Poly-diallyl-dimethyl-ammonium chloride (PDDA) solution was used to disperse carbon nanotubes (CNTs) to form a stable PDDA-CNTs aqueous dispersion. The negatively charged glucose oxidase (GOx) and positively charged PDDA-CNTs composite were used to prepare multilayer biosensing films on glassy carbon electrodes (GCE) via layer-by-layer (LBL) self-assembly technique. The optimum number of layers on GCE was 4. A mixture of 3-dimethyl (methacryloxyethyl) ammonium propane sulfonate (DMAPS) and Graphene (GR) was dropped on the multilayer films to prepare a bacteriostatic glucose biosensor. The results show that CNTs could evenly disperse in the PDDA films and the multilayer PDDA-CNTs films can significantly improve the catalytic current response toward glucose (Glu). The biosensor could detect glucose linearly from 16.5 to 214.3 mM. The bacteriostatic properties of the sensor were ensured by the bacteriostatic characteristic of DMAPS.

© The Author(s) 2017. Published by ECS. This is an open access article distributed under the terms of the Creative Commons Attribution 4.0 License (CC BY, http://creativecommons.org/licenses/by/4.0/), which permits unrestricted reuse of the work in any medium, provided the original work is properly cited. [DOI: 10.1149/2.0841706jes] All rights reserved.

Manuscript submitted December 28, 2016; revised manuscript received March 1, 2017. Published March 31, 2017.

Electrochemical biosensors which utilize immobilized oxidase1–6 or metal/metal oxide7–10 to catalyze the oxidation of target analyte, could respond to the changes of concentration of the analyte, and output electrical signal with some disciplines. It can be used to determine biological, clinical, or environmental substances.11–13 Many works have been carried out to achieve high biosensing performance.14–17 However, less research has been done about the bacteriostatic electrochemical glucose biosensor.

Antimicrobial or bacteriostatic material has been widely used in industries such as textile, food fermentation and medical device industry.18,19 In the process of bio-fermentation, the detection of various biochemical parameters (biomass, cell activity, substrate, nutrition, products and metabolites) forms the basis of controlling process of the fermentation.20 The growth rate of micro-organism can be monitored by the consumption of glucose. Consequently, the abnormal phenomenon of fermentation process can be forecasted by the timely detection of glucose.21 However, bacterium can be easily adsorbed on the surface of the glucose sensors to form a bio-film. The bio-film can block the substrate close to the electrode, and the accuracy of the detection can be affected.22 In these cases, it is especially necessary to use antimicrobial or bacteriostatic glucose biosensors. But there are very few studies reporting the antimicrobial or bacteriostatic electrochemical glucose biosensor. In this paper, a bacteriostatic film is built on GCE via layer-by-layer (LBL) self-assembly method and used to construct a glucose sensor.

It is generally known that chemical compounds contain quarternized ammonium groups and that zwitterionic sulfopropylbetaine shows bacteriostatic or antimicrobial properties.23 Besides the zwitterionic materials show outstanding protein resistance performance.24,25 A typical sulfobetaine, 3-dimethyl(methacryloxyethyl) ammonium propane sulfonate (DMAPS) (Scheme 1), which contains a sulfonate group and a quarternized ammonium separated by an alkyl spacer, has outstanding antimicrobial properties.

Layer-by-layer (LBL) self-assembly method is a useful and versatile technique to fabricate functional molecular assemblies with well-defined architectures.26,27 Poly(diallyldimethylammonium chloride) (PDDA) is a positively charged polyelectrolyte, and the formation of π bond between PDDA and carbon nanotubes thereby improves the dispersibility of carbon nanotubes in water.28–30 In this paper, we fabricate a glucose sensor with DMAPS, PDDA, CNTs, GOx and Graphene via LBL self-assembly technique.

Scheme 1. The chemical structure of 3-dimethyl(methacryloxyethyl) ammonium propane sulfonate (DMAPS), one type of sulfobetaines methacrylate.

Experimental

Chemicals and reagents.— Hydroxy Multi-walled CNTs (MWNT-OH) (OD < 8 nm Length ~ 30 μm Purity > 95 wt%) and Carboxyl Graphene (GR, C = 2 mg mL−1) used in this work were purchased from Nanjing XFNano Material Tech Co., Ltd., Poly(diallyldimethylammonium) chloride (PDDA) (Mw < 100,000, 35 wt% in water) and 3-dimethyl(methacryloxyethyl) ammonium propane sulfonate (DMAPS) were from Aladdin Co. (Shanghai, China). Glucose oxidase (GOx) was bought from Sigma-Aldrich Company, and the enzymatic activity was about 175 U mg−1. The test bacteria escherichia coli (E. coli, 8099) was from Microbial Culture Collection Center of Guangdong Institute of Microbiology.

Electrochemistry measurements were proceeded in 1/15 M phosphate buffer solution (PBS) (pH = 6.98). The buffer solution was made by dissolving 0.04 mol Na2HPO4 and 0.027 mol KH2PO4 in 1.000 L of ultrapure water. Glucose solutions with different concentration were made by dissolving certain amount of anhydrous β-D-glucose in the 1/15 M PBS and were stored at 4 °C (at least 12 hours’ store is needed for mutarotation before use). GOX solution (8.0 mg mL−1) was prepared by dissolving certain amount of GOX powders in PBS and stored at 4 °C.

Instrumentation.—Ultrapure water was obtained by using Merck Millipore Direct-Q 3.5.8. Electrochemical impedance spectroscopy (EIS), and amperometric measurements such as i-t curves, cyclic voltammetry (CV), were tested by using a CHI 660E (Shanghai Chenhua) electrochemical workstation. A three electrode system which contained a saturated calomel electrode (SCE) as reference electrode and a platinum wire (Φ 0.5 mm) as counter electrode was used for electrochemical measurements. A pH meter (PHS-3C, Shanghai Leici) is used to measure the pH value, and a conductivity meter (DDS-307, Shanghai Leici) is used to measure the conductivity of the solution.

The effect of DMAPS toward the bacterial growth was observed by using a ultra-violet–visible spectrophotometer (UV–vis) (UV-2550, Shimadzu). The optical density at a wavelength of 600 nm (OD600) was used to characterize the bacteriostasis property of DMAPS.
Sensor fabrication.—Preparation of PDDA-CNTs and DMAPS-GR suspension.—MWNT-OH (1.0 mg) was dispersed in 1.0 mL of PDDA (10 wt%) solution and was ultrasonicated for 1 min to get stable PDDA-CNTs black suspension (1000 ppm); 1.0 mL DMAPS 10 wt% (PBS PH = 6.98 1/15 M) and 1.0 mL 2.0 mg mL⁻¹ Carboxyl Graphene(GR) were mixed with ultrasonication to prepare a 5 wt% DMAPS-Gr suspension.

Electrode modification.—Glasy carbon electrode (GCE) was polished with 0.3 and 0.05 μm alumina powder, rinsed thoroughly with water, and sonicated for 2 mins in water and in ethonal, respectively. The cleaned electrode was soaked in the PDDA-CNTs suspension and the GOX solution alternately, each for 30 minutes. The weak adsorption was dissociated by water washing. The modified GCE was dried by nitrogen at the end of each assembly deposition. The last layer is PDDA-CNTs with positive charge ((PDDA-CNTS/GOx)n4). Finally, DMAPS-GR was adsorbed into the surface of the PDDA-CNTs, and the above modified electrode modified electrodes immersed in DMAPS-GR dispersion for 30 mins, followed by thoroughly rinsed with water to remove any physically adsorbed components and then dried under nitrogen.

Results and Discussion

The bacteriostasis property of the DMAPS.—PDDA-CNTs are chosen as the anchor layer of the sensor. PDDA is a cationic polymer and is a highly positively charged material because of its amino groups. It can interact with carbon nanotubes by π-π interaction, and improve the dispersibility of carbon nanotubes in water. It can self-assemble on the surface of GCE. The electric charge of DMAPS in solution is variable with the pH value. Fig. 1 shows the conductivity of DMAPS solutions with different pH values. It clearly shows that the isoelectric point (pl) of the polyelectrolyte DMAPS is about 4.1 from which it’s easy to know that both DMAPS (pI 4.1) and GOX (pl 4.2) are negatively charged at pH 6.98. Since the black dispersion of PDDA-CNTs is positively charged, the alternate adsorption of PDDA-CNTs, GOX and DMAPS-GR will result in alternative layers. The self-assemble process is schematically depicted in Scheme 2.

The bacteriostasis property of the sensor depends on the bacteriostasis compounds DMAPS. The bacteriostasis property of DMAPS can be shown by measuring the turbidity of the cell suspension, which is characterized by the optical density at a wavelength of 600 nm (OD600). This is a common method for estimating the concentration of bacterial or other cells in solution. Fig. 2 shows the OD600 curves of bacteria solution (E. Coli) with or without DMAPS. The higher OD600 means the solution is opacity, and has more bacteria. Hence, lower OD600 means better antibacterial activity. If there is no DMAPS, the OD600 will be high, and with DMAPS (10 wt%), the OD600 will be lower. This demonstrates that DMAPS have strong bacteriostasis property against E. Coli.

Electrochemical characterization of the modified GCE electrode.—To study the electrochemical properties of the prepared biosensor, cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) are carried out in 5 mM Fe(CN)₆³⁻/⁴⁻ solution. Cyclic voltammetry in [Fe(CN)₆]⁴⁻/³⁻ solution is a convenient and valuable tool to determine the electrocatalytical activity of the surface modifier on the electrode. In this paper, cyclic voltammetry is carried out in 5 mM [Fe(CN)₆]⁴⁻/³⁻ solution at 50 mV s⁻¹. Fig. 3 shows the CV curves of the three electrodes: bare GCE, PDDA/GCE and PDDA-CNTs/GCE. The reduction peaks and oxidation peaks appear when the redox couples of [Fe(CN)₆]⁴⁻/³⁻ for the bare GCE is +0.27 V vs. SCE in forward scans and +0.04 V vs. SCE in reverse scans. As to the PDDA/GCE, the oxidation current decreases obviously compared with the bare GCE. While for the PDDA-CNTs/GCE, the oxidation peak current of [Fe(CN)₆]⁴⁻/³⁻ is larger than that at bare GCE. Furthermore, the reduction peak potential shifts more positive and the oxidation peak potential shifts more negative compared with the bare GCE. The larger peak current of CDP-CNT/GCE is caused by the larger surface area and electrocatalytic activity of CNTs dispersed in the modifying layer.
EIS is a powerful method for the study of interface properties.\textsuperscript{34} It can provide abundant information about the electrode surface as the impedance changes during the biosensor fabrication process. The charge-transfer resistance (Rct), which can indicate the electron-transfer kinetics of the redox probe (Fe(CN)\textsubscript{6}\textsuperscript{3/4}) on the electrode interface, could be estimated by the diameter of the semicircular part of EIS curve.\textsuperscript{35,36} Fig. 4 shows typical Nyquist plots obtained from six electrodes: bare GCE, PDDA-CNTs/GOX/GCE, (PDDA-CNTs/GOX)\textsubscript{2}/GCE, (PDDA-CNTs/GOX)\textsubscript{3}/GCE, (PDDA-CNTs/GOX)\textsubscript{4}/GCE, and (PDDA-CNTs/GOX)\textsubscript{4.5}/PDDA-DMAPS-GR/GCE. All the EIS curves are composed of a straight line and a semicircle. An equivalent circuit model R\textsubscript{1}(R\textsubscript{2}Cap2)Cap2 is used to describe the electrochemical system. The solid line shows the fitting curves by using the equivalent circuit. The fitting results is coincident with the measured EIS curves, and the error of the fitting results is neglectable. R\textsubscript{2} represents the resistance of the modified layers. As the number of PDDA-CNTs/GOX bilayers increases, the diameter of the semicircles increased drastically, and the value of R\textsubscript{2} decreases monotonously. The four-layer (PDDA-CNTs/GOX)\textsubscript{4}/GCE sensor, which provides direct experimental evidence about the successful deposition of DMAPS-GR on (PDDA-CNTs/GOX)\textsubscript{4}/GCE,\textsuperscript{37} is known to have the highest performance.

These kinds of biosensors are based on the oxidation of hydrogen peroxide generated according to the following reactions: β-D-glucose + O\textsubscript{2} + H\textsubscript{2}O \xrightarrow{\text{glucose oxidase}} D-gluconic acid + H\textsubscript{2}O\textsubscript{2}. Next, one of the products H\textsubscript{2}O\textsubscript{2} is decomposed on the electrode and produces response current. More layers have more enzymes and can produce more H\textsubscript{2}O\textsubscript{2} at the same time under the same condition. This may lead to higher response current. In the meantime, the mass transfer of glucose and H\textsubscript{2}O\textsubscript{2} is sensitive to distance. With the increase of layers, the mass transfer is becoming more and more difficult. This leads to rapid decrease of reaction rate and thus causes lower response current.\textsuperscript{38} These two contrary effects result in the phenomenon that the four layers of biosensor have the highest response current.

Amperometric response to glucose.—The amperometric responses of the biosensor toward successive addition of glucose are measured by using a CHI660E electrochemical workstation. The working electrode is adjusted to be +0.60 V vs. SCE. The i-t curve is shown in Fig. 6. The biosensor can respond to the change of glucose concentration quickly and then reaches to a steady-state signal within 10 s. The resulting calibration plot for glucose over the concentration ranging from 0.0 mM to 253.6 mM is presented in the inset of Fig. 6. It shows that such an biosensor could work linearly from 16.5 to 214.3 mM. The corresponding regression equation of the linear plot is: i/\mu A = 2.50 + 0.07 c/mM, R = 0.99. The sensitivity is thus estimated as 70 nA mM\textsuperscript{−1}. The detection limit is estimated to be 3.5 mM (S/N = 3) according to the calibration curve. Table I shows the linear range (mM), limit of detection (\mu M), and applied potential (V vs. SCE) of some typical glucose sensors. The above parameters at this work are comparable with those mentioned.
The (PDDA-CNTs/GOx)4 films displayed excellent electro catalytic activity to the reduction of Glucose. GR and DMAPS combined with (PDDA-CNTs/GOx)4 is used to construct (PDDA-CNTs/GOx)4.5/DMAPS-GR sensor. The bacteriostatic properties of the sensor are ensured by the bacteriostatic characteristic of DMAPS. The (PDDA-CNTs/GOx)4.5/DMAPS-GR glucose sensor has a linear range from 16.5 to 214.3 mM. This study may provide a new stragtem for online monitor the bio-fermentation process.

Acknowledgments

This work was supported by the Guangdong Natural Science Foundation (2015A030315345) and the Shenzhen Science and Technology Research grant (JCYJ20150324140036855).

References

1. C. Z. Zhu, G. H. Yang, H. Li, D. Du, and Y. H. Lin, Anal. Chem., 87, 230 (2015).
2. C. W. Hou and G. J. Wang, Biosens. Bioelectron., 56, 204 (2014).
3. L. Fotonu, F. Raiei, M. M. Heravi, and D. Nematollahi, J. Electroanal. Chem., 639, 15 (2010).
4. H. P. Yang, Y. F. Zhu, D. C. Chen, C. H. Li, S. G. Chen, and Z. C. Ge, Biosens. Bioelectron., 26, 295 (2010).
5. V. Maseiko, A. Kausaite-Minkstiene, A. Ramanavicius, Z. Balevicius, and A. Ramanavicius, Sens. Actuators B, 189, 187 (2013).
6. K. Tian, S. Alex, G. Siegel, and A. Tiwari, Mater Sci Eng C Mater Biol Appl, 46, 548 (2015).
7. A. Tarlani, M. Fallah, B. Lottl, A. Khazraei, S. Golsanamlou, J. Muzart, and M. Mirza-Aghayan, Biosens. Bioelectron., 67, 601 (2015).
8. M. Miki, S. Iwahara, and S. Uno, Jpn J Appl Phys, 53, 04EL061 (2014).
9. Y. Xing, G. W. Gao, G. M. Zhu, J. H. Gao, Z. C. Ge, and H. P. Yang, J. Electrochem. Soc., 161, B106 (2014).
10. Y. Fan, Z. Yang, X. Cao, P. Liu, S. Chen, and Z. Cao, J. Electrochem. Soc., 161, B201 (2014).
11. X. Ge, W. Zhang, Y. Lin, and D. Du, Biosens. Bioelectron., 50, 486 (2013).
12. J. Yuan, K. Wang, and X. Xia, Adv. Funct. Mater., 15, 803 (2005).
13. J. Kuan, Z. Wang, and X. Xia, J. Mater. Chem. B, 3, 6301 (2015).
14. M. Rengaraj, Y. Haldorai, C. H. Kwak, S. Ahn, K. J. Jeon, S. H. Park, Y. K. Han, and Y. S. Huh, J. Mater. Chem. B, 3, 6301 (2015).
15. P. T. Yin, S. Shah, M. Chhowalla, and K.-B. Lee, Chem. Rev., 115, 2483 (2015).
16. L. Q. Rong, C. Yang, Y. Q. Qian, and X. H. Xia, Talanta, 72, 819 (2007).
17. K. Ohkura, A. Sukeno, H. Nagamune, and H. Kourai, Biosens. Bioelectron., 13, 2579 (2005).
18. V. Dos Santos, M. Dos Santos, G. D. J. Cliciane, S. T. Fujiwara, J. R. Garcia, Y. S. Huh, M. J. Hwang, H. K. Lee, and S. H. Kwak, Int. J. Electrochem. Sci., 10, 10351 (2015).
19. A. Dheilly, I. Linossier, A. Darchen, D. Hadjiev, C. Corbel, and V. Alonso, Appl. Microbiol. Biotechnol., 87, 157 (2008).
20. C. W. Hsu and G. J. Wang, Anal. Chim. Acta, 554, 204 (2006).
21. H. P. Yang, Y. F. Zhu, D. C. Chen, C. H. Li, S. G. Chen, and Z. C. Ge, Biosens. Bioelectron., 26, 295 (2010).
22. F. R. Schmidt, J. Electroanal. Chem., 537, 3 (2014).
23. J. Dastjerdi and M. Montazer, Colloids Surf., B, 189, 5 (2010).
24. P. Kanmani and J.-W. Rhim, Adv. Funct. Mater., 15, 803 (2005).
25. V. Ramanavicius, P. Genys, Y. Oztekin, and A. Ramanaviciene, Nano Lett., 13, 3115 (2011).
26. K. Tian, S. Alex, G. Siegel, and A. Tiwari, Mater Sci Eng C Mater Biol Appl, 46, 548 (2015).
27. V. D. Santos, M. D. Santos, G. J. D. Ciclancie, S. T. Fujwara, J. R. Garcia, C. A. Pesola, and R. W. Wohler, J. Electrochem. Soc., 8, 10601 (2013).
28. W. Yuan, Z. Lu, J. Liu, H. Wang, and C. M. Li, Nanotechnologies, 24, 045605 (2013).
29. R. H. Huyck, M. T. Hunley, M. H. Allen Jr, and T. E. Long, Polymer Preprints, 49, 410 (2008).
30. J. H. Rouse and P. T. Lillehei, Nano Lett., 3, 59 (2003).
31. D. Q. Yang, J. F. Rochette, and E. Sacher, The Journal of Physical Chemistry B, 109, 4481 (2005).
32. G. Shi, Z. Sun, M. Liu, L. Zhang, Y. Liu, Y. Qu, and L. Jin, Anal. Chem., 79, 3581 (2007).
33. J. H. Pauzer and W. A. Wood, Method. Enzymol., p. 82, Academic Press, New York (1996).
34. A. Dheilly, I. Linossier, A. Darchen, D. Hadjiev, C. Corbel, and V. Alonso, Appl. Microbiol. Biotechnol., 79, 157 (2008).
35. C. W. Hsu and G. J. Wang, Anal. Chim. Acta, 554, 204 (2006).
36. H. P. Yang, Y. F. Zhu, D. C. Chen, C. H. Li, S. G. Chen, and Z. C. Ge, Biosens. Bioelectron., 26, 295 (2010).
37. V. Maseiko, A. Kausaite-Minkstiene, A. Ramanavicius, Z. Balevicius, and A. Ramanavicius, Sens. Actuators B, 189, 187 (2013).
38. H. Yang and Y. Zhu, Anal. Chim. Acta, 554, 92 (2005).

---

**Table I. Comparison of analytical parameters for detection of glucose over various glucose biosensors.**

| Electrode matrix | Linear range (mM) | Limit of Detection (μM) | Applied potential | Ref. |
|------------------|-------------------|------------------------|-------------------|------|
| (PPV/Pt)         | -                 | 27.4                   | 1.06              | 26   |
| SiPy + Cl⁻ + GOx |                   |                        |                   |      |
| GOx/SiO₂/Pt      | 0.005–2.5         | 0.3                    | 0.6               | 36   |
| GOx/CNT-CDP/GCE  | 0.004–3.23        | 3.5                    | 0.6               | 4    |
| ITO/ZnO/AuNPs/GOx | 2.5–20            | -                      | 6                 |      |
| Au–Ni coaxial    | 0.0275–27.5       | 5.5                    | 0.4               | 2    |
| nanorod array    |                   |                        |                   |      |
| DMAPS-GR/PDDA-CNTs/GOx/GCE | 16.5–214.3 | 3.5 mM                  | 0.6               | Present work |

---

**Figure 6.** Amperometric responses of the (PDDA-CNTs/GOx)4.5/DMAPS-GR/GCE sensor upon subsequent additions of glucose solution into 1/15 M PBS at 0.6 V vs. SCE. The inset shows the calibration i-c curve for glucose concentrations between 16.5 and 253.6 mM.