces of the etched PC. The depth of nanochannels can be controlled at nanometer level (etching rate: 0.015 nm/s). The generated carboxyl groups improve considerably the wettability and electroosmosis of the fabricated channels, and can be further used to pattern biological molecules.

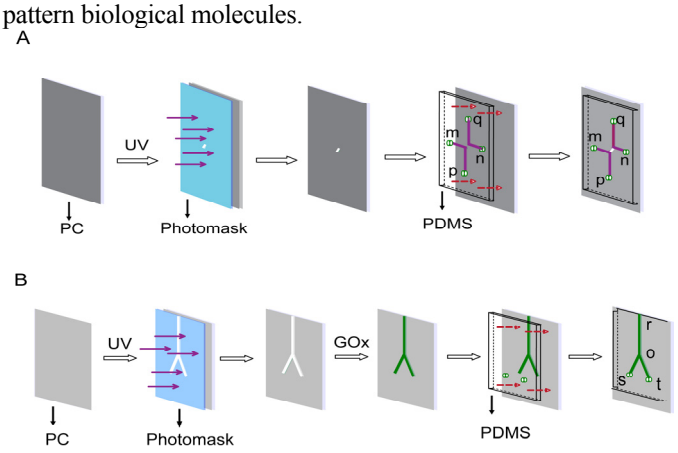


*Figure 1: (A) Fabrication processes of the micro/ nanofluidics chips for protein concentration. (B) fabrication processes of the nanofluidic chip for enzyme kinetics study in spatial confinements with channel-end electrochemical detection.*

To demonstrate the facile and convenient applications of the present UV-ablation technique, two kinds of micro/nanofluidics chips were fabricated respectively for protein concentration (Figure 2A) and enzyme reaction kinetics study (Figure 3A). Figure 2A shows a micro/nanofluidics chip for protein concentration, in which a single nanochannel was integrated in microfluidics chip. The single nanochannel was achieved by exposing a PC substrate to UV-irradiation for 20 min at a lamp-to-plate distance of 2.0 cm. The depth of nanochannel is estimated as ca. 18 nm according to the etching rate.

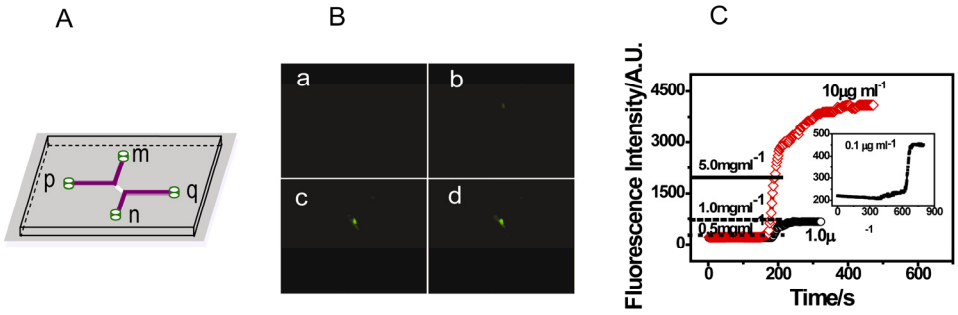


*Figure 2. A: Schematic layout of the micro/nanofluidic chips for protein concentration; B: time sequence photoimages (a-d) of 1 μg ml$^{-1}$ FITC-DSA in 10 mM PBS (pH 7.0) in a nanofluidic device. Images were taken after applying a voltage of 400 V between proteins reservoir and waste reservoir for 0, 180, 200, 300 s. C: Plot of FITC-DSA concentration from three concentration samples (400 V) vs enrichment time. The inset is the enlarged plot of 0.1 μg ml-1 FITC-DSA.*

Upon application of a 400 V high voltage to the protein reservoirs (anode) and waste reservoir (cathode), the negatively charged fluorescein isothiocyanate labeled dog serum albumin (FITC-DSA) sample is concentrated at the anodic electrode side in front of the nanochannel. Figure 2B shows the plot of concentration of FITC-DSA as a function of enrichment time at a voltage of 400 V. The inset shows the enlarged plot of 0.1 μg ml$^{-1}$ FITC-DSA. From the change of the fluorescence intensity, it can be known that at the first 150 s enrichment time, the enrichment effect is not clear. This time period is mainly used to drive samples in the microchannel. Increasing the time further results in rapid enrichment of FITC-DSA in front of the nanochanned and soon reaches saturation. The proteins enrichment results indicate that the present micro/nanofluidics chip has a perfect protein enrichment capacity.

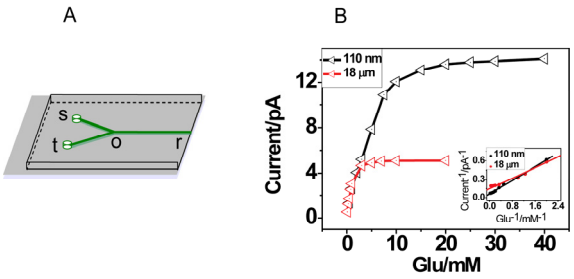


*Figure 3: (A) Schematic layout of the nanofluidic chips for study of enzymatic reaction kinetics. (B) Calibration curves of the steady-state current responses to hydrogen peroxide produced by enzymatic reaction in nanochannel (110 nm height) and microchannel (18 μm height) as functions of glucose concentrations. The inset shows the Lineweaver-Burke plots.*

In the case of enzymatic reaction investigation, after immobilization of GOx on the nanochannel surface, the steady-state current response to hydrogen peroxide generated from enzymatic reaction of the immobilized enzyme was collected. A nanofluidics chip (110 nm in depth, 200 μm in width) and a microfluidics chip (18 μm in depth, 200 μm in width) are both used as the confined enzyme nanoreactors to investigate the enzyme kinetics in different spatial confinements, (Figure 3A). The steady-state electrochemical current responses to various concentrations of glucose solutions are used to evaluate the activity of the immobilized enzymes. The results are shown in Figure 3B. Figure 3B, inset shows the Lineweaver-Burke plots (linear correlation is respectively 0.9977, and 0.9978, with corresponding nanofluidics chip and microfluidics chip). It is clear that the current response firstly increases with the glucose concentration, then levels off gradually at higher glucose concentrations. This is typical for an enzymatic reaction, which is similar to those reported for enzymatic reactions under homogeneous or heterogeneous conditions previously. It is found that with the increase of channel depth from 110 nm to 18 μm, the Michaelis-Menten constant of GOx decreases from 5.2 mM for nanofluidic chip to 1.43 mM for microfluidics chip. In addition, the maximum current increases with decreasing the channel depth, showing a faster reaction rate in a relatively smaller spatial confinement. Thus, higher conversion efficiency of the substrates to production can be expected in a smaller nanochannel depth.

## CONCLUSION

In summary, we have presented a simple and cost-effective method for nanofluidics devices fabrication based on photochemical decomposition reaction. This fabrication technique is simple, fast and low-cost, and is accessible to most laboratories. Obviously, the present approach offers many obvious advantages over other approaches. The photochemical decomposition reaction occurs at molecular level, thus, it allows facile control over the channel depth at nanoscale level. The carboxyl groups generated from photochemical reaction considerably improve the wettability and electroosmosis of the fabricated channels, and can be further used to pattern biological molecules. The observed smoothing effect of the UV-ablation method would be an additional advantage being critical to nanofluidics devices fabrication. Our results have shown that the fabricated nanofluidics devices can be successfully used for proteins concentration and enzymatic reaction kinetics study. The significant influence of spatial confinement on enzymatic reaction kinetics has been for the first time revealed, and will be further studied in our group. These results suggest that nanofluidics devices fabricated using the UV-ablation method are specially promising to the study of chemical and biological reactions on the nanoscale. We expect that the present fabrication method and the resulting nanostructures with functionalized surfaces could provide new platforms for nanofluidics development towards various applications in biology and chemistry.

## ACKNOWLEDGEMENTS

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## CONTACT

*X. H. Xia, tel: +86-25-83597436; xhxia@nju.edu.cn