A New Nitrite-selective Fluorescent Sensor Fabricated from Surface-initiated Atom-transfer Radical Polymerization

Qiang Peng,* Ming-Gui Xie, Koon-Gee Neoh,[†] and En-Tang Kang*[†]
*Department of Chemistry, Sichuan University, Chengdu 610064, P. R. China*
[†]*Department of Chemical and Biomolecular Engineering, National University of Singapore, Kent Ridge, Singapore 119260, Singapore*

A highly selective sensor for nitrite has been developed from surface-initiated atom-transfer radical polymerization on silicon wafer, using PEGMA and pyrene derivatives as spacer and fluorophore, respectively.

Nitrite (NO_2^-) is the most ubiquitous chemical contaminant in food, industrial and physiological systems.[1] This ion can easily interact with amines to form toxic and carcinogenic nitrosamines.[2] Another danger that occurs after nitrite ingestion is that it can convert oxyhemoglobin to methemoglobin in our bloodstream, thereby interfering with oxygen transport in the blood.[3] Nitrite, as its potential toxicity, has intrigued many specialists, thus, many methods for nitrite determination have been developed, including chromatography, potentiometry, amperometry, polarography, spectrophotometry, and colorimetric method.[4–9] Up to now, most of the work to detect nitrite has been carried out in solution. In order to design a sensory system for nitrite in continuous flow, it is a challenging task to immobilize active molecules on a solid substrate. However, it is commonly observed that the response sensitivity will decrease remarkably when a sensor is transferred from the homogeneous to the heterogeneous state. Corma et al.[10] developed a selective senor for iodide in the presence of other halides, using delaminated ITQ-2 zeolite having large external surface as the substrate. Here, we reported the highly selective sensor for nitrite in aqueous phase fabricated from surface-initiated atom-transfer radical polymerization (ATRP), which can afford well-defined sensory film with homogeneous dispersed active molecules.

The preparation procedures of the sensors are shown in Scheme 1. The single crystal silicon wafers were used as the support because of their crucial importance to the development of nano-dimension and molecular electronics in modern microelectronics industry.[11] In this work, Si–H surface was used to instead Si/SiO₂ surfaces because Si–C linkage is much more robust and stable in polymer–Si hybrids compared with Si–O–Si or other SAM interaction,[12] which is important to increase the lifetime of solid-state sensor by preventing the sensory film from desquamation. The ATRP initiators were immobilized according to the previous method[11] via radical-induced hydrosilylation of 4-vinylbenzyl chloride (VBC) with the Si–H surface to afford covalently (Si–C) bonded monolayer Si–VBC surface. For preparation of Si–g-PGMA surface, CuCl₂ was chosen to control the concentration of the deactivating Cu(II) complex during the surface-initiated ATRP process on the Si–VBC surface. Of course, this process is a "controlled" or "living" process. Polyglycidyl methacrylate (PGMA) is chosen here as the spacer and the surface linker because it has excellent environmental stability and biocompatibility, which is suited to be used in advanced biotech-
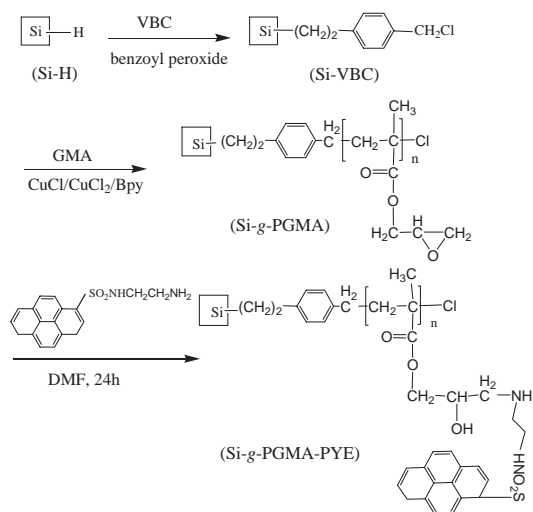
Scheme 1. Schematic diagram illustrating the preparation processes of PYE immobilization on the Si–g-P(GMA) surface.

nologies, such as DNA separation and proteins immobilization.[13] Since the epoxy groups of the PGMA can react readily and irreversibly with nucleophilic groups, such as NH₂ and COOH. Thus, the Si–g-PGMA surface with a high density of epoxide groups is well-suited for the immobilization of the active molecule, N-(1-pyrenylsulfonyl) ethylenediamine (PYE). In addition, the hydroxyl groups can be derived from this nucleophilic coupling reaction to increase the hydrophilic property of the sensory film, which is propitious to apply the resulting device in aqueous phase. The presence of grafted GMA polymer on the Si–VBC surface and PYE immobilized on Si–g-PGMA were confirmed by XPS analysis. Figure S1 gives the C(1s) core-level spectrum of the silicon surface with grafted PGMA brushes (Si–g-PGMA surface) at an ATRP time of 5 h. The C(1s) core-level spectrum can be curve-fitted into three peak components with BEs at about 284.6, 286.2, and 288.4 eV, attributable to the *C*–H, *C*–O, and O=*C*–O species, respectively, in an approximate ratio of 3:3:1 and consistent with the chemical structure of PGMA. The thickness of the grafted PGMA polymer was measured to be 53 nm by an ellipsometer. Also from Figure S1, the C(1s) and N(1s) spectral line shapes of the Si–g-PGMA–PYE surfaces are significantly different from the corresponding spectral line shapes of the original Si–g-PGMA surface. The C(1s) core-level spectra of the Si–g-PGMA–PYE surfaces obtained at the PYE immobilization time of 5 h can be curve-fitted into five peak components with BEs at about 284.6, 285.5, 286.2, and 288.4 eV, attributable to the *C*–H, *C*–N, *C*–O and O=*C*–O species, respectively. The *C*–N peak component is associated
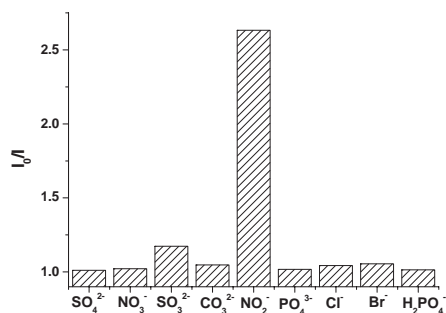
Figure 1. Fluorescence emission response profiles of Si–g-PGMA–PYE ($\lambda_{ex} = 270$ nm, $\lambda_{em} = 380$ nm). The concentration of anions was 50×10^{-4} M.

Figure 2. Changes emission spectra of the aqueous solutions upon addition of nitrite ($\lambda_{ex} = 270$ nm) at room temperature.

with the linkages between PGMA and PYE. From above, the spacer polymer and active molecules have successfully been grafted or immobilized on silicon wafer. The fraction of GMA repeat units coupled with PYE is estimated from the corresponding $[C–O]/[O=C–O]$ peak component area ratio of the Si–g-PGMA–PYE surface to be about 25%.

To investigate the sensing properties, the fluorescence spectra of Si–g-PGMA–PYE sensor were measured at the presence of different anions (at the concentration of 50×10^{-4} M) in aqueous environment. Herein, it was worth noting that all the spectra contained broad peaks and the fine structures of pyrene and its derivatives were not observed in this work. The reason may be that the immobilization limited the mobility of pyrene, further to infect the structure of molecular energy levels and the transition process of electrons.[14,15] This sensor shows no significant change in fluorescence intensity upon contact with NO_3^-, SO_4^{2-}, SO_3^{2-}, CO_3^{2-}, PO_4^{3-}, $H_2PO_4^-$, Cl^-, and Br^-. However, there is a dramatic quenching in fluorescence intensity upon contact with NO_2^- anions, suggesting that the resulting sensor is sensitive and selective to NO_2^- anions. Figure 1 gives a direct comparision of the fluorescence emission response to these anions. Control experiments were carried out to detect the fluorescence property quenched by different concentration of nitrite. From Figure 2, when the concentration is increased, the fluorescent intensity is decreased gradually. The Stern–Volmer constants (Ksv) were determined to be 2.0×10^2. The detection limit was evaluated to be 7.58×10^{-5} M. The high sensitivity is due to the enrichment of Si–g-PEGMA surface to nitrite, because this type of enrichment effect might be caused by formation of hydrogen bonds between the amino groups on the surface and the nitrite, further to form nitrosamine.[16] More important is that the grafted brushes on silicon wafer by ATRP containing OH groups can efficiently increase the hydrophilic property of the film. As a result, it can release more active molecules to contact with nitrite and play a role of amplification effect. The well-defined polymer brushes controlled by ATRP process also homogeneously dispersed the active molecules on the surface, and thus to maintain relatively the stability of the fluorescence detection.

Reversibility is a key factor for evaluation of an excellent sensor. To characterize the reversibility of the response of the sensor to nitrite, the plate was immersed in ultrapure water, and the fluorescent emission intensity was measured at about 380 nm once a minute. Five minutes later, the plate was washed with a sodium nitrite solution (50×10^{-4} M) in water for three

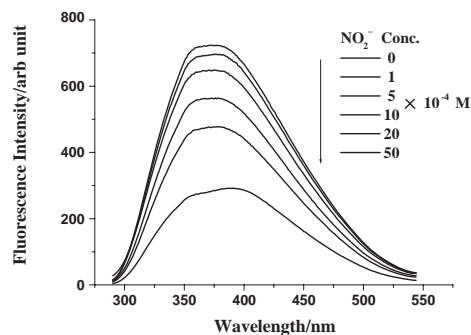times, and then the fluorescent emission was measured again at the same condition for another 5 min. The process was repeated for ten times, and the results are presented in Figure S2. It can be seen from the figure that the response of the plate to nitrite is fast and fully reversible, which is important to extend the lifetime of the sensors. It is also hoped that the findings may be useful for reversible information storage. The fluorescence response remains stable even after 3 month usage. The carrier immobilized on silica wafer by covalent bonding effectively prevented the leakage of carrier. A newly prepared sensor can be used at least 3 months.

In conclusion, a novel and practical heterogenous fluorescent sensing system has been developed from surface-initiated atom-transfer radical polymerization on single crystal silicon wafer, using PGMA and pyrene derivatives as spacer and active molecule, respectively. The sensor is found to be sensitive and selective to nitrite compared with other anions in aqueous solutions, and exhibit good reversibility. Above results are helpful to pave the way to design novel solid sensory systems with excellent physical and chemical properties.

References
1　S. P. Spalding and M. E. Exner, *J. Environ. Qual.*, **22**, 392 (1993).
2　I. A. Wolff and A. E. Wasserman, *Science*, **177**, 15 (1972).
3　E. H. W. Burden, *Analyst*, **86**, 429 (1961).
4　M. A. Koupparis, K. M. Walczak, and H. V. Malmstadt, *Analyst*, **107**, 1309 (1982).
5　J. Z. Li, X. C. Wu, R. Yuan, H. G. Lin, and R. Q. Yu, *Analyst*, **119**, 1363 (1994).
6　M. Bertotti and D. Pletcher, *Anal. Chim. Acta*, **337**, 49 (1997).
7　M. I. N. Ximenes, S. Rath, and F. G. R. Reyes, *Talanta*, **51**, 49 (2000).
8　T. Kawakami and S. Igrashi, *Anal. Chim. Acta*, **333**, 175 (1996).
9　K. Horita, G. F. Wang, and M. Satake, *Analyst*, **122**, 1569 (1997).
10　A. Corma, M. S. Galletero, H. Garcia, E. Palomares, and F. Rey, *Chem. Commun.*, **2002**, 1100.
11　J. M. Buriak, *Chem. Rev.*, **102**, 1272 (2002).
12　J. E. Lim, C. B. Shim, J. M. Kim, B. Y. Lee, and J. E. Yue, *Angew. Chem., Int. Ed.*, **43**, 3839 (2004).
13　G. MacBeath and S. L. Schreiber, *Science*, **289**, 1760 (2000).
14　J. Matsui, M. Mitsuishi, and T. Miyashita, *J. Phys. Chem. B*, **106**, 2468 (2002).
15　D. S. Karpovich and G. L. Blanchard, *Langmuir*, **12**, 5522 (1996).
16　E. Kolb, M. Haug, C. Janzowski, A. Vetter, and G. Eisenbrand, *Food Chem. Toxicol.*, **35**, 219 (1997).