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Real time monitoring of surface chemical events by streaming potentials in microfluidic channels

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Real time monitoring of surface chemical events by streaming potentials in microfluidic channels

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Chemistry at Virginia Commonwealth University.

By

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Abstract

REAL TIME MONITORING OF SURFACE CHEMICAL EVENTS BY STREAMING POTENTIALS IN MICROFUIDIC CHANNELS

By Kenji Nokura, MS

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Chemistry at Virginia Commonwealth University.

Virginia Commonwealth University, 2013

Director: Julio C. Alvarez, Chemistry Department

Zeta potential is one of the tools to measure the surface charge of materials, and Alvarez et al. have developed a microchannel device to measure zeta potentials in real time for label-free sensing using immobilized receptors on microchannel surfaces. However, the challenge has been the charge interference of surface modifiers on analyte detection. Therefore, it was necessary to find the best strategy to regenerate minimal surface charge after modifying the channel with polymer films that would anchor the affinity groups for the analyte.

It was demonstrated that adsorption of positively and negatively charged analytes were monitored via real time zeta potential measurements by using surface-immobilized polystyrene nanospheres, and the best discrimination of analyte binding on the nanoparticles was observed when the underlying film was a non-ionic polymer.

Titanium oxide nanoparticles (TiO₂) were immobilized on microchannels modified with
non-ionic polymers to investigate if the surface charge of the microchannel was induced by the concomitant surface charge reactions of the TiO$_2$ nanoparticles upon UV exposure. Analysis by XPS indicates that desorption of proteins monitored by zeta potential changes are induced by UV exposure.
1.1 - Introduction

The measurement of zeta potential is one of the ways to estimate the surface charge of a material and can be used to detect how a surface interacts with adsorbing species that change its charge. For example, surface accumulation of nanoparticles, folding of bound proteins and surface binding, could in principle be monitored by zeta potential measurements. Likewise, in the case of surface contamination, strategies can be developed to eliminate surface contaminants and monitor their presence via zeta potential measurements. In surfaces, one of the methods to measure zeta potential is streaming potential (SP) (Figure 1.1.1).

![Diagram of zeta potential](Figure 1.1.1: Schematic representation of ion distributions on solid-liquid interface on negatively charged macroscopic surface (top) and principle of Streaming Potential generation (bottom). Counter-ions strongly immobilized on the solid surface and generate a layer, called Stern Layer. Distribution of co-ion increases as counter-ion decreases from solid surface towards bulk solution, which makes electric double layer (EDL). Slipping plane is the point where the distributed ions can be removed by pressure-driven flow. The Ions from...
slipping plane to bulk solution can be removed by pressure-driven flow and that result generates gradient of the potential, which is Streaming Potential, $E_{sp}$.

Streaming potentials elicited by pressure driven flow are related to the surface charge and the zeta potential through the Smoluchowski equation.

$$E_{sp} = \frac{P\varepsilon\varepsilon_0\zeta}{\eta\kappa}$$  \hspace{1cm} eq (1.1)

Where $E_{sp}$ is Streaming Potential. $P$ is applied pressure, $\zeta$ is zeta potential. $\varepsilon$ is solution permittivity. $\varepsilon_0$ is vacuum permittivity. $\eta$ is solution viscosity. $\kappa$ is solution conductivity.

By rearranging equation 1.1, the zeta potential is calculated by following equation:

$$\zeta = \frac{\eta\kappa}{P\varepsilon\varepsilon_0}E_{sp}$$  \hspace{1cm} eq (1.2)

This technique is suitable for measuring the zeta potential of macroscopic surfaces, which can be exposed to the flow of solution streams, such as a plate, a membrane or a fiber. It also allows the estimation of the zeta potential of microscopic particles if they are immobilized on a channel surface.$^{1-3}$ This approach might lead to a more versatile method that can evaluate different type of samples.
1.2 - Development of a streaming potential device

![Image of streaming potential device](fig1.2.1.png)

Fig 1.2.1: Developed streaming potential device. Solution was vacuumed and flowed to auto injector towards microchannel controlled by LABVIEW.

Alvarez et al. have developed a micro channel device for measuring zeta potential via streaming potentials including functions such as real-time monitoring detection of adsorption\(^4\)\(^7\) (Figure 1.2.1, 1.2.2). Pu and Luna-Vera equipped the streaming potential device with the following features:

- Cost efficient plastic material with significant UV transparency as a microchannel substrate.
- The pressure-driven flow to generate the steaming potential was attained by vacuum rather than positive pressure of the solution into microchannel.
The liquid flow was set to be pulsed to generate pulsed steaming potentials (PSP) that no longer required reference electrodes as the analytical signal was encoded in the pulses regardless of any baseline drift.

Development of voltage follower and transistor switch to obtain the voltage signal through a data acquisition (DAQ) card controlled by LABVIEW (Figure 1.2.1, 1.2.2).

Data conversion of the raw voltage signal from PSP was performed with LABVIEW(Figure 1.2.2)

Development of injector to allow real-time monitoring of pulsed streaming potential as illustrated with the label-free detection of the test analyte lysozyme (Figure 1.2.1).

These features are significantly different from other streaming potential devices available in the market, (i.e. as Zeta Cad) and have potential advantages over those types of instruments. The improvements in the streaming potential device that were attained after the work by Luna-Vera and that are described in this thesis are the following:

Instead of reporting the streaming potential, the device now furnishes the zeta potential signal directly, after reprogramming the SP data conversion software written with LABVIEW.

Automation of signal acquisition and data conversion from raw signal to zeta potential (Figure 1.2.2)

A new microchannel design was implemented to allow simultaneous dual channel acquisition (reference and a blank) including the circuit and the injector units.
Fig 1.2: Interface of Streaming Potential software coded by LABVIEW. It controls solenoid valve to open vacuum line to generate square pulse to obtain pulsed streaming potential $E_{sp}$. Automation settings and UV are also programmed to this interface.

- Other than the plastic imprinted microchannel, new interrogation platforms were developed and optimized for pulsed streaming potential measurements. For instance, a silica capillary, a 3D-printed plastic channel and glass plate (Figure 1.2.3).
- Installation of UV exposure function in the streaming potential device for data acquisition via transistor switch to control the UV exposure electronically, (Figure 1.2.2).

These improvements were not chemistry related, however, they can potentially increase the versatility of streaming potential device and its analytical performance.
Fig 1.2.3: New design of microchannel substrates. A-left, silica capillary. A-middle imprinted microchannel. A-right, multi-printed microchannel. B - 3D printed microchannel made by different material (left: ABS, middle: PLA and right: Nylon). C - Streaming potential cell for film or plate analysis.

Continuing challenges persist in relation to the producing sensing surfaces with reproducible surface coverage and charge density, as well as controlling spurious non-specific adsorption during experiments. Nevertheless, this method offers the possibility of label free detection and being an alternative to similar approaches of detecting adsorption in real time, such as Surface Plasmon resonance (SPR) and quartz crystal microbalance (QCM).
1.3 – The zeta potential determined from on pulsed streaming potentials in real-time

One of the biggest challenges for the zeta potential device developed by Alvarez et al. is the competitors in the label-free sensing market by real time approach, such as SPR and QCM. These methods are well known label-free detection techniques that are used for applications monitoring biomolecular interaction, such as antigen-antibody, cell adhesion, and characterization of adsorbed proteins. These methods are based on the mass adsorbed on the sample. For QCM, measuring the resonance frequency change of a piezo crystal (Δf), which is proportional to the mass (Δm) of the adsorbed molecules per unit area, is expressed by the Sauerbrey equation:

$$\Delta f = \frac{1}{C_{QCM}} \Delta m$$  \hspace{1cm} \text{eq (1.3)}$$

where $C_{QCM}$ is the mass sensitivity.

For SPR, The resonance angle shift of thin layer metal (ΔΘ) is what is proportional to the mass (Δm) of the adsorbed molecules, and is given by:

$$\Delta \Theta = \frac{1}{C_{SPR}} \Delta m$$  \hspace{1cm} \text{eq (1.4)}$$

The possible advantages to compete with these other methods is the low cost of the instrument, when comparing to Vendors, such as Biacore® (e.g. ~$133,000 for entry model. ~$300 for one Sensor Chip). It is possible to reduce the material cost of a hand-made streaming potential device down from ~$4000 to ~$200 (excluding the laptop computer) or
even less, once we figure out efficient material choice to assemble the device and programming was coded by free language software, such as JAVA.

We have not demonstrated all the potential versatile functions of this method due to the challenge in reproducibility of the surface modification and the discrimination of the analyte adsorption respect to interferences in underlying charge of the anchoring polymer. Adsorption determination and surface chemical reactions have been widely studied by SPR and QCM and it might make PSP method less competitive, since monitoring chemical surface reactions, such as cross-linking reaction is one desired goal in real-time surface analysis.\(^8\) Once a surface chemical reaction can be monitored by streaming potentials following zeta potential changes, I believe there might be opportunities to make the device more competitive. Given that the PSP output signal is based on surface charge changes, which is different from QCM and SPR (eq1.2-1.3)\(^{10}\), there are some opportunities to observe unique phenomena via surface charge during a surface chemical reaction. In this thesis, studies of surface charge discrimination of analyte binding on nanoparticles is described in Chapter 2, and surface charge control by UV treatment, which might involve photo-radical reactions, is discussed in chapter 3. Although these results are still inconclusive, they will help us select more ideal systems to monitor surface charge changes during surface reactions.
1.4 - References


2.1 – Monitoring surface charge changes

One of the questions this research has intended to answer, was to determine if the streaming potential technique can be used as an adsorption sensor and/or a surface reaction monitoring approach. Therefore, it is necessary to select an adequate system that either incorporates ionic charges or changes the charge during the course of a reaction. To the best of my knowledge, there have been only a few in-situ studies of surface chemical reactions reported.\textsuperscript{1-4} Some of these studies were focused on surface polymerization and it was concluded that the charge form the polymer takes over the entire surface charge making it difficult to detect charge changes, presumably because ion incorporation is limited by Donnan potentials developed on the film-solution interface.\textsuperscript{1,2,23-24} Another study was done by immobilizing latex particles on mica to investigate the correlation between surface charge of a bare latex particle and latex adsorbed on the Mica surface.\textsuperscript{3-4} Since our research group has been modifying microchannel surfaces with surface bonded polymers generated via radical reactions on plastic channels by UV, I decided to investigate the best conditions to detect a surface charge change from this type of reaction and further incorporation of ions. Luna Vera investigated modification of hydroxyl groups of a polyethylene glycol acrylate (PEGA) surface by trifluoroacetic anhydride (TFAA) and it was concluded that only less than 3\% of TFAA was either chemically or physically immobilized, thus this was not a good candidate to detect the modification of hydroxyl groups on PEGA.\textsuperscript{5} One possible explanation is because of the nature of the photo-grafted polymer, which explains the zeta potential shift towards the surface charge of the polymer itself, making the
detection of exogenous ion adsorption harder (Figure 2.1.1). Therefore, having an ionic polymer on the surface could make the detection of ion adsorption more difficult.

To address this problem, I thought of using larger charged species (nanoparticles) and hopefully with better surface coverage to produce detectable changes in the surface charge.
Figure 2.1.2 Strategy for controlling a signal from the target particle. It tackles the selective adsorption of an analyte to the target to explain the signal comes from the target.

To implement the notion above, the streaming potential signal upon adsorption of charged species on the immobilized particles was investigated (figure 2.1.2). Since the material making up the particles is different from that of the substrate, there might be a possibility to distinguish the target signal on the particles than directly on the underlying charged polymer. Channels modified with films of PEGA and N,N'-carboadiimidazole (CDI) termed as CDI-PEGA were used as substrates to reduce the charge within the films given that these polymers are examples of low charge polymers that could be compared with the charged polymer. The composite CDI-PEGA can be modified with amine functional groups via

\[
\zeta = f(\zeta_{\text{substrate}})
\]

\[
\zeta = f(\zeta_{\text{particle}})
\]

\[
\zeta_{\text{substrate}} = \{ \zeta_{\text{CDI-PEGA}}, \zeta_{\text{COC}} \}
\]

\[
\zeta_{\text{surface}} = \{ \zeta_{\text{substrate}}, \zeta_{\text{particle}}, \zeta_{\text{particle-substrate}} \}
\]

\[
\zeta = f(\zeta_{\text{analyte}})
\]

\[
\zeta = f(\zeta_{\text{surface}}, \zeta_{\text{analyte}}, \zeta_{\text{analyte-substrate}})
\]

\[
\zeta = f(\zeta_{\text{surface}}, \zeta_{\text{analyte}}, \zeta_{\text{analyte-particle}})
\]

\[
\zeta = f(\zeta_{\text{surface}}, \zeta_{\text{analyte}}, \zeta_{\text{analyte-substrate}})
\]
cross-linking reaction by making on amide bond (figure 2.1.3). Particles of 60 nm coated with amines were used as a model to determine if zeta potentials could be detected in this type of modified channel. Lysozyme, as a positively charged probe and Heparin, as a negatively charged species were used to study their adsorption behavior.

2.2- Materials and instrumentation

Cyclic olefin copolymer (COC) was obtained from Ticona Inc. Stainless steel wire was obtained from Small Parts. 60 nm amino nanosphere was obtained from Bangs Laboratories, Inc (Fisher, IN, USA, Earth). Polyethylene glycol acrylate (PEGA), benzophenone, N,N'-
carbodiimazole (CDI), Heparin and DMSO were obtained from Sigma-Aldrich (Saint Louis, MO). Microsphere of 340 nm and 2.6 μm diameters were obtained from Invitrogen. Lysozyme was purchased from Worthington Biochemical Corporation (Lakewood, NJ). Dibasic sodium phosphate and monobasic sodium phosphate were from EM Chemicals (Gibbstown, NJ). Streaming potential instrument was developed by Dr. Pu and Luna-vera.\textsuperscript{5,11-12} Hitachi FESEM ultra high resolution Scanning Electron Microscope (SEM) was used for SEM analysis.

2.3 - Experiment

The following numbers in experimental section (e.g. 2.4.1.1) correspond to the number in the results section:

2.4.1.1 - The COC microchannels were made by an imprinting method, followed by photo-radical polymerization of PEGA.\textsuperscript{5,11-12} CDI (150mg/mL or 15mg/mL) dissolved in DMSO was injected into the COC-PEGA microchannel and left for 24 hours. 20 times dilution of the amine-coated nanosphere (3.8 x 10^{12} particles/mL) in PBS was injected into the CDI-activated PEG-PEGA microchannel and was left for 24 hours. The extra microspheres were washed by injection of 2 ml DI water into a microchannel chip, and then the zeta potential was measured via streaming potential. SEM images were taken on an open-microchannel plate as written previously\textsuperscript{5}.

2.4.1.2 - Microchannels were made by an imprinting method, following by photo-radical polymerization of PEGA. CDI (150mg/mL or 15mg/mL) dissolved in DMSO was injected into COC-PEGA microchannel and left for various hour and then zeta potential was
measured. The same concentration of CDI was injected into microchannel after the measurement and left for several hours to repeat the step. After 4 days activation, 20 times dilution of amine-coated nanosphere (3.8 x 10^{12} particles / mL) in PBS was injected into CDI-PEGA microchannel. The same procedure of CDI injection was applied for the injection of amine-coated nanosphere, instead of injection of CDI in DMSO.

2.4.1.3 - 1.0 mM pH 11 phosphate buffer was injected into CDI-PEGA and stored for various hours, and then zeta potential was measured. The pH 11 phosphate buffer was injected into the same microchannel chips and stored and continue the steps up to 6~7 days.

2.4.2.1 - Nanospheres immobilized in CDI-PEGA microchannels were made by using the procedure on 2.4.1.1. Nanospheres immobilized COC-PEGA microchannels were made by injection of amine-coated nanosphere (3.8 x 10^{12} particles / mL) in 1.0 mM phosphate buffer at pH 6.4 into COC-PEGA and stored for 24 hours. Lysozyme was diluted by DI water to and then diluted by appropriate solvent to adjust to desired concentration, such as dilute by DI water to 350 nM and then dilute by phosphate buffer to adjust to 70 nM. The solutions were injected into microchannel via auto injector\(^5\) and zeta potentials were measured in real-time by streaming potential analysis. SEM images were taken after flowed the phosphate buffer for 15 min, and sputtered platinum on opened-microchannel plate\(^5\).

2.4.2.2 - the same procedure as 2.4.2.1 was applied. Heparin was diluted and concentration was adjusted to 3.7m units/mL. ~1mM phosphate buffer at pH 7.5 was used for dispersing nanosphere.
2.4 - Result and discussion

2.4.1 - Studying adsorption through surface charge changes

2.4.1.1 - Screening test

<table>
<thead>
<tr>
<th>Name</th>
<th>Injected compounds</th>
<th>microchannel surface</th>
<th>pH 3.1</th>
<th>pH 7.0</th>
<th>pH 11.0</th>
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<tbody>
<tr>
<td>Expt</td>
<td>60nm sphere (3.8x10^{12}/mL)</td>
<td>150mg/mL CDI-PEGA</td>
<td>31.4</td>
<td>-33.1</td>
<td>-47.1</td>
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<tr>
<td>Ctrl1</td>
<td>PBS</td>
<td>150mg/mL CDI-PEGA</td>
<td>30.2</td>
<td>-33.33</td>
<td>-50.8</td>
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<tr>
<td>Ctrl2</td>
<td>CDI 150 mg/mL PEGA</td>
<td>PEGA</td>
<td>24.7</td>
<td>-30.5</td>
<td>-47.8</td>
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<tr>
<td>Ctrl3</td>
<td>CDI 15 mg/mL PEGA</td>
<td>PEGA</td>
<td>8.8</td>
<td>-14.2</td>
<td>-29.2</td>
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<tr>
<td>Ctrl4</td>
<td>dehydrated DMSO</td>
<td>PEGA</td>
<td>-0.3</td>
<td>-5.4</td>
<td>-16</td>
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<tr>
<td>Ctrl5</td>
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<td>PEGA</td>
<td>-9.1</td>
<td>-18.1</td>
<td>-27.0</td>
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<tr>
<td>Ctrl6</td>
<td>DI water</td>
<td>PEGA</td>
<td>-1.2</td>
<td>-10.7</td>
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<table>
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<tr>
<th>Zeta Potential, (\zeta) (mV)</th>
<th>Standard deviation</th>
<th>Zeta Potential, (\zeta) (mV)</th>
<th>Standard deviation</th>
<th>Zeta Potential, (\zeta) (mV)</th>
<th>Standard deviation</th>
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</thead>
<tbody>
<tr>
<td>Expt</td>
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<td>Ctrl1</td>
<td>0.6</td>
<td>Ctrl2</td>
<td>0.1</td>
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</tbody>
</table>

Table 2.4.1.1.1: Characterization of immobilized 60nm amine-coated sphere on CDI activated PEGA microchannel by zeta potential and control experiments. Standard deviation explains repeatability of each measurement condition (n=>3)

Figure 2.4.1.1.1: SEM images of 60 nm amine-coated polystyrene sphere immobilized CDI-PEGA microchannel (left), carbodiimidazole activated PEGA microchannel (middle) and Polyethylene glycerol acrylate microchannel (right). All of the images were taken after measuring zeta potential.

Zeta potential measurements at three different pH were performed to understand how the zeta potential was changed during CDI activation of PEGA, following by immobilization of
amine-coated microspheres on CDI-PEGA microchannels. Table 2.4.1.1.1 shows the zeta potential of immobilized 60 nm amine-coated Polystyrene nanospheres on a CDI activated surface, the particle-free CDI activated surface and the controls at three different pH. Figure 2.4.1.1.1 shows SEM image of nanospheres immobilized on a CDI-PEGA surface (Figure 2.4.1.1.1. left), which indicates at least 40% of surface covered by immobilized microsphere while 3.8x10^{12} particles / mL nanosphere was injected on 300 mg CDI activated PEGA surface. CDI-PEGA microchannel (Figure 2.4.1.1.1 middle) seems to have rougher surface than PEGA (Figure 2.4.1.1.1 right), which might be a rearrangement of polymer configuration caused by CDI activation^{21,22}. PEGA control groups (ctrl4-ctrl6) shows quite similar surface charge except phosphate buffer saline injection, which will be talked more detail in next section. Injection of CDI changes zeta potential of PEGA and it generates iso-electric points between pH 3- and pH 7 (ctrl2 and ctrl3). That indicates CDI-adsorbed PEGA itself generates surface charges. Although there is presence of microspheres on CDI-PEGA microchannel, there are no zeta potential differences between CDI activated PEGA surface and microsphere immobilized CDI activated surface (Ctrl1, Ctrl2 vs expt.). That result shows there is a possibility that surface charge of immobilized nanosphere was not detected via streaming potentials. This could be due to irreproducibility of microchannel chips, which might produce the ± 3~6mV differences caused by photopolymerization process and/or surface history.^{14}
2.4.1.2 - Time analysis

Since there is a possibility that variation of the chips might interfere in the result of the screening test (Table 2.4.1.1), non-real-time analysis during CDI activation through immobilization of microsphere was investigated by using one microchannel chip (Figure 2.4.1.2.1 top and bottom). It suggests that CDI was adsorbed on PEGA surface and immediately changed its surface charge within 1 hour for pH 3 (■), 7 (■) and 11 (■).

These zeta potentials were stabilized within 1 day and did not significantly change after 24 hours. Since CDI activation usually takes 1 day to be completed,\textsuperscript{7-10} that might be a good indicator that it would be possible to measure chemical reaction in real-time via streaming potential. However, adsorption of buffer ions or storage of microchannels increase variation around ±3–6 mV of zeta potential (e.g. Figure 2.4.1.2.1 top and bottom; 20 < t< 100). After 4 days CDI activation, anime-coated nanospheres were injected into the microchannel chip and zeta potential was measured up to 4 days in non-real-time mode. (Figure 2.4.1.2.1 bottom).
Figure 2.4.1.2.1 - Time analysis of CDI-activation of PEGA (top) and immobilization of amine-coated nanosphere on CDI-activated PEGA (bottom). PEGA was used as a reference of CDI-activation at t=0, and 96 hours CDI-activated PEGA was used as a reference of immobilization of amine-coated nanosphere at t=0. Blue colors (■, ■) show the zeta potential at pH 3, red and pink colors (■, ■) show zeta potential at pH 7, and green colors (■, ■) show zeta potential at pH 11. Error bar indicates the standard deviation of average zeta potential in terms of repeatability.
Zeta potentials for all pH had changed from 4 days CDI activated PEGA microchannel by immobilization of microsphere within 1 hour (e.g. from 39.8 mV to 46.5 mV for pH 3(■), -27.2 mV to -20.6 mV for pH 7 (■) and -45.3 mV to -47.0 mV for pH 11 (■)). The magnitude of this changes were not higher than the zeta potential changes of adsorption of solution ions, therefore, it is hard to conclude the zeta potential changes were caused from amine-coated nanospheres. After the immobilization, the zeta potentials did not significantly change when comparing to CDI-PEGA (ζ for all pH at t=0; figure 2.4.1.2.1 bottom) except at 17 hours immobilization at pH 7 (■). One possibility of these differences might be that nanospheres itself had been stacked and aggregated inside of the microchannel. That might either block the microchannel or generate different zeta potential. Since the zeta potential was changed back to -28mV, which is similar to CDI-PEGA (ζ =27.2mV at t=0) for longer immobilization of nanospheres, that might be counted as an outlier. Therefore, as both screening test and time analysis suggests there are no huge differences between CDI activated surface and nanospheres immobilized on CDI surfaces in terms of surface charge. One possible explanation is the CDI charged polymer dominates surface charge of the microchannel. Another possible reason is that the nanosphere themselves have almost the same surface charge as the CDI surface.
2.4.1.3 - Deactivation of N,N'-carbodiimidazole (CDI)

To address the issues just mentioned in the previous section, CDI deactivation was implemented by injection of basic phosphate buffer solution into CDI-PEGA microchannel\(^8,^{10}\) (Figure 2.4.1.3.1).

![Deactivation of CDI](image)

Figure 2.4.1.3.1; deactivation of CDI from CDI-PEGA surface

Figure 2.4.1.3.2 shows the deactivation of CDI-PEGA surface and Figure 2.4.1.3.3 shows the deactivation of CDI on anime-coated nanospheres immobilized on a CDI-PEGA microchannel. All zeta potentials for CDI-PEGA microchannel tend to decrease when stored in pH 11 phosphate buffer for long periods. That indicates the surface charge of CDI-PEGA surface never changed back to the original zeta potential which was the value for phosphate buffer PEG surface (figure 2.4.1.3.2 at t=0). Several reasons could be proposed to explain the changes observed by adsorption of phosphate buffer, which usually changes the surface charge to negative,\(^17\)

The magnitude of the zeta potential at pH 3 is significantly higher than the microchannel without nanospheres (Figure2.4.1.3.2 ■ vs Figure 2.4.1.3.3 ■ and Figure2.4.1.3.2 ■ vs Figure 2.4.1.3.3 ■). That might indicate that the zeta potential of amine-coated nanospheres
was able to be observed at acidic pH. However, Figure 2.4.1.3.2 shows the zeta potential did not decrease back to the zeta potential of PEGA surface, which indicates the nanospheres were immobilized on a complicated microchannel surface and it is challenging to explain any of these changes until the end. Also, in terms of the grand goal, controlling the signal from adsorbed targets (nanospheres), there are no differences between CDI-activated PEGA charged surface (Table 2.4.1.1.1). And deactivated CDI-activated PEGA "charged" surface (Figure 2.4.1.3.2). Therefore, it may be better to develop a standard method to wash or regenerate the surface and avoid surface charge interference that comes from solution adsorbed species.

Figure 2.4.1.3.2: deactivation of CDI-PEGA microchannel (15mg/mL CDI for left, and 150mg/mL CDI for right). pH 11 phosphate buffer stored PEGA microchannel was used as a reference (t=0) and shown by big square for all pH. blue colors (■, ■) show the zeta potential at pH 3, brown and orange colors (■, ■) show zeta potential at pH 7, and green colors (■, ■) show zeta potential at pH 11. Error bar indicates the standard deviation of average zeta potential in terms of repeatability.
Figure 2.4.1.3.3 deactivation of amine-coated nanosphere immobilized CDI-PEGA microchannel (15mg/mL CDI for left, and 150mg/mL CDI for right). pH 11 phosphate buffer stored PEGA microchannel was used as a reference (t=0) and shown by big square for all pH. blue colors (■, ■) show the zeta potential at pH 3, brown and pink colors (■, ■) show zeta potential at pH 7, and green colors (■, ■) show zeta potential at pH 11. Error bar indicates the standard deviation of average zeta potential in terms of repeatability.

2.4.2 - Adsorption study

Although there are several possibilities of the interference from a charged polymer, adsorption of positive/negative species on these microchannel surfaces was studied to verify this expectation.
2.4.2.1 - Adsorption study of Lysozyme (positively charged species)

Figure 2.4.2.1.2 shows real-time measurement of lysozyme adsorption on different surfaces. It is obvious that lysozyme injection changes zeta potential towards positive for all surfaces, except PEGA. Poly ethylene glycol (PEG) has hydrophilic surface and its surface charge is almost neutral ($\zeta \approx -4\text{mV}$). That might block the adsorption of proteins. SEM images for nanospheres immobilized microchannel plates were taken after measuring zeta potential and are shown in Figure 2.4.2.1.1.

![SEM images](image)

Figure 2.4.2.1.1: SEM images of 60nm amine-coated nanosphere immobilized on PEGA (left) and CDI-PEGA (middle) after the lysozyme adsorption measurement. SEM images of PEGA (right) was taken stored on phosphate buffer.

These SEM images confirm that nanospheres were immobilized even after 1 hour of pressure-driven flow. Comparing the CDI-PEGA microchannel surface (■, ■) and nanospheres attached CDI-PEGA microchannel surface (■), zeta potential increased to -8 mV for both cases when lysozyme was flowed. That indicates that lysozyme adsorption is detectable by zeta potential. Probably electrostatic interaction has a primary role to explain this adsorption behavior. Lysozyme was mostly washed out for CDI-PEGA microchannel surface (■, ■). However, nanospheres coated CDI-PEGA microchannel (■) retain some of
lysozyme attached on the surface even after washing for ~ 15min. One reason could be that lysozyme rearranged the conformation to stick onto amine-coated polystyrene nanospheres by hydrophobic interactions.\textsuperscript{18,19} By the same token, CDI-PEGA microchannel surface (■) may have un-reacted PEGA , which is hydrophilic and has less tendency to be involved in hydrophobic interactions.

Figure 2.4.2.1.2: Real time monitoring of lysozyme adsorption on different microchannel surface. Phosphate buffer at pH 6.4 was flowed until $t = 600$, Lysozyme was injected at $t=600$s and then washed out by phosphate buffer from $t = 1650$s. Red (■) and green (■) are both CDI-PEGA surface. Grey (■) is PEGA surface. Blue (■) is nanosphere attached CDI-PEGA surface and cyan (■) is nanosphere attached PEGA surface. All conductivities were adjust to ~ 540 μS

For PEGA (■) and nanosphere coated PEGA (■) surfaces, it is clear that lysozyme changed the signal towards positive only for the nanosphere coated PEGA (■). By comparing both nanosphere coated surfaces (■, ■), the adsorption behaviors are almost the same. However, the adsorption speed of the nanosphere coated PEGA (■) surfaces is higher than nanosphere coated CDI-PEGA (■) (Figure 2.4.2.1.3). One possibility may be that the majority of the zeta potential was generated by CDI-PEGA surface (■) and adsorption of
lysozyme was blocked by nanosphere via hydrophobic interaction or electric repulsion from amine part of nanosphere. Another possibility may be that the majority of the zeta potential was generated by nanosphere and adsorption of lysozyme was blocked by CDI-PEGA surface (■) via electrostatic interaction. For the both cases, there will be a disadvantage for sensor selectivity, if an application was implemented by using this charged CDI-PEGA surface (■). Therefore, nanosphere coated PEGA (●) surfaces have better performance in monitoring the adsorption of lysozyme or positively charged proteins.

Figure 2.4.2.1.3: Adsorption speed of lysozyme on nanosphere attached CDI-PEGA microchannel surface (■) and PEGA (●) surfaces. The time period between right after the injection of lysozyme and the saturation of the surface (determined by signal depression and fitting) was analyzed by regression analysis.
2.4.2.2 - Adsorption study of heparin (negatively charged species)

Figure 2.4.2.1 shows real-time monitoring of heparin adsorption on different surfaces. It is obvious that heparin injection changes zeta potential towards to negative for both nanosphere coated surfaces (■, ■). SEM images show immobilized nanosphere on CDI-PEGA (middle) and PEGA (top) surfaces, which confirm there are nanosphere on these surfaces after 20 minutes of zeta potential monitoring (figure 2.4.2.2).

![Graph](image_url)

Figure 2.4.2.1: Real time monitoring of heparin adsorption on different microchannel surface. Phosphate buffer at pH 7.4 was flowed until $t = 450$ seconds, heparin was injected at $t = 450$ seconds and then washed out by phosphate buffer from $t = 800$ seconds. Green (■) is both CDI-PEGA surfaces. Grey (■) is PEGA surface. Blue (■) is nanosphere attached CDI-PEGA surface and cyan (■) is nanosphere attached PEGA surface. All conductivities were adjust to ~ 400 μS.
Figure 2.4.2.2.2; SEM images of 60 nm amine-coated nanosphere immobilized on PEGA (top), CDI-PEGA (middle) and PEGA (bottom) after the lysozyme adsorption measurement. All image on left side describes the high magnified images and right side pictures are low magnified images to see the microchannel itself.

It appears there is no interaction between heparin and neutral (■) or negative (■) surfaces, which may be the reason of negligible signal changes obtained by injection of heparin in those surfaces. By comparing both nanosphere coated surface (■, ■), the adsorption behavior is similar to each other. However, the adsorption speed of the nanosphere attached PEGA
surfaces is higher than nanosphere attached CDI-PEGA microchannel surface (■) (Figure 2.4.2.2.3). That may be explained by electrostatic repulsion between negatively charged substrate (■) and negatively charged target. Therefore, both nanosphere coated polymer surfaces would be able to monitor negatively charged species (heparin).

Figure 2.4.2.2.3: Adsorption speed of heparin on nanosphere attached CDI-PEGA microchannel surface (■) and PEGA (■) surfaces. The time period between right after the injection of heparin and the saturation of the surface (determined by signal depression and fitting) was analyzed by regression analysis.

The results of the adsorption study (section 2.4.2.1 and 2.4.2.2) seem to indicate that nanosphere immobilized PEGA shows signal generated by nanospheres themselves (Figure 2.4.2.1.2 blue (■) and 2.4.2.2.2 blue (■)). Possible explanation could be that the slipping plane is located to where nanospheres were immobilized on non-charged polymer (PEGA) and that is what generates the signal being measured as streaming potential (Figure 2.4.2.2.4). On the other hand, nanospheres on charged polymer may not be located on the
slipping plane and they do not contribute as much to the signal. A future possible approach could be to estimate the location of slipping plane because in principle, this is the most effective spot in the interface to produce a change in the zeta potential. According to Eversole, the slipping plane can be obtained by locating the Stern potential.  

![Figure 2.4.2.2.4: Schematic representation of proposing surface charge profiles as a function of distance from surface for non-charged polymer layer and nano-particle attached non-charged polymer layer](image)

Figure 2.4.2.2.4: Schematic representation of proposing surface charge profiles as a function of distance from surface for non-charged polymer layer and nano-particle attached non-charged polymer layer
2.5 - Conclusion

To attain the goal of this chapter, which is detecting changes in the zeta potential induced by the analyte adsorption, several approaches were investigated. The surface charge of various nanosphere coated surfaces were analyzed via streaming potentials and complemented with dynamic light scattering (DLS) and SEM measurements. It was evident that was difficult to control the amount of surface coverage of the spheres to study the correlation between zeta potential via streaming potential and DLS. Target nanospheres were immobilized on CDI activated PEGA surfaces and the effect of deactivation was investigated. However, the surface charge of CDI activated PEGA surface never returned to the original zeta potential values. It was demonstrated that adsorption of positive and negative target species were able to be monitored by using nanosphere coated surfaces. Nanoparticles attached on non-ionic polymeric films could be a good strategy for monitoring adsorption on the particles.
2.6 - References


5 Luna Vera, F. PhD. Dissertation, Virginia Commonwealth University, 2011.


3.1 - Surface charge signal switching by TiO$_2$ via ultraviolet light

The previous chapter discussed how the surface charge could be controlled by immobilized particles on a polymer surface. The results showed some strategies to detect signals from target analytes adsorbing on the surface. Although these phenomena are usually studied in real-time with SPR or QCM,$^{1,2}$ to the best of our knowledge no report has demonstrated the detection of surface charge changes using those methods. Thielbeer indicates that zeta potentials should change upon interaction with other ions and be detectable by DLS,$^3$ which encouraged us about the possibility of monitoring ion adsorption via streaming potentials. One problem with observing this adsorption in real-time is that the surface charge before and after adsorption may not be large enough to conclude such interaction is occurring. Furthermore, if the zeta potential change is ~3-6 mV, that may be a change similar to that by simply flowing a blank solution. Therefore, we wonder if using some external stimuli to control the surface charge could be detected by streaming potentials. Particles of TiO$_2$, which are widely used in inks, food additives, sun protector and other purposes, have several properties that could be triggered by UV irradiation and that could be related with surface charge changes. Semiconductor property of TiO$_2$ particle generates hole-electron separation via UV light, which could re-distribute the surface charge of TiO$_2$ (Figure 3.1.1).$^{16,17}$ TiO$_2$ has been used as photocatalysts activated by UV light. The UV light produces hole-electron pairs, which generates superoxide anions and hydroxyl radicals. Given that these chemical species destroy carbon bonds in organic substances, TiO$_2$ is used for removal of organic pollutants or even used for killing bacteria (Figure 3.1.1 and
eq 3.6-3.9). It was reported the self-sterilizing effect of Copper doped TiO$_2$ activated under ~4 $\mu$W/cm$^2$ UV light (similar to the brightness of surface of a study desk under dim lighting) and killed bacteria on TiO$_2$-Cu coated tiles in one hour.$^{34}$ This material is commercially available as anti-bacterial material by TOTO Ltd and applied in hospital, hotels toilet tiles and so on.$^{34}$ We hoped that the changes generated by UV irradiation of immobilized TiO$_2$ with/without solute could be detected by streaming potentials.

Figure 3.1.1: Schematic representation of electron-hole separation of TiO$_2$ with oxidation and reduction coming from adsorbed materials

### 3.2 - Materials and instrumentation

The same materials obtained from the same vendors were selected for the fabrication of COC-PEGA microchannel chip, as described in the previous chapter. Zeta potentials were measured using home-made streaming potential device developed by Pu and Luna-Vera.$^9$
Particles of TiO$_2$ were obtained from either US research nanomaterial.Inc (Houston,TX) or Nippon Aerosil.Ltd (Tokyo, Japan).

Super spot Mk III was obtained by Lesco (Torrance,CA). BSA, perchloric acid, hydrochloric acid, sodium hydroxide, formaldehyde, ammonium hydroxide, Cadmium perchlorate and polyethylene glycol acrylate (PEGA) were obtained from Sigma-Aldrich (Saint Louis, MO). Lysozyme was purchased from Worthington Biochemical corp. (Lakewood, NJ). A Hitachi FESEM ultra high resolution Scanning Electron Microscope (SEM) was used for SEM analysis.

**3.3 - Experiment**

Experimental setups were summarized on Table 3.3.1 and the following numbers in experiment section (e.g. 3.4.1) correspond to the numbers in result section:

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<th>Section</th>
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<th>Microchannel substrate</th>
<th>Solvent</th>
<th>Solute</th>
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<tr>
<td>3.4.4.3</td>
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<td>COC-PEGA</td>
<td>Sodium Perchlorate</td>
<td>BSA or lysozyme</td>
</tr>
</tbody>
</table>

Table 3.3.1: Summary of experimental setups of surface charge signal changes by TiO2 via ultraviolet light
3.4.1 - COC-PEGA was fabricated by using the same method reported previously. A 0.8 % w/v TiO$_2$ nanoparticles in DI water was injected into COC-PEGA microchannel and left for one day (Figure 3.3.1 left). Super spot Mk III was used to expose UV light to only the microchannel by using black tape covered plastic tube crafted by COC plate (Figure 3.3.1 right). The UV exposure sequence was controlled by Labview while measuring pulsed streaming potentials with a flowing phosphate buffer. The microchannel was opened and dehydration by vacuum was applied for 12 hours to prepare the channel for XPS and SEM analysis. ThermoFisher ESCALab was used for XPS analysis. Platinum was sputtered on opened microchannel chip and a Hitachi FESEM ultra high resolution Scanning Electron Microscope (SEM) was used for SEM analysis. A Hitachi FESEM ultra high resolution Scanning Electron Microscope (SEM) was used for SEM analysis.

![Figure 3.3.1](image)

Figure 3.3.1: Schematic representation of fabrication of TiO$_2$ covered PEGA microchannel chip (left), and experimental set up of UV irradiation experiment (right).
3.4.2 - Polymerization of anime (N-[3- (dimethylamino) propyl] methacryl-amide; DPMA), acid (acrylic acid, PAA) and polyethylene glycol (PEGA) were reported previously\textsuperscript{8-9}. Otherwise, the same procedure was applied as in 3.4.1

3.4.3 - Perchlorate solution or phosphate buffer was used as solvents. Otherwise, the same procedure was applied as in 3.4.1

3.4.4.1 Around 3.5 mM of formaldehyde or ammonium hydroxide were injected into phosphate buffer at pH 3 while UV irradiation. Otherwise, the same procedure was applied as in 3.4.1

3.4.4.2 300 ppm of cadmium perchlorate was injected into perchlorate solution at pH 7.4 and flowed to microchannel during the UV irradiation experiment. Otherwise, the same procedure was applied as in 3.4.1

3.4.4.3 - 100 ppm of BSA or 200 ppm of lysozyme were injected into perchlorate solution at pH 7.4 and flowed to microchannel during UV irradiation experiment. Otherwise, the same procedure was applied as 3.4.1.
3.4 – Results and discussion

3.4.1 - Screening test

Figure 3.4.1.1: Zeta potential measurement with UV exposure of non-UV reactive substrate (left) and TiO₂ covered PEGA substrate (right). pH 3 phosphate buffer with conductivity of ~190 μS was used as solvent for all measurements. For the non-UV reactive substrate, UV exposure for 5 min was repeated every 5 min and red square (■) and green square (■) indicated UV on and off, respectively. Polycarbonate (■), COC (■) and PEGA (■) were selected as examples of non-UV reactive microchannel substrates. For UV reactive substrate (right), UV exposure was applied for 5 min and no light was applied for 10 min after the UV exposure (e.g. UV on at t = ~650s and off at t = ~1000s). That sequence was applied several times to 3 duplicates of TiO₂ covered TiO₂ microchannel chips (■, ■ and ■). The gray color (■) shows the zeta potential of TiO₂ covered PEGA without UV irradiation.

Several experiments were performed to demonstrate that the charge produced during the photocatalytic reaction in Figure 3.4.1.1 could be detected by streaming potentials when the TiO₂ particles were immobilized in the microchannel. According to control experiments with UV exposure without TiO2 particles shown on Figure 3.4.1.1 (left), the zeta potential changed randomly. This indicates the random signal change might be from the adsorption of buffer ions which that usually change the zeta potential by 3-6 mV \(^{10}\) (e.g. COC). On the other hand, TiO₂ covered PEGA on Figure 3.4.1.1 (right) shows correlation between zeta potential changes and UV exposure on the microchannel surface.
Analysis by SEM and XPS was performed to confirm the presence of TiO$_2$ on the microchannel surface (Figure 3.4.1.2). Images by SEM indicate there are small regions with particles immobilized on surface when comparing with PEGA surface (Figure 2.4.1.1.1). Considering the raw surface of PEGA and size of TiO$_2$ nano-particles (30-50 nm from US research nanomaterial .Inc), the TiO$_2$ particles are immobilized on surface even after 30 min UV irradiation. It is challenging to distinguish the UV effect on TiO$_2$ coated PEGA in comparison to the same surface without UV exposure (Figure 3.4.1.2).
Figure 3.4.1.3: XPS spectra of PEGA (−), TiO\textsubscript{2} covered PEGA with UV (−) and without UV exposure (−). C1s scan result is shown on top, O1s scan on bottom left and Ti2P scan result was shown on bottom right.

Figure 3.4.1.3 shows the result of XPS spectroscopy. The Ti peaks on Figure 3.4.1.3 bottom right, indicate the presence of TiO\textsubscript{2} on the surface\textsuperscript{12-13}. Carbon peaks with shoulders were observed on PEGA surface (−), which may indicate the presence of C=C and C=O bonds (Figure 3.4.1.3 top). These peaks were significantly lower on TiO\textsubscript{2} immobilized PEGA surface, especially after the UV exposure (−) (Figure 3.4.1.3 bottom left). One reason may be that X-rays were blocked by TiO\textsubscript{2} nanoparticles and did not reach to PEGA
surface, since X-Rays in XPS would be able to penetrate surface less than 10 nm depth.\textsuperscript{13} Another reason may be the photocatalytic activity of TiO$_2$ destroyed carbon bonds resulting in carbon dioxide and water. Since photo-catalytic reaction via TiO$_2$ has enough energy to break carbon bonds (e.g. C-C, C-H, C=O),\textsuperscript{6,14-15} that could explained why less stable bonds (C=C comparing C-C) are destroyed during the photocatalytic reaction. Based on these results, it could be possible to conclude that there is immobilized TiO$_2$ on the COC-PEGA surface and therefore the changes in zeta potential upon UV exposure might be attributed to TiO$_2$ nanoparticles.
3.4.2 - Reproducibility

Table 3.4.2.1: Summary of reproducibility test. Each case was tested 5 times (n=5).

<table>
<thead>
<tr>
<th>pH3</th>
<th>Polymer</th>
<th>Signal changes from Polymer by UV light</th>
<th>Signal changes from TiO2 by UV light</th>
<th>Reproducibility</th>
<th>Signal changes towards to</th>
<th>Signal switching</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>PEGA</td>
<td>No</td>
<td>Yes</td>
<td>Fair to good</td>
<td>Negative</td>
<td>Y</td>
</tr>
<tr>
<td>3</td>
<td>Amine</td>
<td>No</td>
<td>Yes</td>
<td>Poor</td>
<td>Negative, then positive</td>
<td>Y</td>
</tr>
<tr>
<td>3</td>
<td>Acid</td>
<td>Yes</td>
<td>Yes</td>
<td>Fair</td>
<td>Negative, then positive</td>
<td>Y</td>
</tr>
<tr>
<td>7</td>
<td>PEGA</td>
<td>No</td>
<td>Yes</td>
<td>Poor to Fair</td>
<td>Negative, then positive</td>
<td>No</td>
</tr>
<tr>
<td>7</td>
<td>Amine</td>
<td>No</td>
<td>Yes</td>
<td>Poor</td>
<td>Positive, then negative</td>
<td>No</td>
</tr>
<tr>
<td>7</td>
<td>Acid</td>
<td>Yes</td>
<td>Yes? (3 out of 5)</td>
<td>Poor</td>
<td>Positive, then negative</td>
<td>No</td>
</tr>
<tr>
<td>10</td>
<td>PEGA</td>
<td>No</td>
<td>Yes</td>
<td>Poor to Fair</td>
<td>Negative</td>
<td>No</td>
</tr>
<tr>
<td>10</td>
<td>Amine</td>
<td>No</td>
<td>Yes</td>
<td>Poor to Fair</td>
<td>Negative</td>
<td>No (1 out of 5)</td>
</tr>
<tr>
<td>10</td>
<td>Acid</td>
<td>Yes</td>
<td>Inconclusive</td>
<td>Poorest</td>
<td>Inconclusive</td>
<td>No</td>
</tr>
</tbody>
</table>

UV exposure was applied for 5 min for 4 times. Phosphate buffer at conductivity ~ 190 uS was used as a solvent. Each polymer was fabricated by following compounds: PEGA by PEGA Amine by DPMA and Acid by PAA.

Although several variables were chosen and studied to improve reproducibility and repeatability, it is difficult to demonstrate the origin of the surface charge of TiO2 upon UV exposure. Table 3.4.2.1 shows the summary of the UV switching behavior for different substrates and/or pH. Figure 3.4.2.1-3.4.2.3 shows the detail of the results. PEGA substrate, which has neutral charge, would have best ability to show the repeatability of UV switching. For charged surfaces, which were made by acrylic amine (DPMA) and acid (PAA) monomers, produce interference, especially if the charge of film is the same as the charge of the particle (Figure 3.4.2.2 bottom left and 3.4.2.3 top left). This observation supports the result from chapter 2 (section 2.4.2).
Figure 3.4.2.1: Real-time monitoring of zeta potential with UV irradiation on TiO$_2$-PEGA substrate (left) and PEGA substrate (right). pH 3 (top), pH 7 (middle) and pH 11 (bottom) phosphate buffer, conductivity around 190 $\mu$S was flowed into microchannel and UV was exposed every $\sim$310 seconds for all cases. (e.g., UV was on at $t = 270, 970, 1600...$ off at $t = 640, 1300...$). 5 duplicates were shown in different color on the chart and purple color with shadow (■) shows average value and its standard deviation.
Figure 3.4.2.2: Real-time monitoring of zeta potential with UV irradiation on TiO$_2$ covered acid-polymer substrate (left) and Acid-polymer substrate (right). pH 3 (top), pH 7 (middle) and pH 11 (bottom) phosphate buffer, conductivity around 190 μS was flowed into microchannel and UV was exposed every ~310s for all cases. (e.g. UV was on at $t = 270, 970, 1600...$ off at $t = 640, 1300...$). 5 duplicates were shown in different color on the chart and purple color with shadow (■) shows average value and its standard deviation.
Figure 3.4.2.3: Real-time monitoring of zeta potential with UV irradiation on TiO2 covered amine-polymer substrate (left) and amine-polymer substrate (right). pH 3 (top), pH 7 (middle) and pH 11 (bottom) phosphate buffer, in conductivity around 190 μS were flowed into microchannel and UV was exposed every ~310 seconds for all cases. (e.g. UV was on at t = 270, 970, 1600... off at t = 640, 1300...). 5 duplicates were shown in different color on the chart and purple color with shadow (■) shows average value and its standard deviation.
Because all chips were modified via UV-polymerization, there is the possibility that the polymerization reaction was not completed during the modification of microchannel chip.

For the PEGA substrate, the signal does not go back to the original zeta potential values in neutral and basic solution (Figure 3.4.2.1 middle left and bottom left).

Since the photo-radical reaction is complicated and involve several pathways, many researchers have proposed different mechanism to explain the UV effect. Moorthy and Wang had explained the hole-electron separation pathways of TiO$_2$ in terms of surface charge. Therefore, their proposed mechanism could explain our results.$^{16,17,19}$

The surface of TiO$_2$ nanoparticle is positively charged in acidic media and negatively charged in alkaline media,$^{18}$ which is explained by the following reactions:

\[
\begin{align*}
\text{Ti}^{IV}\text{-OH} + \text{H}^+ & \rightarrow \text{Ti}^{IV}\text{-OH}_2^+, \quad \text{pH} < \text{pH}_{lep} \quad \text{eq (3.1)} \\
\text{Ti}^{IV}\text{-OH} + \text{OH}^- & \rightarrow \text{Ti}^{IV}\text{-O}^- + \text{H}_2\text{O}, \quad \text{pH} > \text{pH}_{lep} \quad \text{eq (3.2)}
\end{align*}
\]

Under UV irradiation the photo-generated holes and electrons diffuse to the surface of TiO$_2$ particles, and undergo the following reactions to decompose organic compounds, for example.$^{6,19}$

\[
\begin{align*}
\text{TiO}_2 + \text{hv} & \rightarrow \text{h}_{VB}^+ + \text{e}_{CB}^- \quad \text{eq (3.3)} \\
\text{e}_{CB}^- + \text{Ti}^{IV}\text{-OH} & \rightarrow \text{Ti}^{III}\text{-OH} \quad \text{eq (3.4)} \\
\text{h}_{VB}^+ + \text{Ti}^{IV}\text{-OH} & \rightarrow \text{Ti}^{IV}\text{-O}^-\text{-H}^+ \quad \text{eq (3.5)} \\
\text{O}_2 + \text{e}_{CB}^- & \rightarrow \text{O}_2^- \quad \text{eq (3.6)} \\
\text{O}_2^- + \text{pollutant} & \rightarrow \rightarrow \rightarrow \text{H}_2\text{O} + \text{CO}_2 \quad \text{eq (3.7)} \\
\text{H}_2\text{O} + \text{h}_{VB}^+ & \rightarrow \text{OH}^- + \text{H}^+ \quad \text{eq (3.8)} \\
\text{OH}^- + \text{pollutant} & \rightarrow \rightarrow \rightarrow \text{H}_2\text{O} + \text{CO}_2 \quad \text{eq (3.9)}
\end{align*}
\]
Since the TiO_2 surface becomes more negative with UV irradiation,\textsuperscript{17} equation 3.5 might not dominate and probably charge carriers (h_{VB}^+) might be trapped as Ti^{3+} inside of TiO_2.\textsuperscript{6} Once UV irradiation is stopped, recombination process takes place:

\begin{align*}
\text{h}_{\text{VB}}^+ + \text{Ti}^{\text{III}}-\text{OH}^- & \rightarrow \text{Ti}^{\text{IV}}-\text{OH} & \text{eq (3.10)} \\
\text{e}_{\text{CB}}^- + \text{Ti}^{\text{IV}}-\text{O}^*-\text{H}^+ & \rightarrow \text{Ti}^{\text{IV}}-\text{OH} & \text{eq (3.11)} \\
\text{e}_{\text{CB}}^- + \text{h}_{\text{VB}}^+ & \rightarrow \text{energy} & \text{eq (3.12)}
\end{align*}

Therefore, a possible reason for the returning of the signal under acidic pH, H\textsuperscript{+}, h_{VB}^+, Ti^{3+} or some other positively charged compound interfere the valence band (hole) related reaction (eq3.8) and deactivate the radical reaction (e.g. eq 8). Some other factors, like in PEGA at pH 7 (Figure 3.4.2.1 middle left) change the reaction trend from negative to positive after several times of UV exposure. Since there are sources of contamination, such as oil from the TiO_2, impurities of PEGA monomer, phosphate solution, some adsorbed organic materials may decompose via photocatalytic reactions to produce the zeta potential changes observed (eq 3.7 and 3.9). According to Figure 3.4.2.1, although each chip made with PEGA shows good repeatability for UV switching, in general it is challenging to attain good reproducibility of the zeta potential observed. Given that 3-6 mV zeta potential difference among microchannel duplicates is common for our streaming potential instrument,\textsuperscript{10} there are big challenges to improve the quality of microchannel chips or understand the source of the lack of reproducibility in some of the conditions studied.
3.4.3 – Signal behavior in different solutions

Figure 3.4.3.1: Real-time monitoring of zeta potential with UV irradiation on TiO₂-PEGA substrate perchlorate solution (top) and comparison of zeta potential changes between perchlorate solution (■) and phosphate buffer solution (●) (bottom) with conductivity around 1900 μS. UV was exposed every ~310 seconds for all cases. (e.g UV was on at t= 600, 1300, 2000... off at t= 1000, 1600...). 4 duplicates were shown in different color on the chart and purple color with shadow (■) shows average value and its standard deviation. For the right chart, $\Delta \zeta$ was calculated by $\Delta \zeta = \zeta_0 - \zeta$; $\zeta_0$= $\zeta$ before 1st time UV exposure. Perchlorate solution data were obtained from TiO₂ covered PEGA at pH 7.4 perchlorate solution (Figure 3.4.3.1 top) and phosphate buffer data was obtained from TiO₂ covered PEGA at pH 3 phosphate buffer (Figure 3.4.2.1 top left). The time length for two datasets were trimmed and adjusted to make a comparison. Therefore, time scales are not exactly correct.

Different behavior was observed when using perchlorate solution instead of phosphate buffer. The left side of Figure 3.4.3.1 shows the real-time monitoring of zeta potential with/without UV exposure and the right side Figure 3.4.3.1 shows relative zeta potential change ($\Delta \zeta = \zeta_0 - \zeta$; where $\zeta_0$= $\zeta$ before 1st time UV exposure) comparing between perchlorate and phosphate buffer solution. It is clear that the zeta potential of TiO₂ covered PEGA in perchlorate increased around 5 mV in 1 minute after UV exposure, except 1st cycle of UV exposure (Figure 3.4.3.1 left). The reason for signal variation in the 1st cycle might be the adsorption of some contaminants, such as oil from the industrially made TiO₂.
According to the right side of Figure 3.4.3.1, the speed of signal switching in phosphate buffer was much slower than the case in perchlorate solution (0.168 ± 0.003 mV/s for perchlorate solution and -0.0027 ± 0.006 mV/s for phosphate buffer UV exposure; 1st UV cycle was omitted). Also, surface charge under phosphate buffer kept decreasing even after 5 minutes of UV exposure, in contrast to the stable signal observed during 1 minute for perchlorate solution. The signal direction was also different; the signal tends to increase for perchlorate solution and decrease for phosphate buffer solution. According to Abdullah et al., phosphate buffer solution might interfere with photocatalytic reactions of TiO₂, in comparison to perchlorate solution. Another researcher group claimed that there is the formation of phosphate modified TiO₂ nanoparticles called P-TiO₂. Since UV radical reaction would bring high energy (2.8-3.2eV), TiO₂ could make a bond to phosphate molecules on the surface, given that P-TiO₂ was made by simple adsorption followed by heat-treatment. The reason of increase signal in perchlorate solution is explained by Wang et al, as a few cations on the TiO₂ surface or inside polymer holes might be neutralized by the photo-generated O₂⁻. Another possible reason might be the conductivity change within the film of TiO₂ particles during UV exposure. Xie et all reported that conductive membranes reduce magnitude of streaming potential towards to zero, which will explain the increase signal from negative to positive under perchlorate solution.
3.4.4 Signal switching behavior with adsorbed compounds

Several organic and inorganic compounds were injected into TiO$_2$-PEGA microchannel to study the UV behavior in the presence of the injected compounds. Since several researchers have reported the UV Photo-radical reaction of TiO$_2$ with organic and inorganic compounds, the effect of these compounds on the immobilized particles was studied to determine if surface charge changes could be detected via streaming potentials. Several investigators also reported that TiO$_2$ UV photocatalytic reactions change by addition of inorganic compounds.$^{25,26}$

3.4.4.1 – organic compounds

Figure 3.4.4.1.1: Real-time monitoring of zeta potential of TiO$_2$ covered PEGA microchannel at pH 3 phosphate buffer with conductivity around 180-220 $\mu$S, depending on additives. 3.5 mM formaldehyde (left) and 3.4 mM ammonium solution (right) were flowed from $t = 700$ seconds for formaldehyde and 900 s for ammonia solution until 2300 seconds for formaldehyde and 3500 seconds for ammonia. UV exposure (blue color for both cases) was applied while flowing these compounds.

Figure 3.4.4.1.1 shows the real-time monitoring of zeta potential with injection of simple organic compounds into TiO$_2$ coated PEGA microchannel with and without UV exposure. For aldehyde injection (Figure 3.4.4.1.1 left), both conditions (with/without UV) increase
the signal while flowing the compound. However, $\Delta \zeta$ without UV exposure was roughly twice higher than with UV exposure. Also, the signal after washing formaldehyde without UV decreased. On the other hand, UV exposure with addition of ammonium solution decreases the signal whereas without UV the signal increases. The signals for either UV treatment increased after flowing the original phosphate buffer solution. These behaviors for the injected compounds are different from the behavior in blank phosphate buffer. The possible reason to explain the signal difference is the photocatalytic reaction of TiO$_2$ involving decomposition of aldehyde and ammonium. Some researchers reported that most of the compounds containing carbon will be decomposed to water and carbon dioxide,$^{28,29}$ whereas ammonia solution will be converted to $\text{N}_2$, $\text{NO}_2$ and $\text{NO}_3$. It may be necessary to investigate by chromatography the effluent after the UV irradiation to identify the decomposition compounds generated. Degassing of the solution with Nitrogen was also investigated, because the presence of Oxygen determines the reaction pathways (eq 3.6).$^{30}$ However, no difference was observed.
3.4.4.2 – inorganic compound

Figure 3.4.4.2.1; Sensorgram of cadmium perchlorate injection experiment by real-time monitoring of zeta potential. Perchlorate solution at pH 7.4 was used as a blank. The experiment was studied as following procedure; 5 minutes UV switching (0 < t < 5000), UV exposure durability test (5000 < t < 12000), 5 minutes UV switching (12000 < t < 16000), flowing cadmium perchlorate without UV (16000 < t < 16500), flowing cadmium perchlorate with UV (16500 < t < 22500), flowing cadmium perchlorate without UV (22500 < t < 23000), washing cadmium perchlorate with UV switching (23300 < t).

Only cadmium was studied as an example of inorganic species in the role of coreactant for the photocatalytic reaction by TiO$_2$. Cadmium is harmful to humans and the environment. Particles of TiO$_2$ have enough energy to reduce ionized Cadmium cations to metal via photocatalytic reduction and generate cadmium-doped TiO$_2$ nanoparticles.$^{25,26}$ This is one of the common methods to generate metal-doped TiO$_2$ and that will change the photocatalytic activity of TiO$_2$. $^5$ Zeta potential changes of TiO$_2$ coated PEGA via UV switching in perchlorate solution, followed by injection of Cadmium ion with UV exposure, was investigated and is shown in Figures 3.4.4.2.1 and 3.4.4.2.2.
Figure 3.4.4.2.2; Magnification and comparison of complete sensorgram of Figure 3.4.4.2.1. The time length was rescaled to make a comparison. The left chart shows the UV activation of TiO$_2$ with (■) and without (■) cadmium ion. Right side shows the comparison of UV switching activities as an output of $\Delta \zeta$. The 1st switching was shown in blue (■), 2nd switching after 1 hour UV exposure was shown in pink (■), and 3rd UV switching after cadmium flow with UV was shown in green (■).

According to the result, there was minor zeta potential difference between initial UV exposure ($0 \leq t < 5000$ in Figure 3.4.4.2.1) and UV treatment after 1 hour ($5000 \leq t < 12000$ in Figure 3.4.4.2.1). That indicates the surface charge of TiO$_2$ coated PEGA was stable at least for 1 hour of continuous UV exposure. There is no major zeta potential difference between TiO$_2$ coated PEGA before (-41.6mV) and right after (-45.3mV) Cadmium cation solution with UV (Figure 3.4.4.2.2). The zeta potential after Cadmium ion flow was slightly higher (-43.2mV) than after UV treatment. The magnitude of the change was quite similar to the one produced by adsorption. Also, UV switching activity produces similar change in the zeta potential for all types of microchannel surfaces, Figure 3.4.4.2.2 ($\Delta \zeta = 6 \pm 1$ mV for 1st trial, $6.0 \pm 0.6$ mV for 2nd trial and $4.5 \pm 0.6$ mV for 3rd trial). Although it may be that these 1mV differences are differences from cadmium doping, it also may be too small to be
explained by the mechanism of charge separation in the doped TiO$_2$ particle well (Figure 3.4.4.2.3), because the separated electron will be collected by doped-metal$^{6,32}$.

These results indicate that cadmium either did not react with TiO$_2$, or it did not change the surface charge of TiO$_2$. Another explanation is that the charge change is undetectable via streaming potential. Nguyen et al. reported that Cadmium-doped TiO$_2$ has different surface charge from TiO$_2$ nanoparticle, measured by DLS.$^{25,26}$

3.4.4.3 – Protein Adsorption

Lysozyme and BSA were also investigated to determine if there is signal change caused by UV treatment of adsorbed proteins on TiO$_2$ coated PEGA, since Zheng et all claimed these protein adsorbed onto TiO$_2$. $^{33}$
Figure 3.4.4.3.1: Sensorgram of 200 ppm Lysozyme injection experiment recorded by real-time monitoring of zeta potential. Perchlorate solution at pH 7.4 was used as a blank. The experiment was investigated as following procedure; 5minutes UV switching (0 < t < 50), injection of 200 ppm lysozyme (69 < t < 74), washing lysozyme by blank solution (74 < t < 248), UV exposure (248 < t < 305) and then stopping UV exposure (305 < t).

Figure 3.4.4.3.1 shows real-time monitoring of zeta potential when injecting lysozyme with UV exposure. It is clear that lysozyme changed the zeta potential of TiO$_2$ coated PEGA surface to positive values (+10 mV). Desorption of lysozyme was observed after flowing the blank solution and signal decreased and stabilized to -27 mV in 90 minutes. That indicates residual lysozyme was attached to TiO$_2$-PEGA surface presumably by hydrophobic and/or electrostatic interaction. The signal increased slightly by 1.5 mV for the first 30 seconds once UV was initiated and then decreased continuously after that for the next 60 min. The signal decreased by 1.5 mV once UV exposure was stopped. These trends are different from the UV switching behavior in perchlorate solution, indicating a difference in the underlying mechanism.
Figure 3.4.4.3.2 shows the XPS spectra of UV treated TiO$_2$-PEGA microchannel after washing with lysozyme and several control experiments. When comparing to lysozyme without UV treatment (■) it seems that lysozyme adsorption on TiO$_2$ (■) produces a higher signal of the peaks for carbon (~290 eV), nitrogen (402 eV), sulfur (165 eV) and a lower signal of TiO$_2$ around 460 eV.$^{12-13}$ Lysozyme washing with UV treatment (■) significantly decreases the peak for carbon, nitrogen and sulfur. The TiO$_2$ peak increased for UV treated samples, which may indicate removal of the compounds adsorbed on TiO$_2$. These results indicate that the effect produced by adsorption of lysozyme was affected by the UV treatment of TiO$_2$. A possible explanation is that the TiO$_2$ destroyed the PEGA substrate and the organic material surrounding the TiO$_2$ particles was removed including the adsorbed lysozyme by decomposition via photocatalytic reaction of TiO$_2$.$^{6,14-15}$
Figure 3.4.4.3.2: XPS spectra of TiO$_2$ covered PEGA surface (■), with adsorption of lysozyme (■), after washing lysozyme without UV (■), and after washing with UV (■). Survey spectra (top left), C1s (top right), N1s (middle left), O1s (middle right), S2p (bottom left) and Ti2p spectra (bottom right) were investigated.
Figure 3.4.4.3.3; Durability test of Lysozyme injection experiment measured by real-time monitoring of zeta potential. Perchlorate solution at pH 7.4 was used as a blank. The experiment was investigated as following procedure; flowing blank (0 < t < 8), injection of 200ppm Lysozyme (8 < t < 13), washing BSA by blank solution (13 < t < 23), UV exposure (23 < t < 93) and then stopping UV exposure (93 < t). Blue color (■) shows 1st trial, pink color (■) shows 2nd, yellow color (■) shows 3rd and green color (■) shows 4th trial of UV treatment of microchannel after Lysozyme adsorption. All trial was done by using the same microchannel chip.

UV treatment after adsorption of lysozyme on TiO₂ coated PEGA was repeated several times by using the same microchannel chip to verify the reproducibility of the measurements (Figure 3.4.4.3.3). All trials show quite similar result, except the 4th trial, which may indicate that the microchannel was not durable enough to resist prolonged UV treatment due to destruction of PEGA and lysozyme. 6,14-15 This result indicates that one microchannel is able to withstand the UV treatment of lysozyme several times, however, the durability of TiO₂ covered PEGA has to improve.
Figure 3.4.4.3.4: Sensorgram of BSA injection experiment generated by real-time monitoring of zeta potential. Perchlorate solution at pH 7.4 was used as a blank. The experiment was investigated as following procedure; 5 minutes UV switching (0 < t < 80), injection of 200 ppm BSA (81 < t < 86), washing BSA by blank solution (86 < t < 104), UV exposure (104 < t < 160) and then stopping UV exposure (160 < t).

Figure 3.4.4.3.4 shows real-time monitoring of zeta potential with injection of BSA with UV exposure. The BSA changed the zeta potential of TiO$_2$ coated PEGA surface to less negative value (-27 mV to -24 mV). Although XPS shows some adsorption of BSA during injection, no desorption was observed according to the negligible zeta potential change observed when flowing blank solution. That indicates the residual BSA was attached to TiO$_2$-PEGA surface probably by hydrophobic interaction$^{33}$. The signal gradually increased to -20 mV after 10 minutes of UV exposure, and then decreased continuously with further UV treatment (45 min). The signal decreased around 3 mV once UV exposure ceased, which is similar behavior as observed with perchlorate solution. (Figure 3.4.3.1)
Figure 3.4.3.5: XPS spectra of TiO$_2$ covered PEGA surface (■), with adsorption of BSA (●), after washing BSA without UV (▲), and after washing with UV (▲). Survey spectra (top left), C1s (top right), N1s (middle left), O1s (middle right), S2p (bottom left) and Ti2p spectra (bottom right) were investigated.
Figure 3.4.4.3.5 shows the XPS spectra of UV treatment of TiO$_2$ coated PEGA microchannel surface after flowing BSA and several control samples. It seems BSA adsorption on TiO$_2$ (■) produce higher signal on the peaks for carbon (~290eV), nitrogen (402eV) and similar signal to TiO$_2$ around 460eV,\textsuperscript{12-13} in comparison to BSA without UV treatment (■). BSA flow with UV treatment (■) decreases the XPS peaks of carbon and nitrogen which may indicate BSA damage upon UV treatment. The Ti peak increased with BSA and UV treatment, which is consistent with destruction and removal of the adsorbed BSA. These results indicate the effect generated by adsorption of BSA was completely changed by UV treatment of TiO$_2$. The same explanation given for lysozyme might be applied to BSA.

![XPS spectra of UV treatment of TiO$_2$ coated PEGA microchannel surface](image)

Figure 3.4.4.3.6: Durability test of BSA injection experiment measured by real-time monitoring of zeta potential. Perchlorate solution at pH 7.4 was used as a blank. The experiment was investigated as following procedure; flowing blank ($0 < t < 10$), injection of 100 ppm BSA ($10 < t < 15$), washing BSA by blank solution ($15 < t < 30$), UV exposure ($30 < t < 85$) and then stopping UV exposure ($85 < t$). Blue color (■) shows 1st trial, pink color (■) shows 2nd, yellow color (■) shows 3rd and green color (■) shows 4th trial of UV treatment of microchannel after BSA adsorption. All trial was done by using the same microchannel chip.

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UV treatment after adsorption of BSA on TiO$_2$ coated PEGA was repeated several times by using the same microchannel to study the repeatability of the results. Figure 3.4.4.3.6 shows the different trial with quite similar result, except 1st and 3rd trial in which significant signal change was observed during UV treatment. This behavior may indicate some blocking or aggregation in the microchannel. Destruction of PEGA that generates cavities by UV treatment may be a possible explanation.\textsuperscript{6,14-15} The result indicates although one microchannel chip is able to measure the UV treatment of BSA several times, the signal during UV treatment experiment is not completely reproducible.

![Figure 3.4.4.3.7: Comparison of UV switching under flowing protein solution as an output of $\Delta \zeta$. UV was turn on and off every 5 minutes started from 7 minutes. Blue color (■) indicates UV switching behavior of TiO$_2$ covered PEGA microchannel in perchlorate solution, Pink color (■) shows UV switching behavior of TiO$_2$ covered PEGA microchannel in perchlorate with 200 ppm lysozyme, green color (■) shows UV switching behavior of TiO$_2$ covered PEGA microchannel in perchlorate with 100 ppm BSA solution and grey color (■)shows UV switching behavior of PEGA microchannel in perchlorate solution as a reference. The initial zeta potentials were -46 mV for perchlorate, 15 mV for lysozyme, -24 mV for BSA and -21 mV for perchlorate on PEGA.](image)
The behavior in perchlorate solution with/without protein were studied and shown in Figure 3.4.4.3.7. It seems that the presence of the protein in solution may change the surface charge behavior under UV exposure, No significant changes were observed for the presence of BSA during UV irradiation. For BSA, it could be that during UV irradiation of TiO$_2$ turns the microchannel surface to be hydrophilic,$^{34}$ making difficult to interact with BSA and thus the protein is not able to strongly adsorbed in the Stern layer of TiO$_2$ coated PEGA, which in turn would not produce a zeta potential change.$^{33}$ This explanation fits the result of Figure 3.4.4.3.8, which shows almost the same zeta potential after BSA flow with and without UV treatment in perchlorate solution. To continue further investigation, as mentioned in section 3.4.4.1, it might be needed to use chromatography to analyze the products of the photochemical reactions.

Figure 3.4.4.3.8: Comparison of UV switching behavior of TiO$_2$ covered PEGA between injection of 100 ppm BSA solution in perchlorate (■) and perchlorate solution right after the UV switching with 100 ppm BSA experiment (■) as an output of zeta potential. UV was turn on and off every 5 minutes started from 6 minutes. The time scale was adjusted to make a comparison, since these two experiments were done in the same microchannel chip.
3.5 - Conclusion

Nanoparticles of TiO$_2$ were immobilized on COC-PEGA microchannel surfaces to investigate if changes in the surface charge of the microchannel induced by UV exposure could be detected by streaming potentials. Although it is challenging to achieve appropriate reproducibility in zeta potential, the UV treatment caused surface changes that were detected by streaming potentials. No particular surface charge was found to be the to immobilize TiO$_2$ nanoparticles. The interaction of several organic, inorganic compounds and proteins on the photocatalytic activity of TiO$_2$ was study with and without UV treatment. It was demonstrated that adsorption of proteins shows clear zeta potential changes upon UV exposure, supported by XPS analysis. Further investigation needs to be done to confirm if any reaction was involved possibly by chromatographic techniques.
3.6 - References


Vi, N. N. H. PhD. Dissertation, University of New South Wales, 2005.


