Reliability of a Compact and Portable Chemiluminescence Detector

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Abstract: Two reliability issues (the lowering of voltage and the recovery of Teflon) for a compact chemiluminescence detector for glucose measurement based on a single planar transparent EWOD (electrowetting-on-dielectrics) device are studied. Several dielectrics for lowering the manipulation voltage are investigated and a low voltage of 20-27V is realized. An on-chip heater is designed and manufactured for restore the damage to the hydrophobic surface of the EWOD following the chemiluminescence reaction. Glucose measurements show detector sensitivity reaches $0.12\text{V}/\mu\text{M}$, with a detection range of $1\mu\text{M}$ to 20mM and a detection limit of $1\mu\text{M}$, indicating it's the detector's potential as a portable immuno-detector offering prompt response and low cost measurement compared with expensive and bulky traditional instruments.

Keywords: EWOD; Chemiluminescence; Glucose detection; Portable

Introduction

Electrowetting-on-dielectric (EWOD) devices have been widely applied for chemical and biological detection [1-7]. EWOD based chemiluminescence detectors are a key research focus in fields related to food preparation and storage, industrial processes, environmental monitoring, and clinical diagnostics [8, 9]. Combined with EWOD, the detectors are compact, portable and automatically controllable, consuming only tiny amounts of expensive reagents and samples for bio-chemical detection like glucose[10-16].

This paper reports a compact chemiluminescence detector for glucose measurement based on a single planar transparent EWOD device. It features high sensitivity, a large detection range, and a low detection limit. The detector has high potential to serve as a portable immuno-detector, providing prompt responses and low cost measurement compared to expensive and

bulky traditional instruments.

Figure 1 provides a schematic of the construction of the chemiluminescence detector system. The droplets on the EWOD chip are mixed by the control circuit. In a dark box, the droplets then react and emit fluorescence, which can be received by a photo detector and converted into voltage signals.

Figure 1. Structure of the chemiluminescence detector system.

This system can be used to detect glucose concentrations based on the oxidation of luminol (3-aminophthalhydrazide) in an alkaline medium emitting light in a wavelength range of 425–435 nm. This method is widely used for detecting oxidants as MnO_4^- , $[\text{Fe}(\text{CN})_6]^{3-}$, IO_4^- , H_2O_2 , and reductase such as SO_3^{2-} , NO or organic compounds [17]. The chemical equation shown in Fig. 2 illustrates the glucose detection process: Glucose-oxidase (GOD) oxidizes the blood glucose and produces H_2O_2 . In a phosphate buffered saline (PBS) solution (pH=8~9), HRP catalyzes the H_2O_2 to oxidize luminol, producing 3-aminophthalate and emitting fluorescence, the intensity of which reflects the glucose concentration.

Figure 2. Chemiluminescence reaction for glucose detection

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As is shown Fig. 3, a typical EWOD chip is constructed of electrodes, a dielectric layer and a hydrophobic layer (e.g., Teflon as shown in Fig. 3). When we apply a certain voltage to an electrode, the contact angle (CA) between the droplet and the chip surface will change and the droplet will be pulled towards the electrode.

Figure 3. Schematic of single planar EWOD chip and droplet motion.

Good photo detection sensitivity requires maximizing light exposure, and this is accomplished through two strategies. First, a transparent EWOD chip is fabricated on a glass substrate with a layer of ITO on its surface. The transparent dielectric layer can be Ta_2O_5 , Al_2O_3 , HfO_2 or SU-8, etc. Second, a single planar EWOD chip guarantees that droplets maintain an approximately spherical shape, which focus the fluorescence and significantly enhance the chemiluminescence signal [12].

The variation of the contact angle along with the applied voltage can be described by the Lippmann equation:

$$\cos \theta_v - \cos \theta_0 = \frac{\epsilon \epsilon_0}{2 \gamma_{LV} t} V^2 \quad (1)$$

In Eq. (1), θ_v and θ_0 are the CAs when the applied voltage is respectively on and off. ϵ and ϵ_0 are respectively the permittivity of the dielectric layer and the permittivity of vacuum. t is the thickness of the dielectric layer and γ_{LV} is the interface energy between the air and the droplet.

To make the detector compact and portable, the driving voltage on the EWOD should be as low as possible so as to reduce the complexity of peripheral circuits and allow for IC integration. From Eq. (1), it is clear that this can be accomplished by using high dielectric material and by reducing the thickness of the dielectric layer. However, breakdown of the dielectrics should be avoided in the meantime. So the dielectric layer cannot be too thin.

In addition, the Teflon surface becomes hydrophilic after chemiluminescence reactions, thus impeding droplet motion and reducing the sensitivity and accuracy

of chemiluminescence detection. Our previous work studied this phenomenon and proposed a solution involving annealing at above 200°C for 5min [13]. Thus, to ensure the portable detector works properly, a heater is integrated into the chip.

This paper investigates two reliability problems: the driving properties related to different high dielectric materials and Teflon recovery through the use of an on-chip heater.

Chemiluminescence detector for glucose

Chemicals and Apparatus

The reagents used include 10 μ L glucose with concentrations ranging from 1 μ M to 20mM, 10 μ L 100mg/L HRP, 10 μ L 10mM GOD, and 10 μ L pre-mixing solution including 1mM luminol and 2.5mM p-Indophenol (PIP). PIP is utilized to enhance the fluorescence and shorten the reaction time.

The chemiluminescence signal is detected by a photomultiplier (SenSL, MiniSL-30035-X08, the active area was 9 mm²)

The contact angles are measured using a Drop Shape Analyzer DSA30 (KRÜSS, GmbH).

Fabrication process of the EWOD chip

The fabrication process of the transparent single planar EWOD chip starts from cleaning a 1mm thick ITO glass substrate. The ITO electrodes are patterned using photolithography and wet etching in HNO₃:HCl:H₂O=1:3:6 solution at 65°C for 1 min. After removal of the photoresist and cleaning, a layer of thin film with a high dielectric constant is deposited, *e.g.*, 300nm Ta₂O₅ by PVD, or 50nm HfO₂ or Al₂O₃ by ALD or 1.5 μ m SU-8 by spin coating. The last step is to spin coat 80nm of Teflon® AF2400 at 4000rpm for 30s.

A heater is integrated into the EWOD chip for glucose detection. The chip measures 4cm by 2.5cm. Droplets with volumes ranging from 5 μ L to 40 μ L can be driven smoothly from the periphery to the center on the chip where they are mixed prior to reaction.

Figure 4. Layout of the single planar EWOD chip integrated with a heater for mixing four droplets.

Comparison of different dielectric layer

From Eq. (1), the magnitude of the voltage mainly relies on the thickness and the dielectric constant of the dielectric layer. To reduce the driving voltage, different dielectric layers coated with 80nm Teflon are compared. A DC voltage is applied to the droplet by a tiny probe and the contact angle is recorded using a contact angle meter (Fig. 5).

Figure 5. Contact angle vs. applied voltage of single planar EWOD chips with different dielectric layers

From an initial CA of about 115°, the droplet can be continuously driven as the CA declines to about 90°. From Fig. 5, we can see that the contact angle of the EWOD chips with Ta₂O₅, HfO₂ or Al₂O₃ can decline to about 90 degrees when a voltage of 20V is applied, while the chip with SU-8 needs 60V or more. Though the EWOD chip with HfO₂ exhibits a relatively lower driving voltage, it unfortunately breaks down when higher voltages are applied.

Further experiments were conducted to verify the actual driving ability of each EWOD chip with a different dielectric layer. Figure 6 shows the driving velocity vs driving voltage (1kHz sine wave). The effective value (root-mean-square, r.m.s) of the driving voltage is recorded when the droplet cannot keep up with the signal. The volume of the droplet is 10 μ L for all chips.

Figure 6. Driving velocity vs. applied voltage of the single planar EWOD chip with different dielectric layer

Figure 6 shows that the droplet on EWOD chip with Al_2O_3 , HfO_2 or Ta_2O_5 layers starts to move (1mm/s) at a very low voltage of about 18-20V, and that the velocity can reach 40mm/s when the voltage rises to 25-27V, while the chip with the SU-8 layer requires significantly higher voltages to achieve such rates (1mm/s at 65V and 40mm/s at 73V). Taking the manufacturing process into account, the Ta_2O_5 layer made by PVD is obviously the best solution. It is cheaper to fabricate and provides faster droplets movement than the ALD dielectrics, while still providing high reliability at low driving voltages.

On-chip heater

To allow the Teflon surface to recovery automatically, a heater is integrated into the EWOD chip. The heater is constructed from the ITO electrodes, which are made of the same material as typical EWOD controlling electrodes. This maintains the simplicity of the original processing step.

In a single-planar EWOD chip model with glass substrates, electrodes, dielectric layer and Teflon, a simplified approximate formula of the relationship between the temperature and the heating power in a thermal equilibrium state can be deduced as follows:

$$T = T_0 + \frac{V^2}{hRA} = T_0 + \frac{P}{hA} \quad (2)$$

Here, A is the area of the heater, and h is a simplified coefficient related to the material, the layer thickness and other factors. In this model, for the sake of simplicity, the heater is assumed to cover the whole area of the chip and the temperature is uniform at the surface.

The heater model is built using Comsol multiphysics. Given that the square resistance of our ITO glass substrate is $15\Omega/\square$, the heater electrode with a resistance of $1\text{k}\Omega$ is designed as shown in Fig. 7. The layout of the heater identical to that shown in Fig. 4.

Figure 7. Comsol model of the on-chip $1\text{k}\Omega$ heater and the temperature distribution when a voltage of 30V is applied. The center temperature is 265.9°C .

In the Comsol model, the applied voltage is changed to simulate the relationship between the

heating power and temperature, as shown in Fig. 7.

Figure 8. Simulation results and linearity fitting of heating power vs peak temperature

T_{peak} here means the temperature at the center of the chip surface. The simulated relationship between T_{peak} and the heating power (P) can be described as:

$$T_{\text{peak}} = 80 + 0.20 * P \quad (3)$$

The good linearity presented here proves that the theoretical formula of the simplified EWOD model fits well.

According to Eq. (3), the heating power and temperature are positively correlated. Figure 9 presents the experimental results for CA recovery. Both on-chip heating and direct heating by hot plate are tested. The on-chip heater's effect on recovery corresponds closely to the hot plate temperature.

Figure 9. Relationship between recovered CA and heating power (by on-chip heater, the blue line) or temperature (by hot-plate, the red line) after being heated for 5 min.

In Fig. 9, the red line is a little “higher” than the blue line, especially in the low temperature area. This is because the temperature distribution is not uniform, as is shown in Fig. 7. In addition, time is needed to warm up the on-chip heater. For comparison purposes, data is only recorded after the heater has been on for 5 min. CA

recovery improves when heated for a longer time, and can recover to approximately 120° when heated at 200°C for 10 min. The on-chip heater facilitates Teflon layer recovery and device reusability by applying a voltage of 33V to the heater for 5 min.

Glucose detection

Glucose and other reagents are driven to the center of the EWOD chip for reactions. The fluorescence is received and converted to a voltage signal. Figure 10 shows the successful detection of glucose concentrations ranging from 1μM to 20mM. The detected voltages show a good linear correspondence with the level of concentration. By tuning the amplifier, very low concentrations ranging from 1μM to 100μM can be detected. After each reaction, the droplet is drawn away and DI water is driven to the center to clean the mixing and detection area. Voltage is then applied to the heater to recover the CA for the next detection iteration.

The relationship between voltage and concentration can be described through linear fitting. In ranges from 250μM to 20mM, it is:

$$V = 5.8 \times 10^{-5} \cdot C + 0.14 \quad (4)$$

In ranges from 1μM to 100μM, it is:

$$V = 0.12C - 0.08 \quad (5)$$

C refers to the glucose concentration in terms of μmol/L, and V is the detected voltage with unit V.

Figure 10. Chemiluminescence detector linear measurement of glucose concentrations: (a) ranging from 250μM to 20mM. (b) ranging from 1μM to 100μM.

In the high concentration range from 250μM to 20mM, the detector exhibits a high degree of linearity with a sensitivity of 58μV/μM, while in the low

concentration range from the 1μM to 100μM, the sensitivity reaches 0.12V/μM. The detection limit reaches 1μM, which is 1000 times lower than many home blood glucose biosensors, and much lower than the latest reported electrochemical glucose biosensors [18-19].

In addition, in our experiment, the received signal is displayed on an oscilloscope and shows that the response is immediate and will decay after about 20s, indicating a prompt detection response.

Conclusion

A compact chemiluminescence detector for glucose measurement with high sensitivity and a large detection range is reported and evaluated in terms of reliability. The driving voltage can be as low as 20-27V using 300 nm Ta₂O₅ by PVD as the dielectric layer, and a 1kΩ heater is integrated into the chip to maintain the Teflon's hydrophobic properties. The detector can be reused after applying a voltage of 33V for 5 min. The detector's limit can be 1μM and the range is from 1μM to 20mM. Future work will focus on improving glucose detection and further system integration.

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References

- [1] S. K. Fan, C. Hashi and C. J. Kim, "Manipulation of multiple droplets on N×M grid by cross-reference EWOD driving scheme and pressure-contact packaging," in *16th IEEE Annual International Conference on Micro Electro Mechanical Systems*, Kyoto, 2003, pp. 694-697. doi:[10.1109/MEMSYS.2003.1189844](https://doi.org/10.1109/MEMSYS.2003.1189844)
- [2] F. Mugele and J. C. Baret, "Electrowetting: from basics to applications," *Journal of Physics: Condensed Matter*, vol. 17, pp. 705, 2005. doi: [10.1088/0953-8984/17/28/R01](https://doi.org/10.1088/0953-8984/17/28/R01)
- [3] R. B. Fair, "Digital microfluidics: is a true lab-on-a-chip possible?" *Microfluidics and Nanofluidics*, vol. 3, pp. 245-281, 2007. doi:[10.1007/s10404-007-0161-8](https://doi.org/10.1007/s10404-007-0161-8)
- [4] Y. H. Chang, G. B. Lee, F. C. Huang, Y. Y. Chen and J. L. Lin, "Integrated polymerase chain reaction chips utilizing digital microfluidics," *Biomedical Microdevices*, vol. 8, pp. 215, 2006. doi:[10.1007/s10544-006-8171-y](https://doi.org/10.1007/s10544-006-8171-y)
- [5] R. Sista, Z. Hua, et al., "Development of a digital microfluidic platform for point of care testing," *Lab on a Chip*, vol. 8, pp. 2091, 2008. doi:[10.1039/B814922D](https://doi.org/10.1039/B814922D)

- [6] I. A. Eydelnant, U. Uddayasankar, B. B. Li, M. W. Liao and A. R. Wheeler, "Virtual microwells for digital microfluidic reagent dispensing and cell culture," *Lab on a Chip*, vol. 12, pp. 750, 2012.
doi:[10.1039/C2LC21004E](https://doi.org/10.1039/C2LC21004E)
- [7] Y. Yu, J. Chen and J. Zhou, "Parallel-plate lab-on-a-chip based on digital microfluidics for on-chip electrochemical analysis," *Journal of Micromechanics and Microengineering*, Vol. 24, pp. 015020, 2014.
doi:[10.1088/0960-1317/24/1/015020](https://doi.org/10.1088/0960-1317/24/1/015020)
- [8] L. J. Kricka, "Clinical applications of chemiluminescence," *Analytica Chimica Acta*, vol. 500, pp. 279, 2003.
doi:[10.1016/S0003-2670\(03\)00809-2](https://doi.org/10.1016/S0003-2670(03)00809-2)
- [9] L. Gámiz-Gracia, A. M. García-Campaña, J.F. Huertas-Pérez and F.J. Lara, "Chemiluminescence detection in liquid chromatography: Applications to clinical, pharmaceutical, environmental and food analysis—A review," *Analytica Chimica Acta*, vol. 640, pp. 7, 2009.
DOI: [10.1016/j.aca.2009.03.017](https://doi.org/10.1016/j.aca.2009.03.017)
- [10] M. G. Pollack, V. K. Pamula, V. Srinivasan and A. E. Eckhard, "Applications of electrowetting-based digital microfluidics in clinical diagnostics digital microfluidics in clinical diagnostics," *Expert Review of Molecular Diagnostics*, vol. 11(4), pp. 393, 2011.
doi:[10.1586/erm.11.22](https://doi.org/10.1586/erm.11.22)
- [11] K. Choi, A. Ng, et al., "Automated Digital Microfluidic Platform for Magnetic-Particle-Based Immunoassays with Optimization by Design of Experiments," *Analytical Chemistry*, vol. 85, pp. 9638, 2013.
doi: [10.1021/ac401847x](https://doi.org/10.1021/ac401847x)
- [12] X. Zeng, K. Zhang, J. Pan, G. Chen, A.Q. Liu, S.K. Fan and J. Zhou, "Chemiluminescence detector based on a single planar transparent digital microfluidic device," *Lab on a Chip*, vol. 13, pp. 2714, 2013.
doi:[10.1039/C3LC50170A](https://doi.org/10.1039/C3LC50170A)
- [13] X. Zeng, K. Zhang, G. Tao, Z. Zeng, S. K. Fan, and J. Zhou, "Recoverable electrowetting-on-dielectric device in chemiluminescence enzymatic detector," *Japanese Journal of Applied Physics*, vol. 53(6), pp. 060304, 2014.
doi: [10.7567/JJAP.53.060304](https://doi.org/10.7567/JJAP.53.060304)
- [14] V. Srinivasan, V. Pamula, M. Pollack and R. Fair, "a digital microfluidic biosensor for multianalyte detection," in *16th IEEE Annual International Conference on Micro Electro Mechanical Systems*, Kyoto, 2003, pp.327-330.
doi:[10.1109/MEMSYS.2003.1189752](https://doi.org/10.1109/MEMSYS.2003.1189752)
- [15] V. Srinivasan, V. Pamula and R. Fair, "An integrated digital microfluidic lab-on-a-chip for clinical diagnostics on human physiological fluids," *Lab on a Chip*, vol. 4, pp. 310, 2004.
doi:[10.1039/B403341H](https://doi.org/10.1039/B403341H)
- [16] G. Perry, F. Lapierre, Y. Coffinier, V. Thomy and R. Boukherroub, "Superhydrophobicity and Graphene Oxide Nanosheets to Prevent Biofouling in EWOD based Lab-on-chip Devices," in *222nd Meeting of ECS*, Honolulu, 2012, pp. 2777.
- [17] J. Ocaña-González, M. Ramos-Payán, et al., "Application of chemiluminescence in the analysis of wastewaters – A review," *Talanta*, vol. 122, pp. 214–222, 2014.
doi:[10.1016/j.talanta.2014.01.028](https://doi.org/10.1016/j.talanta.2014.01.028)
- [18] W. Zhao, Y. Ni, Q. Zhu, R. Fu, X. Huang and J. Shen, "Innovative biocompatible nanospheres as biomimetic platform for electrochemical glucose biosensor," *Biosensors and Bioelectronics*, vol. 44, pp. 1, 2013.
DOI: [10.1016/j.bios.2012.12.036](https://doi.org/10.1016/j.bios.2012.12.036)
- [19] J. D. Newman, and A. P. F. Turner, "Home blood glucose biosensors: a commercial perspective," *Biosensors and Bioelectronics*, vol. 20, pp. 2435, 2005.
doi:[10.1016/j.bios.2004.11.012](https://doi.org/10.1016/j.bios.2004.11.012)