

High-Sensitive Biosensor for Urea Detection in Urine via Hydrogel Whispering-gallery Mode Microresonators

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ABSTRACT

Urea is a critical biomarker in biomedical applications, therefore sensitive detection for urea has been an attractive topic and has provided the basis in diseases diagnosis. In recent years, increasing attention has been paid to developing sensitive and reliable sensing strategies, however, conventional detecting methods often suffer from limited sensitivity and long response time. Hydrogels, with their unique three-dimensional network structures, are regarded as promising sensors. Nevertheless, detecting low levels of urea still remains challenging, as the signal outputted by hydrogel is weak. In this paper, we propose a hydrogel-based microfluidic biosensor that incorporates an enzyme-functionalized, pH-sensitive hydrogel within a whispering-gallery-mode (WGM) microresonator. By leveraging the ultrahigh quality (Q) factor of WGMs, the sensor significantly enhanced sensitivity. Upon exposure to urea, urease immobilized in the hydrogel catalyzes its hydrolysis, increasing the pH value and inducing hydrogel swelling. This swelling leads to a decrease in the refractive index (RI), producing a measurable blue shift in the WGM resonance spectrum. Furthermore, the biosensor exhibits excellent selectivity, maintaining robust performance even in the presence of structurally similar compounds, indicating its potential for working under complex environments.

Keywords: hydrogel, whispering-gallery mode, urea detection, microfluidic, biosensor

1. INTRODUCTION

Urea plays a critical role in diagnosing blood- and urine-related diseases [1], [2], making the precise and reliable detection of urea levels essential to biomedical diagnostics. Conventional urea biosensors, including chromatography [3]–[5], spectrometry [6]–[8], fluorescence [9]–[11], and electrochemical [12]–[14], predominantly rely on urease-catalyzed enzymatic reactions. Despite these methods are effective, they often suffer from limitations such as low sensitivity, prolonged response time, and are complicated in sample preparation or labeling procedure.

To address these limitations, hydrogels have emerged as promising materials for developing novel urea biosensors, owing to their unique three-dimensional (3D) network structures. This structure offers an increased surface area for immobilizing recognition elements and a hydrated environment that mimics biological tissues, facilitating biomolecular interactions [15]. Hydrogels also exhibit sensitivity to pH value and other biomedical changes, enabling the detection of target substances. Nevertheless, detection in low urea concentrations still remains challengeable, due to the subtle swelling is hard to be observed by naked eye or measured directly. In this case, whispering-gallery-mode (WGM) microresonators provide a promising solution to these challenges. These optical devices boast ultrahigh quality (Q) factors and small mode volumes, enabling enhanced light-matter interactions and high sensitivity to minor biological changes. [16], [17].

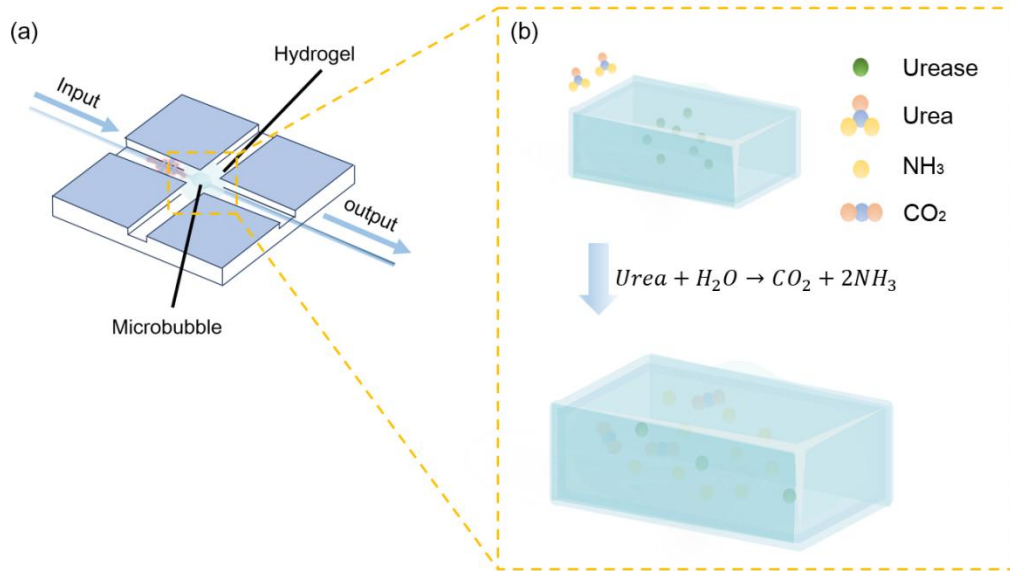


Figure 1. The hydrogel-based optofluidic platform for urea detection. (a) The sensing mechanism. (b) When encountering urea, the pH value was changed, inducing hydrogel swelling and RI alteration.

In this study, we proposed a hydrogel-based WGM microfluidic biosensor by integrating pH-sensitive hydrogels with WGM resonators, which can achieve highly sensitive and selective urea detection. As shown in Figure 1a and 1b, upon exposure to urea, the enzymatic reaction increases the pH, causing the hydrogel to swell. This swelling reduces the refractive index (RI), leading to a measurable blue shift in the WGM resonance spectrum. The biosensor demonstrates a detection limit of 0.3 mM, with exceptional selectivity, offering a novel solution for optical biosensing in complex environments.

2. RESULTS AND DISCUSSIONS

The proposed biosensor employs a pH-sensitive polyacrylic acid-co-dimethylaminoethyl methacrylate hydrogel functionalized with urease. When exposed to urea, urease catalyzes its hydrolysis into ammonia (NH_3) and carbon dioxide (CO_2), while simultaneously, the NH_3 dissolving in the water, thus raising the pH value. This process ionizes the carboxyl groups within the hydrogel, increasing hydrophilicity, water uptake, and hydrogel swelling. This swelling finally induces a decrease in RI, leading to a measurable blue shift in the WGM resonance spectrum.

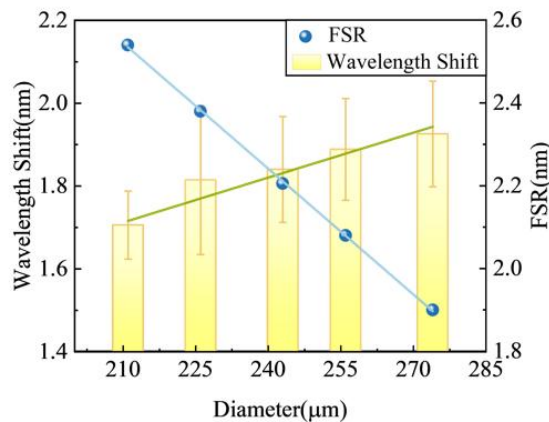


Figure 2. FSR and wavelength shift with various diameters of microcavities at urea concentration of 4 mM.

The diameter of the WGM microbubble resonator significantly influenced the performance of biosensors. We fabricated a batch of microbubbles with various diameters, and Figure 2 demonstrated their free spectral range (FSR) and wavelength shift at a urea concentration of 4mM, respectively. It can be observed that larger diameters enhanced sensitivity, which attributed to the increase within the Q-factor, extending the evanescent field, and further improved the interaction between the mode field and surrounding medium. However, excessively large diameters reduced the FSR, complicating the interpretation of spectral shifts. Finally, a microbubble diameter of 226 μm was selected to balance sensitivity and accuracy.

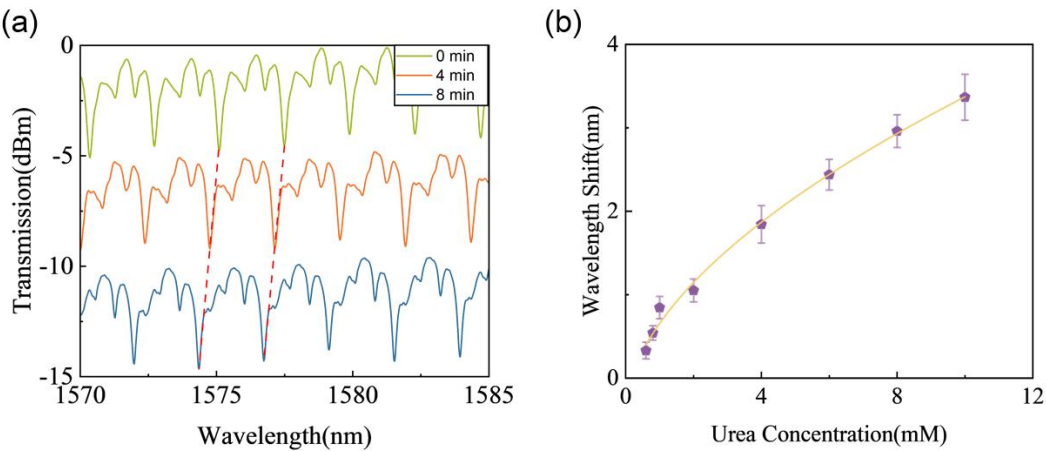


Figure 3. (a) The blue shift tendency until stabilized under 0min, 4min, 8min, respectively. (b) The linear relationship between the wavelength shift and the urea concentration.

The WGM spectral response under different urea concentrations were further investigated. Firstly, Figure 3a showed the spectral response at 1 mM, where an apparent blue shift occurred. It was also observed that the magnitude of the shift increased with higher urea concentrations. This is attributed to the enzymatic hydrolysis of urea, which generates ammonia and further raises the pH value, resulting in more pronounced swelling. However, when the concentration lower than 0.3 mM, the blue shift was almost disappeared. Overall, the biosensor exhibited a detection limit of 0.3 mM for urea, and the platform stabilized within 8 minutes after exposure to urea. Figure 3b illustrated the calibration curve, which demonstrated a linear relationship between urea concentration and spectral shift, confirming the sensitivity of the biosensor.

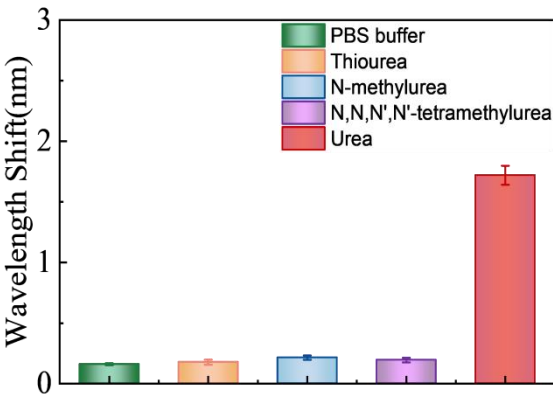


Figure 4. Wavelength shift of the biosensor when test similar structure to urea (all concentrations are at 4 mM).

In the end, the biosensor was confirmed have excellent selectivity, as significant spectral shifts were observed only in the presence of urea. As shown in Figure 4, structurally similar compounds, such as thiourea, N-methylurea, and N, N, N', N'-tetramethylurea, did not cause significant but negligible spectral shifts. This phenomenon can be attributed to hydrogen bonds with water molecules in the solution, which led to a slight swelling, and the same phenomenon can also be observed in control groups.

3. CONCLUSIONS

In this work, we proposed a hydrogel-based WGM microfluidic biosensor for ultrasensitive and selective urea detection. The enzymatic reaction between urease and urea triggered hydrogel swelling, increasing the pH value, while simultaneously, leading to a measurable blue shift in the WGM resonance spectrum. The biosensor exhibited a detection limit of 0.3 mM, with remarkable selectivity and rapid stabilization within 8 minutes. These features make it a robust, label-free, and rapid-response solution for urea detection, even in complex environments. Future work could focus on improving the hydrogel durability and optical properties to broaden its applications in biomolecular sensing.

4. ACKNOWLEDGMENTS

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REFERENCES

- [1] S. N. Botewad, D. K. Gaikwad, N. B. Girhe, H. N. Thorat, and P. P. Pawar, "Urea biosensors: A comprehensive review," *Biotechnology and Applied Biochemistry*, vol. 70, no. 2, pp. 485–501, **2023**, doi:10.1002/bab.2168.
- [2] S. M. Laville, A. Couturier, O. Lambert, M. Metzger, N. Mansencal, C. Jacquelinet, M. Laville, L. Frimat, D. Fouque, C. Combe *et al.*, "Urea levels and cardiovascular disease in patients with chronic kidney disease," *Nephrology Dialysis Transplantation*, vol. 38, no. 1, pp. 184–192, **2023**, doi:10.1093/ndt/gfac045.
- [3] J. Zhou, C. Liu, Y. Chen, X. Luo, and D. Deng, "Determination of urea in swimming pool water using high-performance liquid chromatography with online postcolumn derivatization by xanthidrol," *Journal of Chromatographic Science*, vol. 61, no. 4, pp. 339–346, **2023**, doi:10.1093/chromsci/bmac025.
- [4] T. Sakurai, T. Irii, and K. Iwadate, "Simultaneous quantification of urea, uric acid, and creatinine in human urine by liquid chromatography/mass spectrometry," *Legal Medicine*, vol. 55, p. 102011, **2022**, doi:10.1016/j.legalmed.2021.102011.
- [5] T. Yuan and O. Voznyy, "Guidelines for reliable urea detection in electrocatalysis," *Cell Reports Physical Science*, vol. 4, no. 8, **2023**, doi:10.1016/j.xcrp.2023.101521.
- [6] X. Liu, Y. Wang, H. Cong, Y. Shen, and B. Yu, "A review of the design of packing materials for ion chromatography," *Journal of Chromatography A*, vol. 1653, p. 462313, **2021**, doi:10.1016/j.chroma.2021.462313.
- [7] T. A. Minetto, B. D. Franc, a, G. da Silva Dariz, E. A. Veiga, A. C. Galvao, and W. da Silva Robazza, "Identifying adulteration of raw bovine milk with urea through electrochemical impedance spectroscopy coupled with chemometric techniques," *Food Chemistry*, vol. 385, p. 132678, **2022**, doi:10.1016/j.foodchem.2022.132678.
- [8] N. Passornraprasit, T. Siripongpreda, S. Ninlapruk, N. Rodthongkum, and P. Potiyaraj, " γ -irradiation crosslinking of graphene oxide/cellulose nanofiber/poly (acrylic acid) hydrogel as a urea sensing patch," *International Journal of Biological Macromolecules*, vol. 213, pp. 1037–1046, **2022**, doi:10.1016/j.ijbiomac.2022.06.053.
- [9] A. Z. Alanazi, K. Alhazzani, A. M. Mostafa, J. Barker, H. Ibrahim, M. M. El-Wakil, and A.-M. B. H. Ali, "A novel urease-assisted ratiometric fluorescence sensing platform based on ph-modulated copperquenched near-infrared carbon dots and methyl red-quenched red carbon dots for selective urea monitoring," *Microchimica Acta*, vol. 191, no. 8, p. 505, **2024**, doi:10.1007/s00604-024-06573-0.

- [10] K. Alhazzani, A. Z. Alanazi, A. M. Mostafa, J. Barker, H. Ibrahim, M. M. El-Wakil, and A.-M. B. H. Ali, "Exploiting the pH-responsive behavior of zinc-dithizone complex for fluorometric urea sensing utilizing red-emission carbon dots," *Microchemical Journal*, vol. 204, p. 111129, **2024**, doi:10.1016/j.microc.2024.111129.
- [11] S. Zheng, Y. Fang, Y. Chen, Q. Kong, F. Wang, and X. Chen, "Benzothiazole derivatives based colorimetric and fluorescent probes for detection of amine/ammonia and monitoring the decomposition of urea by urease," *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, vol. 267, p. 120616, **2022**, doi:10.1016/j.saa.2021.120616.
- [12] N. Ezzat, M. A. Hefnawy, S. A. Fadlallah, R. M. El-Sherif, and S. S. Medany, "Synthesis of nickel-sphere coated Ni-Mn layer for efficient electrochemical detection of urea," *Scientific Reports*, vol. 14, no. 1, p. 14818, **2024**, doi:10.1038/s41598-024-64707-z.
- [13] V. Sanko, A. S. enocak, S. O. Tumay, and E. Demirbas, "A novel comparative study for electrochemical urea biosensor design: Effect of different ferrite nanoparticles (mFe₂O₄, m: Cu, Co, Ni, Zn) in urease immobilized composite system," *Bioelectrochemistry*, vol. 149, p. 108324, **2023**, doi:10.1016/j.bioelechem.2022.108324.
- [14] T. S. K. Naik, S. Singh, P. Narasimhappa, R. Varshney, J. Singh, N. A. Khan, S. Zahmatkesh, P. C. Ramamurthy, N. Shehata, G. Kiran *et al.*, "Green and sustainable synthesis of CaO nanoparticles: Its solicitation as a sensor material and electrochemical detection of urea," *Scientific Reports*, vol. 13, no. 1, p. 19995, **2023**, doi:10.1038/s41598-023-46728-2.
- [15] E. M. Ahmed, "Hydrogel: Preparation, characterization, and applications: A review," *Journal of advanced research*, vol. 6, no. 2, pp. 105–121, **2015**, doi:10.1016/j.jare.2013.07.006.
- [16] Ruijie Wu, Ziyihui Wang, Yaixin Fu, Junfeng Jiang, Yu-Cheng Chen, and Tiegeng Liu. *ACS Sensors* **2025** 10 (3), 2330-2338. doi: 10.1021/acssensors.5c00103
- [17] Y. Zheng, Z. Wu, P. P. Shum, Z. Xu, G. Keiser, G. Humbert, H. Zhang, S. Zeng, and X. Q. Dinh, "Sensing and lasing applications of whispering gallery mode microresonators," *Opto-Electronic Advances*, vol. 1, no. 9, p. 180015, **2018**, doi:10.29026/oea.2018.180015.