Preparation and properties of a novel polystyrene solid-phase fluorescence pH sensor based on a naphthalimide derivative

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Abstract
A novel naphthalimide-based solid-phase fluorescence pH sensor (PS-Acyl-II) is prepared by immobilization of a small molecule probe (II) on polystyrene microspheres through an ester bond and is characterized by Fourier-transform infrared spectroscopy, optical microscope, scanning electron microscope, and conductiometric titrations. The sensor can determine the pH of a solution within the pH 4.0–7.0, free from interference of common metal ions, and can be reused several times. The geometries of II, PS-Acyl-II, and its product with H⁺ are optimized at the B3LYP/6-31G** level by density functional theory. The charge distribution, orbital interactions, and bonding characteristics are analyzed and compared in detail to discuss the recognition mechanism and structure–fluorescence property relationships.

Keywords
Solid-phase pH sensor, fluorescence properties, naphthalimide derivatives, recognition mechanism, theoretical calculations, structure–fluorescence property relationships

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Introduction
One of the most important physical and chemical parameters of an aqueous solution is its pH. Chemical, biological, and biochemical processes need to be analyzed for pH.¹,² The common methods of pH detection are by visual colorimetry and the use of glass electrodes and sensors. Glass electrodes are the most commonly used pH analysis instruments in industry, agriculture, scientific research, and other fields.³-⁶ However, glass electrodes have some disadvantages, such as electrochemical interference, their insuitablity for use under high-pressure conditions, fragility, and high internal resistance.⁷,⁸ In recent years, more and more chemical fluorescence sensors have been developed to detect pH due to their high sensitivity and rapid response.⁹-¹² However, most of them cannot be reused. Thus, the development of recyclable solid-phase pH sensors is of significant importance for practical applications.

Our group has performed numerous research on polystyrene microspheres. These polystyrene microspheres exhibit regular shapes, strong chemical resistance, and diverse functional groups, such as Cl and COOH, on the surface for further chemical modifications.¹³ Naphthalimide and its derivatives have high fluorescence quantum yields, large Stokes shifts, and good stability, making them suitable as fluorescence sensors.¹⁴ The naphthalimide structure is a well-known building block that is often employed for the formation of luminescent sensors based on photoinduced electron transfer (PET) design. These molecules are very sensitive to substitution at the 4-position of the naphthalimide ring due to the “push–pull” nature of the internal charge transfer excited state, giving rise to a charge separated excited state. This property favored PET quenching of the excited state of the naphthalimide by electron-rich receptors located at the 4-position.¹⁵-¹⁸ Based on this property, several naphthalimide-based sensors have been reported to detect various ions, including Hg²⁺,¹³,¹⁹ Cu²⁺,²⁰ Zn²⁺,²¹ Ag⁺,²² Pd²⁺,²³ Cd²⁺,²⁴ Al³⁺²⁵ and Cr³⁺²⁶. The pH-sensitive
naphthalimide derivatives were immobilized on the outer surface of rhodamine-doped silica nanoparticles, gel, TentaGel MB NH₂, by Trupp and Matthew groups, respectively. However, small-molecule naphthalimide-based fluorescence sensors have low sensitivity and are difficult to recover. If the free fluorescence sensors can be linked to polystyrene microspheres through chemical bonds, the practical performance of these sensors would be improved, which is convenient for solid–liquid separation and reuse.

Herein, a novel polystyrene solid-phase fluorescence pH sensor (PS-Acyl-II) has been designed and synthesized (Figure 1). Fourier-transform infrared spectroscopy (FTIR) and conductimetric titrations were used to characterize the structure and grafting degree on the surface of PS-Acyl-II. Its fluorescence properties, such as the pH effect, selectivity, and reversibility, have been explored, and the detection mechanism has been discussed from a theoretical level based on density functional theory of quantum chemistry at the B3LYP/6-31G** level.

**Results and discussion**

**Preparation of small molecule probe II**

The small molecule probe II was synthesized from 6-bromo-1H,3H-benzo[de]isochromene-1,3-dione through two steps, involving nucleophilic addition–elimination and substitution of bromine (Scheme 1). Sensor PS-Acyl-II was prepared by the reaction of PS-Acyl-Cl polystyrene microspheres with the potassium salt of II in a molar ratio of 1:1 in hot dimethyl sulfoxide (DMSO). Water and methanol were used to wash the free probe from the surface of the microspheres, and FTIR was used to verify that II had been successfully immobilized on the polystyrene microspheres. As shown in Figure 2, the stretching vibrations of saturated C–H and amide C=O bonds appeared at 2928 and 1695 cm⁻¹, respectively. A new peak at 1736 cm⁻¹ was attributed to the stretching vibration of the ester C=O. According to the images of modified and unmodified microspheres by optical microscope and scanning electron microscope (SEM; Figures 3 and 4), the prepared microspheres have good sphericity and monodispersity. The particle size distributions of the microspheres were measured through dynamic light scattering (DLS). According to Figure 4c, the diameter of PS-Acyl-Cl was about 340 μm. After the modification of II, the diameter of PS-Acyl-II (Figure 4d) was about 360 μm. Compared with the size before modification, the diameter increases, and the surface had many recesses. FTIR and obvious surface changes of the microspheres indicate that II was successfully immobilized on the surface of PS-Acyl-Cl. These results indicated that solid-phase sensor PS-Acyl-II had been synthesized successfully.

The average Cl content on the surface of the microspheres was determined by conductimetric titrations, and the results
of the before and after reaction were 4.7 and 2.1 mmol g⁻¹, respectively, implying the immobilization of II was 2.6 mmol g⁻¹ and the conversion of chloromethyl was 55%.

Response time and fluorescence properties

PS-Acyl-II was mixed with phosphate buffer at pH 5, the fluorescence intensity was measured by excitation at 400 nm to study the influence of time. As shown in Figure 5, fluorescence analysis showed that the recognition of PS-Acyl-II and H⁺ was basically completed within 30 min and reached the maximum fluorescence intensity at 90 min. However, 30 min was chosen as the measurement time. Thus, the sensor had potential utility in H⁺ real-time monitoring.

To explore the pH sensitivity, fluorescence titrations of PS-Acyl-II under different pH conditions were conducted. The maximum absorption of II at wavelength 400 nm was used as the excitation wavelength for the determination of the fluorescence. As shown in Figure 6, the emission wavelength of PS-Acyl-II appeared at 529 nm and the fluorescence intensity decreased gradually on increasing the pH value from 4.0 to 7.0. It can be seen from the plot of the fluorescence intensity ratio versus pH value that when the pH was between 2.0 and 3.0, the fluorescence intensity decreased, this was caused potentially by the cleavage of the ester bonds in PS-Acyl-II. So, we determined that the working range of PS-Acyl-II is pH 4.0–7.0. To explore the anti-interference ability of PS-Acyl-II, so as to apply to a complex environment, the effects of metal ions, such as Ag(I), Ca(II), Cd(II), Co(II), Cu(II), K(I), Mg(II), Na(I), Ni(II), Ba(II), Bi(II), Al(III) and Pb(II) ions, were studied. As shown in Figure 7, when the pH value was 5.0, almost no reduction of fluorescence intensity was detected after adding metal ions, indicating that the presence of metal ions had little effect on the pH response, and therefore PS-Acyl-II is expected to be useful for pH detection in practical samples.

To verify the reversibility of PS-Acyl-II, a base titration was carried out. The addition of sodium hydroxide, which had stronger binding ability for H⁺, caused fluorescence quenching and reformed the free sensor. More interesting is the fact that the sensor is recovered by adding further H⁺. As shown in Figure 7, PS-Acyl-II could be reused more than three times. However, a slight reduction of the fluorescence intensity was observed as the number of uses increased, implying some loss of probe II from the surface of the microspheres.

Detection mechanism

The PET occurs between the electron-donating group and the lumophore. For PS-Acyl-II, the combination of N-methylpiperazine with naphthalene and an electron-withdrawing imide resulted in the push–pull electronic mode, enabling PET along with fluorescence quenching (Figure 8). When the sensor was in constant contact with H⁺, the lone pair electrons on the N-atom bonded with H⁺ to prevent PET, and the delocalized π-electrons on the aromatic ring were excited, leading to a stronger fluorescence. As was evident from the discrete Fourier transform (DFT) results, the positive phase and negative phase were symbolized with red and green, respectively. PS-Acyl-II had a similar configuration, HOMO–LUMO distribution, and molecular electrostatic potential as small molecule probe II, indicating the solid-phase sensor PS-Acyl-II still retained the identification characteristics of II. However, the conformation of PS-Acyl-II changed before and after the recognition. Although both the naphthalimide and
The piperazine rings adopted the envelope configuration, the dihedral angles between them varied significantly, with 65.75° for PS-Acyl-II and 43.18° for PS-Acyl-II + H⁺. They had similar spatial configurations concerning the plane of the alkyl chain and naphthalimide ring, with a dihedral angle of 88.48° for PS-Acyl-II and 88.19° for PS-Acyl-II + H⁺. The energy gap between the HOMO and LUMO was less for PS-Acyl-II than II. The narrow gap meant a relatively higher reactivity, implying that the electronic transfer in PS-Acyl-II was easier and that its interaction with H⁺ demonstrated a relatively higher reactivity than that of II. From this point of view, the immobilization of II on polystyrene microspheres was conducive to improve its sensing performance. In the HOMO of PS-Acyl-II, the electrons were mainly delocalized on the naphthalimide and piperazine rings, which made it difficult for the electrons excited on the naphthalimide LUMO to return to the ground state, leading to the occurrence of PET along with weak fluorescence. With the addition of H⁺, the electrons were no longer concentrated on the naphthalimide and piperazine rings, but instead on the styrene ring, which made the electron transfer cut off and the excited electrons on the naphthalimide LUMO return to the ground state, thereby preventing the PET along with strong fluorescence.

**Conclusion**

In this paper, a novel, solid-phase fluorescence pH sensor PS-Acyl-II has been prepared from 6-bromo-1H,3H-benzo[de]isochromene-1,3-dione and 6-aminohexanoic acid through four steps nucleophilic addition–elimination, substitution, conversion into the potassium salt, and immobilization on the surface of polystyrene microspheres. The structure of PS-Acyl-II was confirmed by FTIR, optical microscope, SEM, and conductometric measurements.
Figure 6. Fluorescence titrations of PS-Acyl-II under different pH conditions: (a) pH 5–9 by phosphate buffer; (b) pH 2–4 by HCl; (c) the working range of PS-Acyl-II was pH 4.0–7.0.

Figure 7. Anti-interference (left) and reversibility (right) of PS-Acyl-II.

titrations. Sensor PS-Acyl-II displayed high sensitivity to H⁺ within the pH 4.0–7.0, good anti-interference, and environmental friendliness by regeneration. The detection mechanism and structure–fluorescence property relationships were discussed based on quantum chemistry calculations at the B3LYP/6-31G** level. With the addition of H⁺, the electrons in the HOMO delocalized on the styrene ring rather than on the naphthalimide and piperazine rings, which prevented the electron transfer and made the excited electrons on the naphthalimide LUMO return to the ground state, causing strong fluorescence. Sensor PS-Acyl-II is expected to be used for determining pH in real samples.

Experimental

General information

Melting points were measured on an X-4 microscope electrothermal apparatus (Taike China) and are uncorrected. Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker AV-400 spectrometer with CDCl₃ as the solvent and tetramethylsilane (TMS) as the internal standard. FTIR were recorded as potassium bromide (KBr) disks using a Thermo Nicolet 380 spectrophotometer. Mass spectra were obtained on a Thermo TSQ Quantum Access MAX mass spectrometer. Micrographs were obtained by optical microscope Nexcope NE620. Scanning electron
micrographs were obtained using a JEOL JSM 5900 LV SEM. Fluorescence emission spectra were recorded on a Shimadzu RF-5301PC spectrofluorometer using cuvettes (path length = 1 cm) at r. t. Chloroacetylated polystyrene microspheres (PS-Acyl-Cl, ~350 μm, 4.7 mmol g⁻¹ Cl) and other reagents were purchased from Aladdin or Nanjing WANQING chemical company.

Preparation of 6-(6-Bromo-1,3-dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)hexanoic acid (I)

6-(6-Bromo-1,3-dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)hexanoic acid (I) was synthesized according to the reported method. 6-Bromo-1H,3H-benzo[de]isochromene-1,3-dione (0.55 g, 2 mmol) and 6-aminohexanoic acid (0.26 g, 2 mmol) were dissolved in ethanol (50 mL) with stirring. The reaction was monitored by silica sheets of TLC (Ethyl acetate: Petroleum ether = 2:3, v/v) until the raw materials had been consumed completely. The reaction mixture was transferred to a beaker and frozen in ice water. The precipitate was filtered and washed with ethanol (three times) to afford 0.69 g of yellow solid (yield 88%). m.p. 151~153 °C; 1H NMR (400 MHz, CDCl₃): δ 8.56 (q, 2H, J = 8.0 Hz, ArH), 8.33 (d, 1H, J = 7.6 Hz, ArH), 8.22 (d, 1H, J = 8.0 Hz, ArH), 8.00 (t, 1H, J = 7.6 Hz, ArH), 4.02 (t, 2H, J = 7.2 Hz, CH₂), 2.20 (t, 2H, J = 7.2 Hz, CH₂), 1.63–1.53 (m, 4H, CH₂), 1.38–1.33 (m, 2H, CH₂); ESI-MS: m/z calcd for C₁₈H₁₅BrNO₄ [M–H]⁻: 388.02, [M + 2–H]⁻: 390.02; found: 387.97 [M–H]⁻, 389.97 [M + 2–H]⁻.

Preparation of sensor PS-Acyl-II

To a solution of I (0.25 g, 0.6 mmol) in methanol (40 mL) was added KOH (0.03 g, 0.54 mmol), and the mixture was stirred at reflux for 1 h. The MeOH was removed under reduced pressure to give the potassium salt of I. DMSO (35 mL) was added to dissolve the salt, and then dried PS-Acyl-Cl microspheres (0.1 g, 0.47 mmol of Cl) were added. The mixture was reacted at 50 °C for 10 h, then filtered, washed several times with water and methanol, and then dried under vacuum for 24 h to afford the immobilized sensor PS-Acyl-II. The amount of I loaded on the microspheres was determined by conductimetric titrations, (three runs). The average content of Cl on the microspheres was 2.1 mmol g⁻¹, indicating the loaded amount of I was 2.6 mmol g⁻¹.

Fluorescence determination

Sensor PS-Acyl-II (0.01 g) was mixed with phosphate buffer (3 mL, pH 5.0), then fluorescence intensity was measured by excitation at 400 nm to study the influence of time. Sensor PS-Acyl-II (0.01 g) was mixed with...
quantum chemistry calculations
The quantum chemistry calculations were carried out in the ground state (in vacuo) with Gaussian 03 software using the B3LYP/6-31G** method. The optimized structures and corresponding electronic and energy parameters were extracted from the GaussView 3.0 program. The charge distribution, bonding characteristics, and orbital interactions were analyzed using natural bond orbital (NBO) theory.

Declaration of conflicting interests
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References
1. Amadu M and Pegg MJ. *J Petrol Sci Eng* 2019; 174: 844.
2. Tomaszewski M, Cema G and Ziembinska B A. *Chemosphere* 2017; 182: 203.
3. Anes B, Bettencourt da S RJN, Oliveira C, et al. *Talanta* 2019; 193: 118.
4. Golan R, Gavrieli I, Lazar B, et al. *Limnol Oceanogr-Meth* 2014; 12: 810.
5. Bargrizzan S, Smenik RJ and Mosley LM. *Soil Sci Soc Am J* 2017; 81: 1350.
6. Georgiev N I, Krasteva P V and Bojinov V B. *J Lumin* 2019; 212: 271.
7. Iken H, Bronder TS, Goretzki A, et al. *Phys Status Solidi A*, 2019; 216: 1900114.
8. Doi K, Asano N and Kawano S. *Sci Rep-UK* 2020; 10: 4110.
9. Evan PSP, Kelsie JA, Justin KT, et al. *ACS Sensors* 2020; 5: 3879.
10. Radunz S, Andresen E, Würth C, et al. *Anal Chem* 2019; 91: 7756.
11. Steinegger A, Wolbeis OS and Borisov SM. *Chem Rev* 2020; 120: 12357.
12. Al-Qaisy WW and Duerkop A. *Sensors & Actuators: B Chemical* 2019; 298: 126848.
13. Liu Y, Zhang J, Feng T, et al. RSC Adv 2020; 10: 25281.
14. Thapa P, Byrnes NK, Denisenko AA, et al. *ACS Sensors* 2021; 6: 192.
15. Daly B, Ling J and Silva A P. *Chem Soc Rev* 2015; 44: 4203.
16. Wang S, Cao J, Cheng Y, et al. *Chem Res Chinese Universities* 2019; 35: 967.
17. Martínez CM, Bright SA, Veale EB, et al. *Front Chem Sci Eng* 2020; 14: 61.
18. Trupp S, Hoffmann P, Henkel T, et al. *Org Biomol Chem* 2008; 6: 4319.
19. Muzeby B and Naseem A. *Photochem Photobiol* 2020; 391: 112354.
20. Liu YL, Yang L, Li P, et al. *Spectrochim Acta A* 2020; 227: 117540.
21. Liu D, Zhang T, Zhang M, et al. *Bioorg Med Chem Lett* 2020; 30: 127073.
22. Ye F, Liang XM, Xu KX, et al. *Talanta* 2019; 200: 494.
23. Zhou L, Hu S, Wang H, et al. *Spectrochim Acta A* 2016; 166: 25.
24. Zhang Y, Chen X, Liu J, et al. *New J Chem* 2018; 42: 19245.
25. Li Z, Chen W, Dong L, et al. *New J Chem* 2020; 44: 3261.
26. Adhikari S, Ta S, Ghosh A, et al. *Photochem Photobiol* 2019; 372: 49.
27. Doussineau T, Trupp S and Mohr GJ. *J Colloid Interf Sci* 2009; 339: 266.
28. Kriltz A, Loeser C, Mohr G, et al. *J Sol-gel Sci Techn* 2012; 63: 23.
29. Refalo MV, Farrugia NV, Johnson AD, et al. *J Mater Chem C* 2019; 7: 15225.
30. Liu Y, Li Y, Zong L, et al. *Chem Res Chinese Universities* 2020; 36: 781.
31. Frisch MJ, Trucks GW, Schlegel HB, et al. *Revision B*. Pittsburgh PA: Gaussian Inc, 2003.
32. Neupane LN, Kim JM, Lohani CR, et al. *J Mater Chem* 2012; 22: 4003.
33. Hu B, Hu L, Chen M, et al. *Biosens Bioelectron* 2013; 49: 499.
34. Qiu M and Liew KM. *J Phys Chem C* 2012; 116: 11709.
35. Li Y, Xiong J, Li S, et al. *Spectrochim Acta A* 2020; 234: 118277.