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Journal of Photochemistry and Photobiology A: Chemistry 171 (2005) 137–144
Journal of Photochemistry and Photobiology A:Chemistry
www.elsevier.com/locate/jphotochem

Optical sensor for lithocholic acid based on multilayered assemblies from polyelectrolyte and cyclodextrin

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Received 20 July 2004; received in revised form 17 August 2004; accepted 7 October 2004
Available online 11 November 2004

Abstract

Multilayered thin films were prepared by a layer-by-layer (LbL) deposition of sulfonated β-cyclodextrin (s-β-CD) and cationic poly(allylamine) hydrochloride (PAH) on the surface of a quartz slide. A self-assembled fluorescent host, s-β-CD/neutral red was formed by the inclusion interaction of neutral red (NR) with the s-β-CD immobilized on the multilayered films. An optical sensor for lithocholic acid (LA), based on the fluorescence quenching of s-β-CD/NR complex immobilized on the multilayered films, has been developed, in which NR served as a sensitive fluorescence indicator probe. The decrease of fluorescence intensity of s-β-CD/NR complex in the presence of LA was attributed to the formation of an inclusion complex between s-β-CD and LA, which has been utilized as the basis of the fabrication of a LA-sensitive fluorescence sensor. The response mechanism of sensor has been discussed in detail. The analytical performance characteristics of the proposed LA-sensitive sensor were investigated. The sensor can be applied to the quantification of LA with a linear range covering from 2 uM to 60 uM and a detection limit of 1 uM. The sensor exhibits excellent reproducibility, reversibility and selectivity.

Keywords: Sulfonated β-cyclodextrin; Poly(allylamine) hydrochloride; Neutral red; Multilayered films; Optical sensor

1. Introduction

The layer-by-layer (LbL) assembly method, introduced by Decher et al. in 1992, has emerged as a promising and versatile approach to fabricate functional molecular assemblies with well-defined architectures and with nanoscale-level control over the film thickness [1,2]. This technique relies on an alternate deposition of oppositely charged species from solution onto a solid surface to form multilayer thin films through an electrostatic force of attraction. The resulting ultrathin polyelectrolyte multilayer (PEM) films have been investigated for a wide range of applications such as electro-optic devices [3], microcapsules [4,5], sensors [6–8], and separation membranes [9]. Strong polyelectrolytes such as poly(diallyldiammonium) chloride (PDADAC) and poly(sodium-4-styrene sulfonate) (PSS) are often used because of their ability to be charged over a large pH range [10,11]. However, more recent studies have shown that preparing multilayer thin films from weak polyelectrolytes, such as poly(allylamine) hydrochloride (PAH) and poly(acrylic acid) (PAA), can produce systems with a rich suite of properties because the behavior of this class of polyelectrolytes is sensitive not only to the ionic strength of the solution but also to its pH [12–15].

Cyclodextrins (CDs) are cyclic oligosaccharides which have hydrophobic cavities capable of forming inclusion complexes with a variety of organic molecules in aqueous solution. They have attracted widespread interest as enzyme mimics and as hosts for molecular recognition, and much

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doi:10.1016/j.jphotochem.2004.10.006

efforts has been made to modify the CDs to improve their catalytic and molecular recognition properties [16–18]. CDs have often been used as the recognition part of molecular sensors owing to their ability to include molecules in their cavities [19–21]. The choice of CDs as sensing materials for chemical sensors is particularly interesting in that they are able to complex in a reversible way a large number of compounds, not only metal ions but also neutral molecules. Furthermore, they can be chemically modified by different substituted functional groups or structurally tuned to fit different chemical species to reach desired selectivity and sensitivity [22]. Recently, CDs and modified CDs have been used as the material for constructing surface self-assembled monolayers (SAMs) [23,24], multilayered thin films [25,26], polymer films [27,28], all containing selective binding sites. CD-containing thin films are attracting much attention because of their possible applications to detection and separation of organic compounds through a host–guest complexation. Suzuki et al. [29] reported a thin-film assembly composed of a CD dimer and ferrocene-modified PAH. The binding activity of the CD dimer was actually masked in the film as the film was constructed through complexation between the CD dimer and the ferrocene moieties in PAH and, thus, the CD cavity was occupied by ferrocene residues in the film. If the CD cavity was available in the film, it would be possible to make the film functional by modifying the film with various kinds of guest molecules. Sato et al. [30] described the preparation of CD-containing polyelectrolyte multilayer films and the binding properties of the film toward azoaromatic dyes.

Lithocholic acid (LA), a naturally occurring bile acid, is known to be a liver-toxic metabolite. It can damage the DNA, the genetic material in cells to cause cancer [31]. Colon cancer is thought to arise from the accumulation of mutations in a single cell in the epithelial cell layer of the colon and rectum. High level of LA in the colon caused by high fat diets is thought to play a role in the carcinogenesis process [32]. Thus, the establishment of LA assay method is of great significance for the disease diagnosis. The interaction of LA with β-CD has been investigated by using titration calorimetry [33]. Very strong binding existed between them, which was much higher than that of many other substrates. To our knowledge, no fluorescent sensor for LA was reported in the literature. Hence, the optical sensor for LA based on a competitive host–guest complexation was designed. However, LA is spectroscopically inert. An appropriate dye with its optical property closely associated with the polarity of environment was searched. As far as we know, the inclusion behaviors of β-CD with some dyes, including nile red [34], methylene blue [35], neutral red [36], cyanine dye [37], coumarin dye [38], have been reported. Among them, the photochemical property of neutral red (NR) is the most sensitive to the microenvironment around it. NR is a readily available biological dye. In the nonpolarity cavity of CD, a great enhancement of fluorescence intensity of NR was observed as compared to NR in aqueous solution. β-CD can bind many hydrophobic species into its cavity in water solution. This inclusion capacity depends on the size fit of the substrate into the cavity and the hydrophobicity of the substrate. Comparing to NR, LA is more hydrophobic and size-matched to the cavity of β-CD. LA would compete with NR to enter into the cavity of CD and thus result in the change of system fluorescence signal.

In this paper, a multilayered thin film containing sulfonated β-CD (s-β-CD) and PAH was built based on the method described by Sato et al. [30]. Sulfonated β-CD capable of binding organic species was deposited as a functional component into thin film. This unique approach incorporates polymer and molecular element into the sensing film and thus results in film with polymer's physical properties and molecular receptor's selectivity. By incorporating molecular recognition functions into polymer film, a stable, sensitive and selective optical sensor was fabricated for the LA assay in which NR served as a probe. Due to strong host–guest complexation between s-β-CD and target analyte (LA), the quantification of LA was realized with the inclusion complex by the proposed sensor. The sensing mechanism and the response characteristics of the sensor were discussed in detail. The sensor can be used for the determination of LA with a linear range covering from 2 uM to 60 uM and a detection limit of 1 uM, and it shows excellent reproducibility, reversibility and selectivity.

2. Experimental

2.1. Materials

Poly(allylamine) hydrochloride (PAH) (average Mw ~ 70,000), poly(vinyl sulfate) potassium salt (PVS) (average Mw ~ 170,000) and lithocholic acid (LA) were purchased from Aldrich and used without further purification. Sulfonated β-CD (s-β-CD), in which 8–9 and 12–14 primary and secondary hydroxyl groups in parent β-CD are sulfonated, were obtained from Aldrich. Neutral red (NR) was provided by Shanghai Chemical Reagents (Shanghai). The chemical structures of the polyelectrolytes, LA, NR and s-β-CD are depicted in Fig. 1. All reagents were of analytical reagent grade. Doubly distilled water was used throughout.

2.2. Apparatus

UV–vis absorption spectra were measured using Lambda 800 UV–vis Spectrometer (PerkinElmer). Fluorescence spectra were recorded on a Hitachi F-4500 fluorescence spectrometer. Excitation and emission slits were set at 5.0 nm and 10.0 nm, respectively. All measurements were carried out at room temperature (ca. 20 °C).

2.3. Preparation of multilayered assemblies

The optical sensor was prepared according to the procedure described by Sato et al. [30]. The layered assemblies were prepared on the surface of a quartz slide

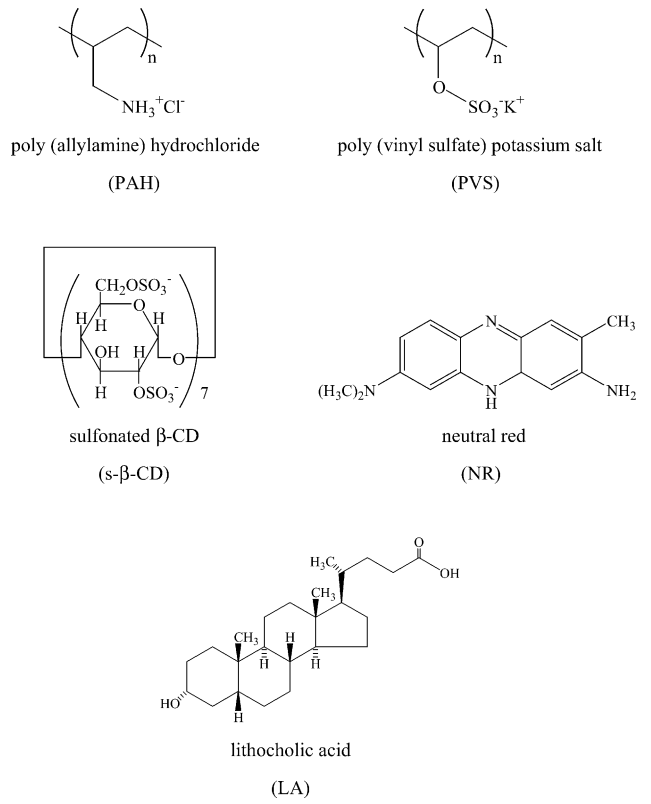

Fig. 1. Chemical structures of polyelectrolytes (PAH and PVS), sulfonated β-CD, NR and LA.

(1.2 cm × 1.0 cm × 0.1 cm) for the spectroscopic evaluation of the adsorption of dye (NR). The quartz slide was first treated in dichlorodimethylsilane (10% solution in toluene) overnight at room temperature to make the surface hydrophobic and was washed with toluene, acetone, and distilled water. The silylated quartz slide was immersed in a PAH solution (100 ug mL^{-1}, in PBS) for 30 min to deposit the first layer through a hydrophobic force of attraction. After being rinsed in PBS for 10 min to remove any weakly adsorbed PAH, the quartz slide was immersed in a s-β-CD solution (100 ug mL^{-1}, in PBS) for 30 min to deposit the second layer. This process provides both sides of the quartz slide with a PAH-s-β-CD layer. The deposition was repeated to prepare multilayered assemblies. The PBS buffer used contains 0.2 g L^{-1} KCl, 0.2 g L^{-1} KH_2PO_4, 1.15 g L^{-1} Na_2HPO_4, and 8.0 g L^{-1} NaCl, and the pH was adjusted to 5.4 by adding HCl. All assemblies were prepared at room temperature (ca. 20 °C).

2.4. Adsorption of NR to the layered assemblies

The PAH-s-β-CD film-modified quartz slide was immersed in a 40 uM NR solution (in 10 mM Tris–HCl buffer, pH 7.5) to deposit the NR to the film. After being rinsed with water, the UV–vis absorption spectra and the fluorescence spectra of the NR-adsorbed slide were recorded. The slide was measured in a quartz cell filled with distilled water.

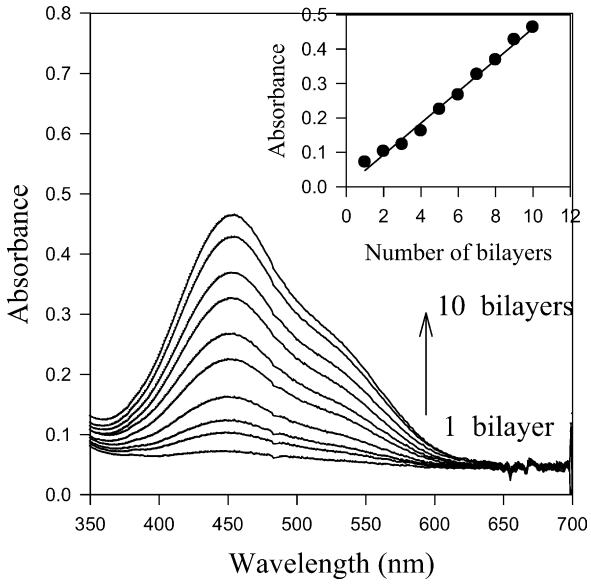

Fig. 2. UV–vis absorption spectra of NR-adsorbed $(PAH\text{-}s\text{-}\beta\text{-}CD)_n$ film. The inset plots absorbance at λ_{max} as a function of the number of layers.

3. Results and discussion

3.1. Preparation of PAH-s-β-CD films

According to the film preparation method proposed by Sato et al. [30], a structural model for the PAH-s-β-CD assembly was proposed. PAH is assumed to form a globular conformation to accommodate s-β-CD not only on the surface but also into the inner domain. The high ionic strength compels PAH to form the globular conformation, and thus, more s-β-CyD molecules are adsorbed upon deposition.

The PAH-s-β-CD films, which was prepared from PBS solutions, was immersed in a 40 uM NR solution (in 10 mM Tris–HCl buffer, pH 7.5) for 1 h to bind NR. The preparation of multilayer films was evaluated by measuring the UV–vis absorption of NR adsorbed to the film. Fig. 2 shows typical absorption spectra of PAH/s-β-CD films and the change in absorbance of the films as a function of the number of depositions after being treated in the NR solution (40 uM). A clear absorption peak arising from π–π^* transition of NR chromophore appeared, and the intensity of the absorption band was enhanced linearly with increasing number of depositions. These results suggest that a much amount of NR is adsorbed upon each deposition due to the accumulation effect of more s-β-CD, which indicated that s-β-CD was successfully built into the PAH multilayer films as anionic counterparts.

3.2. Binding of NR to the assemblies

The PAH-s-β-CD film, which was prepared from PBS solutions, was immersed in a 40 uM NR solution (in 10 mM Tris–HCl buffer, pH 7.5) for 1 h to bind NR. Fig. 3 illustrates the UV–vis absorption spectra and fluorescence spec-

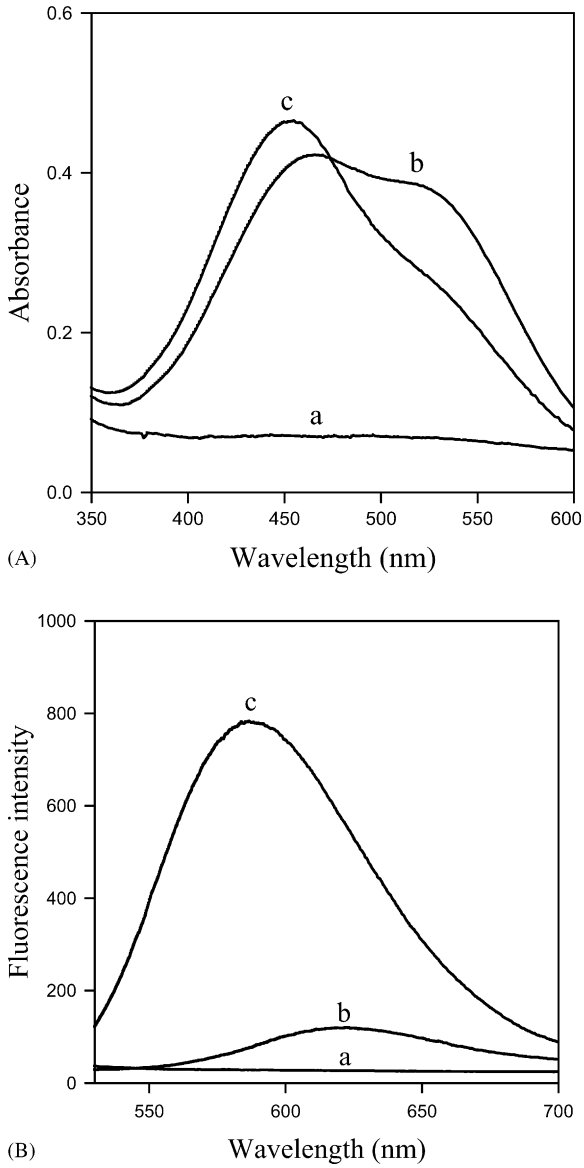

Fig. 3. The UV–vis absorption spectra (A) and fluorescence spectra (B) of 10-bilayer (PAH-s-β-CD)$_{10}$ film after being treated in the NR solution (40 uM). (a) Blank (PAH-s-β-CD)$_{10}$ film; (b) the NR-adsorbed (PAH-PVS)$_{10}$ film; (c) the NR-adsorbed (PAH-s-β-CD)$_{10}$ film.

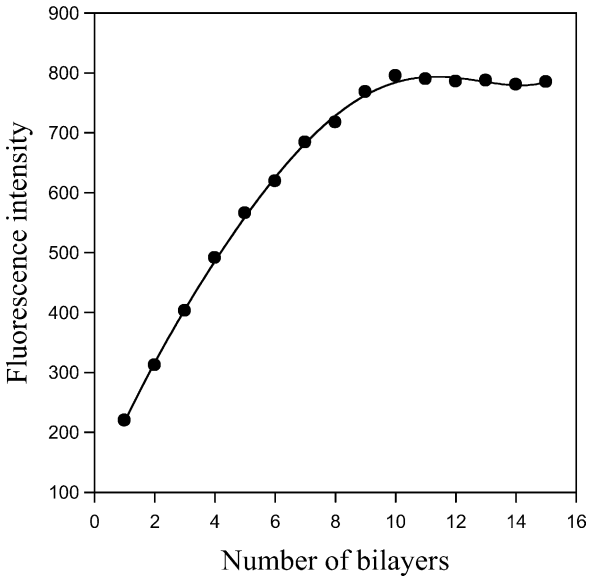

Fig. 4. Effect of the number of bilayers of the film on the fluorescence intensity of NR-adsorbed multilayer film.

tra of 10-bilayer (PAH-s-β-CD)$_{10}$ film after being treated in the NR solution (40 uM). As is shown in Fig. 3A, the film showed absorption band in the region, confirming adsorption of NR to the film because the film exhibited no absorption band in this region before being treated with NR. In addition, the NR-adsorbed (PAH-PVS)$_{10}$ film gave an absorption spectrum quite different from that of (PAH-s-β-CD)$_{10}$ film. Two absorption peaks appeared on the NR-adsorbed (PAH-PVS)$_{10}$ film, which resulted from the neutral and acidic form of NR. A unique absorption peak which is ascribed to the neutral form of NR appeared on the NR-adsorbed (PAH-s-β-CD)$_{10}$ film. The difference of the two films can be explained by the suppression of the acidic form of NR by preferential inclusion of the neutral form of NR in the s-β-CD cavity which influenced the acid–base equilibrium between the acidic and neutral forms of NR. The similar experimental result was also observed from Fig. 3B. As compared to the case of the film untreated, a fluorescence emission peak appeared in the case of the film treated with NR, which further verified the adsorption of NR to the film. However, the NR-adsorbed (PAH-PVS)$_{10}$ film gave a fluorescence emission much weaker than that of (PAH-s-β-CD)$_{10}$ film, which indicated that an extra interaction, inclusion interaction, existed between NR and s-β-CD in the case of (PAH-s-β-CD)$_{10}$ film except for electrostatic interaction. Generally, the hydrophobic and protective microenvironment of CD can shield the excited state of guest included in the cavity of CD from water molecules and other species presented in bulk aqueous solution, thus result in the enhanced fluorescence emission of guest.

3.3. Effect of number of layers on the fluorescence intensity of (PAH-s-β-CD)$_n$ film

One of the merits of polyelectrolyte multilayer films is that the thickness of the film can be precisely controlled by regulating the number of layers. Therefore, it is interesting to evaluate the effects of the thickness of the film on the NR binding properties. Fig. 4 plots the fluorescence intensity originating from NR in the film as a function of the number of layers after being treated in the NR solution (40 uM) for 4 h. As can be seen from Fig. 4, the fluorescence intensity of NR-adsorbed (PAH-s-β-CD)$_n$ film gradually enhanced with the increase of number of layers. This indicated that the loading of NR complexed with s-β-CD increased with the increasing number of layers, as a result of the enhanced loading of s-β-CD in the thicker film. These phenomena confirm that s-β-CD involved in the complexation with NR is lo-

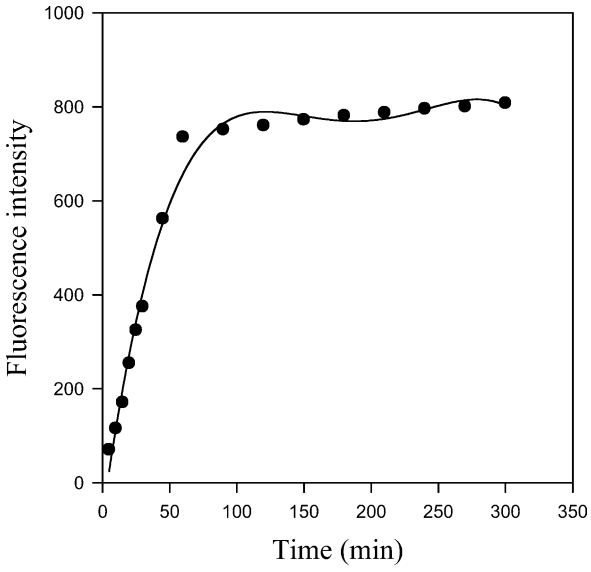

Fig. 5. Effect of adsorption time of NR on the fluorescence intensity of $(PAH\text{-}s\text{-}\beta\text{-}CD)_{10}$ film.

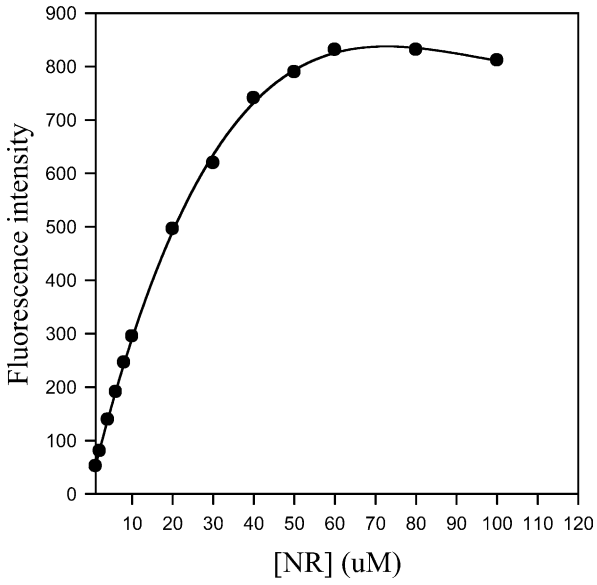

Fig. 6. Effect of NR concentration on the fluorescence intensity of $(PAH\text{-}s\text{-}\beta\text{-}CD)_{10}$ film.

cated not only on the surface of the film but also at the inner layers.

3.4. Effect of adsorption time of NR on the fluorescence intensity of $(PAH\text{-}s\text{-}\beta\text{-}CD)_{10}$ film

The effect of the adsorption time of NR (40 uM) on the fluorescence intensity of $(PAH\text{-}s\text{-}\beta\text{-}CD)_{10}$ film was investigated (Fig. 5). The adsorption of NR to the film was rather slow probably because of a limited rate of transport of NR from the solution to the interior of the film. Fig. 5 shows that the initial rate of the formation of the s-β-CD-NR complex is relatively fast and the fluorescence intensity of $(PAH\text{-}s\text{-}\beta\text{-}CD)_{10}$ film reached saturation within 1 h. Therefore, the optimal adsorption time of NR to the $(PAH\text{-}s\text{-}\beta\text{-}CD)_{10}$ film is chosen as 1 h.

3.5. Effect of NR concentration on the fluorescence intensity of $(PAH\text{-}s\text{-}\beta\text{-}CD)_{10}$ film

The effect of NR concentration on the fluorescence intensity of the multilayered film was investigated after being treated in the NR solution for 4 h. The concentration of NR was varied from 1 uM to 100 uM (Fig. 6). As can be seen from Fig. 6, the fluorescence intensity of the multilayered film was gradually enhanced with increasing NR concentration till the maximum adsorption equilibrium at film saturation. In this paper, NR served as a fluorescence probe. So, the selection of its concentration was very crucial. If the NR concentration selected was low, the sensitivity of the multilayered film would be low. On the other hand, a too high concentration would not beneficial for obtaining an optimum detection limit of the analyte. Considering the reasons mentioned above, the optimal NR concentration was selected as 40 uM.

From the viewpoint of practical applications, it is important to evaluate stability of the NR-adsorbed $(PAH\text{-}s\text{-}\beta\text{-}CD)_{10}$ film. No desorption was observed when the film was rinsed in pure water even for a few days. After the $(PAH\text{-}s\text{-}\beta\text{-}CD)_{10}$ film contacted with a 40 uM NR solution, the fluorescence intensity of the NR-adsorbed $(PAH\text{-}s\text{-}\beta\text{-}CD)_{10}$ film in pure water was recorded over a period of 4 h. The fluorescence intensity was recorded with a 30 min interval, the fluorescence intensity value of the film did not change and a standard deviation of 4.42 was obtained.

3.6. Response to lithocholic acid (LA)

Fig. 7 shows the fluorescence spectra of the NR-adsorbed $(PAH\text{-}s\text{-}\beta\text{-}CD)_{10}$ film in distilled water before and after being treated in different concentration of LA solution. Obviously, the fluorescence intensity of NR-adsorbed $(PAH\text{-}s\text{-}\beta\text{-}CD)_{10}$ film decreases after being treated in LA solution for 40 min. The decrease in the fluorescence intensity of NR-adsorbed $(PAH\text{-}s\text{-}\beta\text{-}CD)_{10}$ film is proportional to the amount of LA, which provides a basis for the optical chemical LA sensor. The $\Delta F/F_0$ value was plotted as a function of the LA concentration and the linearity is excellent. As shown in Fig. 8, linear calibration concentration curve covered the range from 2 uM to 60 uM with a detection limit of 1 uM. The observed decrease in fluorescence intensity of the NR-adsorbed $(PAH\text{-}s\text{-}\beta\text{-}CD)_{10}$ film can be explained by the possibility that NR was expelled from the s-β-CD cavity which was occupied by LA.

3.7. The apparent binding constants

The apparent binding constant (K) of s-β-CD with NR or LA was evaluated by nonlinear curve-fitting analysis us-

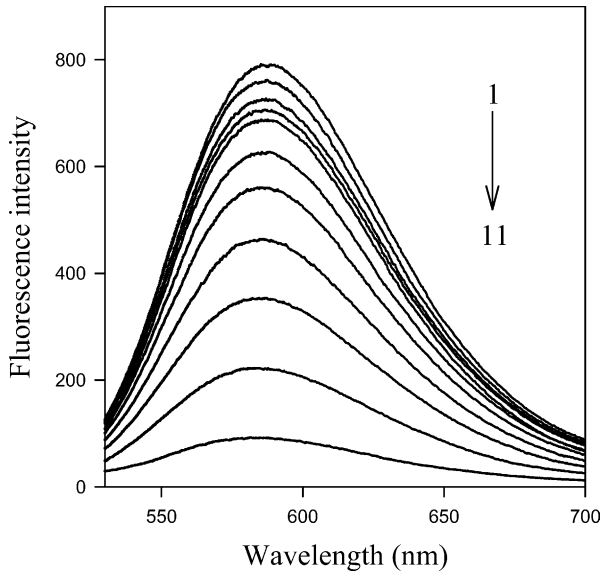

Fig. 7. The fluorescence spectra of the NR-adsorbed (PAH-s-β-CD)$_{10}$ film in distilled water before and after being treated in different concentration of LA solution [LA] (uM): (1) 0; (2) 2; (3) 4; (4) 6; (5) 8; (6) 10; (7) 20; (8) 30; (9) 40; (10) 50; (11) 60.

ing the following equation to the guest-induced fluorescence variations [39]:

$$\Delta F = \left(\left(\frac{K[\mathrm{G}]_0 + 1 + K[\mathrm{H}]_0 - \sqrt{(K[\mathrm{G}]_0 + 1 + K[\mathrm{H}]_0)^2 - 4K^2[\mathrm{H}]_0[\mathrm{G}]_0}}{2K} \right) [\mathrm{H}]_0 \right) \Delta F_{\max} \quad (1)$$

where $[\mathrm{G}]_0$ represents the initial concentration of guest; $[\mathrm{H}]_0$, the initial concentration of s-β-CD; ΔF, the difference in the fluorescence intensity at 587 nm between (PAH-s-β-CD)$_{10}$ film (or NR-adsorbed (PAH-s-β-CD)$_{10}$ film alone) and in the presence of the guest; and $\Delta F_{\max}$, the difference in the fluorescence intensity at 587 nm between (PAH-s-β-CD)$_{10}$ film (or NR-adsorbed (PAH-s-β-CD)$_{10}$ film alone) and the one totally complexed with the guest. In the case of (PAH-s-β-CD)$_{10}$ film (or NR-adsorbed (PAH-s-β-CD)$_{10}$ film), when the guest concentration is in excess ($[\mathrm{G}]_0 \gg [\mathrm{H}]_0$), free guest concentration is almost the same as that of initial guest concentration. Thus the apparent binding constant (K) is approximated to the following equation.

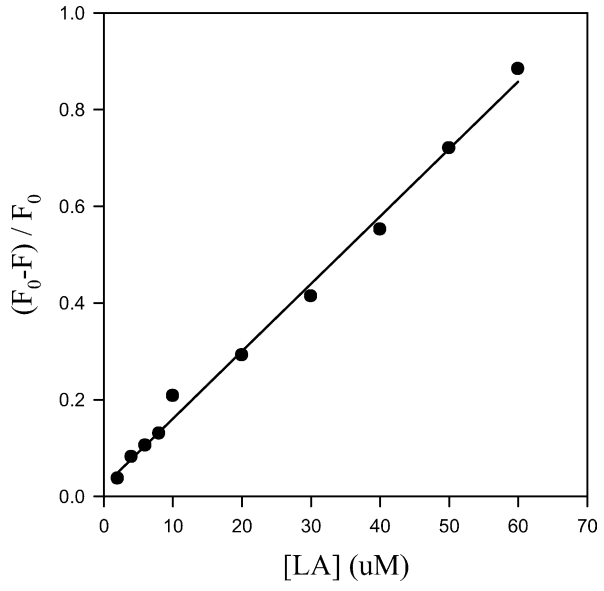

Fig. 8. The $\Delta F/F_0$ value of the NR-adsorbed (PAH-s-β-CD)$_{10}$ film as a function of the LA concentration.

$$K \approx \frac{[\mathrm{HG}]}{([\mathrm{H}]_0 - [\mathrm{HG}])[\mathrm{G}]_0} \quad (2)$$

where [H], [G], and [HG] represent free host, free guest, and host–guest complex, respectively. The concentration of the complex, [HG], is reflected in the magnitude of ΔF. In this case, ΔF is the difference in the fluorescence intensity at 587 nm. Using the values of $[\mathrm{H}]_0$, $[\mathrm{G}]_0$, ΔF, and $\Delta F_{\max}$, Eq. (2) leads to the following representation [40]:

$$\Delta F = \frac{K[\mathrm{G}]_0}{1 + K[\mathrm{G}]_0} \Delta F_{\max} \quad (3)$$

According to Eq. (3), which does not include $[\mathrm{H}]_0$, K values can be estimated without knowing the amount of s-β-CD immobilized to the polyelectrolyte multilayer film.

The apparent binding constant of s-β-CD with NR or LA was calculated based on the aforementioned reasoning. The apparent binding constant of the (PAH-s-β-CD)$_{10}$ film for NR was 3333 M^{-1} and that for LA was 19569 M^{-1}.

3.8. Response characteristics of the optical sensor

The reproducibility and reversibility of the NR-adsorbed (PAH-s-β-CD)$_{10}$ film in the determination of LA were evaluated by repetitively exposing the film to NR solution (40 uM), distilled water and LA solutions of different concentrations. The standard deviations of response of the NR-adsorbed (PAH-s-β-CD)$_{10}$ film to different LA solutions were found to be 4.69 (4 uM), 4.36 (10 uM), and 4.51 (50 uM). The standard deviation of the fluorescence intensity of the NR-adsorbed (PAH-s-β-CD)$_{10}$ film in distilled water was 4.18 for 12 determinations. The LA molecules complexed with s-β-CD could be eluted out of the CD cavity quickly and completely by NR solution for 1 h, which demonstrated the excellent reproducibility and reversibility of the sensor.

For the NR-adsorbed (PAH-s-β-CD)$_{10}$ film after being treated with a 10 uM LA solution, the fluorescence intensity at 587 nm was recorded over a period of 4 h. The fluorescence intensity was recorded with a 30 min interval, a standard deviation of 4.72 was obtained. After a series of 100 times

Table 1
Interference of different species to the fluorescent determination of LA with the proposed sensor

Interferent	Concentration[a] (M)	Fluorescence intensity ($\Delta F = F_i - F_{i0}$)[b]	Relative error (%) ($\Delta F/F_{i0}$) × 100
Methanol	1.0×10^{-4}	4.4	0.56
Ethanol	1.0×10^{-4}	5.0	0.63
Propanol	1.0×10^{-4}	5.4	0.68
Butanol	1.0×10^{-4}	−6.0	−0.76
Acetic acid	1.0×10^{-3}	4.1	0.52
Fructose	1.0×10^{-4}	3.5	0.44
Phenol	1.0×10^{-5}	−6.6	−0.84
Oxalate	1.0×10^{-3}	5.8	0.74
Aniline	1.0×10^{-5}	−6.2	−0.79
Citrate	1.0×10^{-3}	−5.5	−0.70
Carbonate	1.0×10^{-3}	4.9	0.62
Ascorbic acid	1.0×10^{-4}	−5.9	−0.75
Salicylate	1.0×10^{-5}	−6.3	−0.80
Saccharose	1.0×10^{-4}	4.7	0.60
Glucose	1.0×10^{-4}	5.2	0.66
L-Lysine	1.0×10^{-3}	−4.2	−0.53
L-Leucine	1.0×10^{-3}	3.0	0.38
L-Glutamic acid	1.0×10^{-3}	−4.6	−0.58

[a] The concentration of LA was fixed at 10 uM.
[b] F_i and F_{i0} are the fluorescence intensities of the NR-adsorbed (PAH-s-β-CD)$_{10}$ film contacting with 10 uM LA solution with and without the addition of the interferent (F_{i0} = 789.0).

measurements of LA solution, the stability of the sensor was kept constant.

3.9. Selectivity

Some inorganic and organic species were chosen for the study on selectivity of the LA sensor. A foreign species was considered not to interfere with measurement if a relative standard deviation caused by it was less than 5% in the determination of 10 uM LA. The results presented in Table 1 reveal that all species tested caused no interference when existed in specified molar excesses.

3.10. The response mechanism of the multilayered film

NR itself exhibits weak fluorescence emission in aqueous solutions. However, its fluorescence intensity was notably enhanced when NR molecule entered into the polyelectrolyte-film containing s-β-CD. The fluorescence enhancement can be interpreted as due to the formation of an inclusion complex between s-β-CD and NR. The hydrophobic and protective microenvironment of s-β-CD can shield the excited state of NR from water molecules and other species presented in bulk aqueous solution, and therefore, lead to a greater enhancement of the fluorescence intensity of NR. As is expected, when a third component (e.g. LA) with suitable polarity and dimension was added to the host–guest system of s-β-CD/NR, LA and NR would compete for the s-β-CD cavity. The partial NR molecules would be expelled from the s-β-CD cavities due to the introduction of LA. Because the photochemical property of NR strongly depended on its local microenvironment, the addition of LA would make NR lose the protection of s-β-CD hydrophobic cavity, which resulted in the decrease of fluorescence intensity of NR.

4. Conclusions

A sensitive and simple optical sensor for the determination of LA has been developed based on the fluorescence quenching of NR/s-β-CD supramolecular complex immobilized in the polyelectrolyte multilayer film. The preparation of the s-β-CD/NR inclusion complex can be realized in NR aqueous solution with hydrophobic and electrostatic interactions. The degree of the quenching of NR fluorescence in s-β-CD/NR system is proportional to LA concentration. The study of the quenched fluorescence processes enables a better understanding of cyclodextrin-based supramolecular chemistry and the design of novel assay methodology for optically inert guest molecules.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (Nos. 20375012, 20075006 and 29975006), the Foundation for Ph.D. Thesis Research (no. 20010532008) and the Foundation of Scientific Committee of Hunan Province.

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