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Confinement effect of protonation/deprotonation of carboxylic group modified in nanochannel

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ABSTRACT

Protonation and deprotonation processes are the key step of acid–base reaction and occur in many biological processes. Study on the deprotonation process of molecules and/or functional groups in confined conditions would help us understand the acid–base theory and confinement effect of biomolecules. In this paper, we use a recently established approach to the study of protonation and deprotonation processes of functional groups in porous anodic alumina array nanochannels by measuring the flux of electrochemical active probes (ferricyanide ions) using an Au film electrochemical detector sputtered at the end of nanochannels. The protonation and deprotonation processes of surface functional groups in nanochannels will change the surface charges and in turn modulate the transportation of charged electroactive probes through nanochannels. The titration curve for the deprotonation of carboxylic groups in nanochannel confined conditions is obtained by measuring the current signal of ferricyanide probe flowing through an carboxylic-anchored PAA nanochannels array at different solution pH. Results show that the deprotonation of carboxylic group in nanochannel occurs in one step with a $pK_{1/2}$ = 6.2. The present method provides an effective tool to study the deprotonation processes of various functional groups and biomolecules under confined conditions.

1. Introduction

Recent progresses have shown that the study on interfacial interaction of charged nanopores or nanochannels with transporting molecule or ions have become the hot research area due to the increased surface-to-volume ratio of the nanomaterials. Considering molecules or ions flowing through a functionalized nanochannel, two interactions should be considered: volume effect [1,2] and charge effect [3–5]. When the structure size of the modified molecules is comparable to the size of nanochannel, the flux of ions or molecules through the nanochannel/nanopore will be significantly reduced because of the reduced effective free-transport area caused by the modified molecules. Based on this volume effect, Martin et al. successfully separated proteins with different size using Au nanotubes within the nanochannels of track-etched polycarbonate template membranes [1]. Charge effect has a long-range effect at the solid–liquid interface and is mainly manifested by electric double layer (EDL), resulting from the surface charges of the fixed-size nanoporous materials compensated by the counter ions in solution. The EDL thickness depends on ionic strength of the solution. Taking negatively charged surface of a nanochannel as an example (Scheme 1), the EDL thickness is ca. 1 nm in a high ion strength of solution (100 mM). In this case, the free diffusive region is much larger than the confined diffusive region (EDL), thus, the flux of negatively charged ions is high. However, when the ion strength of a solution decreases to 1 mM, the EDL thickness increases to a comparable value with respect to the diameter of nanochannel. Under extreme conditions, the EDLs may overlap completely, and thus the free diffusive region shrinks or disappears. Under this circumstance, only a small quantity of the negatively charged ions can pass through the nanochannel. According to this charge effect, Wang et al. [6] developed a lable-free DNA sensor via studying the surface charge effect on ionic conductance through a nanoporous alumina membrane. Recently, we observed that the surface charge of nanochannels plays a determining role in manipulating the transport of neutral molecules. Transport of phenol through nanochannels array of anodic porous alumina (PAA) membrane can be manipulated by changing the EDL thickness via solution ion strength [3]. In addition, a quantitative label-free DNA analysis approach has been designed using the charge effect [4]. In this case, neutral morpholino (analog of DNA) was immobilized on the inner wall of nanochannels of PAA. As complementary DNA in solution hybridizes with the immobilized morpholino, large amount of negative charges will be introduced into the nanochannels, which significantly reduces the flux of negatively charged electrochemical probes through the morpbolino-DNA functionalized nanochannels. Therefore, label-free detection of DNA can be easily realized and single base mismatching of DNA can be

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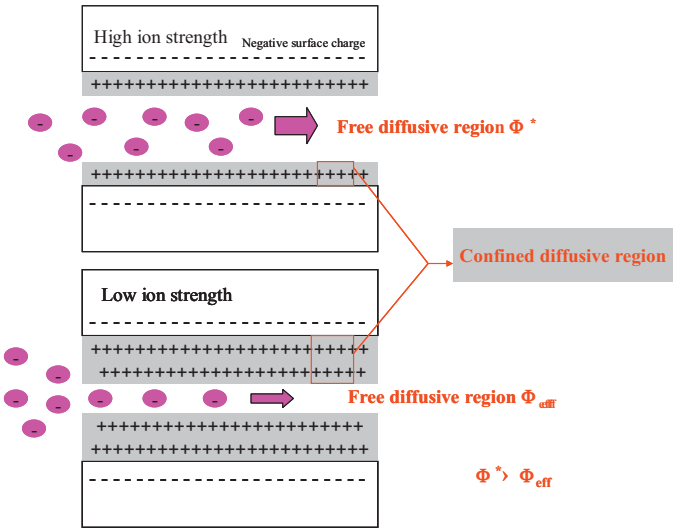
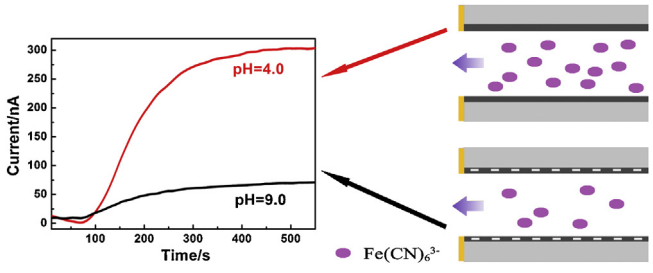


Scheme 1. Schematic sketch of the exclusion effect on the anionic transport. At high ionic strength, free diffusion region (Φ^*) is large, so anions can freely diffuse through the nanochannel. At low ionic strength, the EDL thickness increases and free diffusion region decreases (Φ_{eff}), resulting in a lower effective flux.

distinguished [4]. This charge effect has also been used to studied the confinement effect on the isoelectric point of proteins [7] and protonation of the amino functional groups in nanochannels [8]. Both these two effects can change the flux of transported molecules or ions through the nanochannel, and have been widely used for achieving separation or enrichment of biomolecules or ions [9,10], ion current rectification [11,12], and constructing smart biomimetic interfaces [13,14], biosensors [15,16], and nanofluidic devices [17,18].

In this work, a nanochannels array based device has been proposed for studying confinement effect on deprotonation of the carboxylic functional groups by monitoring the solution pH dependent mass transport of negatively charged electrochemical probe of ferricyanide. The charges carried by the immobilized carboxylic groups in nanochannels are determined by solution pH, and in turn modulate the mass transport of ferricyanide probe across the nanochannels due to the strongly electrostatic interactions. Mass transport of the probe is measured by monitoring its electroreduction current at an Au film electrochemical detector sputtered at the end of a PAA membrane. From the variation of the probe mass transport as a function of solution pH, the $pK_{1/2}$ of the immobilized carboxylic functional groups in nanochannels of PAA can be determined. As shown by Scheme 2, the immobilized carboxylic functional groups will be uncharged in solution pH < pK_a,



Scheme 2. Schematic representation of the principle for the determination of $pK_{1/2}$ of carboxylic group immobilized in nanochannels coupled with an electrochemical detector. At a low solution pH (<pK_a), carboxylic group is uncharged and the flux of probe through the nanochannels is high. Increasing the solution pH > pK_a makes the carboxylic group deprotonated, resulting in a decrease of probe flux through the nanochannels due to the electrostatic repulsion of the negatively charged nanochannels to the negatively charged ferricyanide.

while it carries negative charges in solution pH > pK_a. Thus, the mass transport of the electrochemical probe decreases significantly as the solution pH increases as indicated by the significant decrease of maximal steady-state current due to the strongly electrostatic interactions between the negatively charged ferricyanide and the carboxylic functional groups immobilized nanochannels surface. This method provides an effective tool to study the protonation and deprotonation processes of various functional groups and biomolecules.

2. Experimental

2.1. Materials

Porous alumina membranes (60 μm thickness) with pore diameter of 20 nm was purchased from Whatman International Ltd. (Maidstone, England). 3-Aminopropyltrimethoxysilane (APTMS) was from Sigma–Aldrich. 25% Glutaraldehyde (GA) aqueous solution was from Sinopharm Chemical Reagent Co., Ltd. Phosphate buffers were prepared using analytical grade from the chemicals and deionized water (>18.2 MΩ, Millipore, France). Solutions with low and high pH values were prepared by adding dilute HCl or NaOH, respectively. Solutions with intermediate pH values were obtained by mixing A and B with different ratios. When pH values above 9.5 or pH values below 3.5, porous alumina membranes will dissolve, so the solution pH values from 3.5 to 9.5 were used in the present work.

2.2. Surface modification

Porous anodic alumina (PAA) membrane from Whatman with 20 nm pore diameter and 60 μm thickness was first cleaned as described in our previous work [7]. The cleaned PAA was then immersed into a 10 mL acetone solution containing 1% 3-aminopropyltrimethoxysilane (APTMS) for about 4 h for grafting aminopropyl functional groups in nanochannels. After that, excess silane solution was removed from the PAA nanochannels by rinsing with copious amounts of acetone, followed by deionized water. The samples were then baked at 120 °C for 1 h. The remaining modifications (rooting surface-bound amines) were performed after sputtering an Au film at the 200 nm side of the commercial PAA membranes and the membranes were assembled in the electrochemical detection cell.

2.3. Fabrication of Au film working electrode on PAA membrane

An Au film working electrode on PAA membrane was fabricated as follows: A 100 nm thick Au film was sputtered on the 200 nm side of a commercial PAA membrane. The Au film sputtering was performed using a current of 15 mA in a vacuum chamber with a pressure of 5×10^{-4} mbar (Ar plasma) for 600 s. To dispose the Au film electrode with ease, the Au film-coated PAA membrane was sandwiched between two poly(ethyleneterephthalate) (PET) sheets (100 μm thick, DIKA Official Limited Company, Suzhou, China) with prepunched 2 mm holes. The holes defined the area of the membrane exposing to the solution phase. Then, a 0.2 mm diameter of Au wire was placed in electrical contact with the Au film coated PAA membrane. Finally, the membrane assembly was laminated together by a heating laminator (Beijing Zhongtian Feiniao Science and Technology Limited Company, Beijing China) at 150 °C with the two prepunched holes well aligned.

2.4. Cell assembly and electrochemical measurements

The APTMS-grafted PAA membrane with an Au film electrode was further functionalized by leaving it overnight in a 5% aqueous