A hydrophobicity recoverable EWOD (electrowetting-on-dielectric) based chemiluminescence detector with an integrated signal and heater electrode was developed. X-ray-photoelectron-spectroscopy (XPS) was used to reveal the wetting and dewetting mechanism of Teflon on the EWOD device. It was found that the C-O bond formed on the surface of Teflon after the chemiluminescence reaction led to the surface permanent wetting. To recover the contact angle of the Teflon surface, the recovery threshold time and heating temperature were proposed experimentally for dewetting to release C-O bond.

**INTRODUCTION**

Chemiluminescence is one of the most important immuno-detection methods which can be widely used in the fields of food, industry, environment, clinical diagnosis and medicine. It has many advantages, including high detection sensitivity with simple instrument configuration, wide linear range of signal response, and rapid measurement [1-10].

But conventional chemiluminescence analyzers are not suitable for cheap, compact and portable detector as the bulky analysis machines and high volumes of samples and bio-reagents consumption for measurement.

Compared to conventional chemiluminescence analyzers, we reported an EWOD based chemiluminescence detector for rapid and automatic H$_2$O$_2$ measurement with high sensitivity, wide linear detection range and low sample consumption, which showed many advantages for highly precise portable diagnosis of blood glucose potentially [11].

However, we found that Teflon on the EWOD turned into hydrophilic after chemiluminescence reaction. A similar phenomenon was reported in Richard Far’s research. They considered that the immuno-detection would contaminate the Teflon surface, and the protein adsorption would render the surface permanently hydrophilic, which was detrimental to the droplet transport [12]. Therefore, they immersed the EWOD chip in the silicone oil to isolate the droplet from the Teflon surfaces to prevent any enzymes contamination and protein adsorption.

Differing from their work, we found that the Teflon surface wetting phenomenon could be dewetted by annealing. It was verified by analysis of XPS. We designed an EWOD device with an electrode for both heating and droplet controlling to make its Teflon surface automatically recoverable.

**EXPERIMENT AND ANALYSIS**

**Material and chemicals**

For the device fabrication, the Teflon$^\text{®}$ AF 2400 was purchased from DuPont. SU-8 2002 with developer was purchased from Microchem, and indium tin oxide (ITO) glass from Weisi Technology Co. Ltd., Guangdong, China.

For the chemiluminescence detection, Horse-radish peroxidase (HRP), luminol, 4-iodophenol (PIP) were purchased from Yuanye Biological Technology Co. Ltd., Shanghai, China. The H$_2$O$_2$ solution was purchased from Pengshen Chemical Reagent Co. Ltd., Jiangsu, China.

**System building up and chemiluminescence detection**

A diagram of the chemiluminescence detection system is shown in Figure 1(a). The photomultiplier (SenSL, MiniSL-30035- X08, 9 mm$^2$ active area) below the single planar transparent EWOD device is used for detecting the chemiluminescence signal, and the periphery circuits can provide control signals for the EWOD.

In the field of chemiluminescence detection, the main method is utilizing the luminol-HRP system to measure the concentration of H$_2$O$_2$, which is the most common intermediate product in immuno-detection.

![Chemiluminescence Droplet](image)

The droplets of four reagents (10µL HRP, 10µL luminol, 10µL PIP(4-iodophenol) and 10µL H$_2$O$_2$ were mixed together at the center of the EWOD device, shown in Figure 1(b). After reaction, the mixed droplet will emit blue chemiluminescence signal which can be detected by the photomultiplier [11].

![Figure 1: (a) Diagram of chemiluminescence detection system; (b) Diagram of four reagents mixing process and driving electrode pattern.](image)
It should be noticed that the hydrophilic surface after chemiluminescence reaction in our detection not only will be detrimental to the transport of the droplet, causing the permanently failure of the EWOD device, but also will induce a small contact angle and thus lower the detection sensitivity [11], as shown in Fig.2. Therefore, study on hydrophobic recovery and the mechanism of Teflon wetting and dewetting on the EWOD device are carried out experimentally.

**Figure 2**: Experimental results of contact angle vs. output voltage of photomultiplier. The original contact angle is 120°. It demonstrates that the more hydrophobic surface, the stronger optical signal the photomultiplier received, the larger photoelectric voltage the photomultiplier output, the higher sensitivity of the chemiluminescence detection. The chemiluminescence reagents were 10µL 100mg/L HRP, 10µL 2mmol/L luminol, 10µL 5mmol/L PIP and 10µL 40mmol/L H2O2.

The mechanism of Teflon wetting and dewetting

In Richard Fair’s research [12], they considered that the protein adsorption would render the surface permanently hydrophilic. However, we found that the ‘permanently hydrophilic’ surface could be recovered by annealing, and we considered that the phenomenon of Teflon wetting was just related to the reaction itself.

We tried to put the EWOD device with wetting Teflon surface after chemiluminescence reaction onto a 200°C hot plate. 10min later, the surface of Teflon could dewet to hydrophobic as its initial state, as shown in Figure3.

**Figure 3**: Photos of DI (de-ionized) water on (a) initial hydrophobic Teflon surface; (b) wetting surface of Teflon after chemiluminescence reaction; (c) dewetting surface of Teflon after annealing.

Since enzymes and proteins cannot be removed on 200°C, we inferred that the wetting of Teflon after chemiluminescence reaction was not caused by the adsorption of those bio-chemicals, but by the bond fractures which was induced by reaction intensity.

In order to verify that the hydrophilic modification was determined by the intensity of chemiluminescence reaction, the contact angle on different devices with different concentration reaction of reagents were tested after the chemiluminescence reactions. We kept the concentration of HRP(100mg/L), luminol (2mmol/L) and PIP (5mmol/L) and mixed them with different concentration solutions of H2O2 from 0.25mmol/L to 20mmol/L. Figure 4 shows the results that the stronger the reaction intensity, the smaller the contact angle.

**Figure 4**: Experimental results of concentration solutions of H2O2 vs. contact angle on Teflon after chemiluminescence reaction.

Further surface analysis was used to reveal the essential mechanism of Teflon wetting and dewetting.

Teflon® AF [chemical name: fluorinated (ethylenic-cyclo oxyaliphatic substituted ethylenic) copolymer] is a family of amorphous fluoropolymers based on copolymers of 2,2-bistrifluoromethyl-4,5-difluoro-1,3-dioxole(PDD)[13]. According to its chemical structure which is shown below[14-16], we can get that C, F, O are the three main elements on the surface of our device.

![Chemical structure of Teflon AF](image)

We used XPS to analyze the elements on Teflon surface of our device before reaction, after chemiluminescence reaction (cleaned by DI water and dried in nitrogen airbrush) and after annealing. The XPS spectra are shown in Figure 5. Comparing the three curves in Figure 5, we can see that the peak of element O after reaction is much higher than that before reaction, and it returns to its original state after annealing. The peak of element F after reaction is much lower than that before reaction, and it recovers to the initial high level after annealing as well.
The C-F bond broken in chemiluminescence detector in practical use. After annealing the device after reaction is necessary for the chemiluminescence reaction, which induced the hydrophobic fluorocarbons surface turning into hydrophilic. By washing the surface with DI water and annealing with the central heater, the hydrophilic surface could be recovered to hydrophobic.

Figure 5: XPS spectra of Teflon surface. The rate of element O vs. element F is much higher after chemiluminescence reaction than that before reaction and after recovery.

We also used XPS spectra to analyze the C bond on the Teflon surface before and after chemiluminescence reaction, as well as after recovery, as shown in Figure 6. We can see that the number of F-C-O bond after reaction is more than that before reaction and after recovery. Comparing the three curves, the two strengthened peaks with 290.5eV and 287.6eV after reaction are all relative with C-O bond [17], which induces the surface of Teflon hydrophilic.

Figure 6: High-solution C1s XPS spectra of Teflon surface. The peak of F-C-O bond is much higher after chemiluminescence reaction than that before reaction and after recovery.

From the analysis of all the XPS spectra, we can conclude that the C-F bond on the surface was broken and the C-O bond was formed after the chemiluminescence reaction, which induced the hydrophobic fluorocarbons surface turning into hydrophilic. After annealing, the element O in C-O bond was released and the surface property recovered to hydrophobic. Therefore, on-chip annealing the device after reaction is necessary for the chemiluminescence detector in practical uses.

The chemiluminescence chip with ITO heater

In order to recover hydrophobic surface of Teflon automatically, an ITO heater was directly integrated at the center of the EWOD chip, as shown in Figure 7. When the two pads of the electrode were connected to the signal (30V, 1kHz) and the ground separately, it would be the heating source; while the two pads were both connected to the signal (30V, 1kHz) or ground at the same time, it would be a typical EWOD controlling electrode.

Figure 7: EWOD device for chemiluminescence reaction with heater at center.

When the central heater was used as a typical EWOD controlling electrode, the four reagents could be mixed together and reaction. After chemiluminescence reaction, the Teflon surface underneath the reaction droplet would turn into hydrophilic. By washing the surface with DI water and annealing with the central heater, the hydrophilic surface could be recovered to hydrophobic.

The Figure 8(a) shows the recovered contact angles changing with the annealing time in different annealing temperature after chemiluminescence reaction. Fig.8 (b) illustrates the relationship of the recovered contact angles with different annealing temperature and time after chemiluminescence reaction. It demonstrates that the higher annealing temperature, the better recovery result, and the shorter annealing time.

Since enzyme and protein do not decompose in low temperature such as 100°C, the recovery result also reveals that the essential mechanism of the surface permanent hydrophilic after bio-reaction has nothing to do with the protein adsorption, just because of the C-F bond broken in the chemiluminescence reaction.

SUMMARY

In this work, the wetting mechanism of Teflon in an EWOD based chemiluminescence detector was revealed. A series of experiments and XPS analysis demonstrated that the breaking of C-F bond and forming of C-O bond on the surface of Teflon after the chemiluminescence reaction led to the surface wetting. By annealing the EWOD chip, the C-O bond could be released, and the Teflon surface could be dewetted.
To recover the hydrophobicity of Teflon, an integrated heater and annealing process on EWOD device were proposed. The recovery relationships between the recovering contact angle, the recovery threshold time and heating temperature were studied.

Figure 8: (a) Recovering contact angle of Teflon vs. heating time under different heating temperature after reaction. (b) Relationship between recovering contact angle, recovery threshold time and heating temperature.

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REFERENCES

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