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Molecularly imprinted fluorescent chemosensor synthesized using quinoline-modified-β-cyclodextrin as monomer for spermidine recognition

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Abstract:

Polyamines are polycationic amines and playing important functions in the cellular growth and proliferation. The abnormal levels of polyamines in the biological fluids have been related to different diseases including cancers. However, polyamine analysis is a difficult task because there are no chromphore in the polyamine structures. In this study, a novel molecularly imprinted fluorescent chemosensor for spermidine detection has been synthesized using quinoline modified-β-cyclodextrin as the functional monomer. The imprinted receptors were formed by the interaction between the spermidine and β-cyclodextrin (β-CD). The fluorescence of the chemosensor has shown a "Turn-on" response mode which was resulted from the increase of the environmental hydrophobicity around quinoline group due to the inclusion of spermidine in the CD cavity. The chemosensor has selectivity for the spermidine and its structural analogue spermine due to the imprinting effect. In the research, the binding constant of the imprinted membrane was evaluated and binding mechanism of the MIP was studied by 2D ^{1}H NMR experiment. The research of spermidine analysis in serum demonstrated the imprinted chemosensor has good application potential in the biological sample analysis.

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Keywords: molecularly imprinted chemosensor; spermidine; quinoline modified-β-cyclodextrin; fluorescent detection

1. Introduction

The natural polyamines, including putrescine, spermidine and spermine, are polycationic amines produced in vivo metabolism. The significance of polyamines in cellular growth and proliferation is well recognized.[1] The important roles of polyamines in the stabilization of negative charges of DNA, RNA transcription, protein synthesis, and apoptosis have been studied. Research has found that increase in polyamines and polyamine synthesis enzymes are often related to the tumor growth.[2] The level of some polyamines can be indicative of the presence of malignant tumors, and was proposed as a tool for the cancer therapy effectiveness evaluations.[3]

Due to their multiple functions in cell biology, determination of the polyamines becomes an important task in the biological and pharmaceutical research. However, analysis of polyamines has been a challenge task because polyamines have neither chromophores and fluorophores nor the electrochemical activities. As the result, they cannot be readily analyzed by spectrophotometric or electrochemical methods.

Immunoassays (RIA and ELISA),[4-6] high performance liquid chromatography (HPLC)[7, 8] and thin layer chromatography (TLC)[9] have been used for the analysis of polyamines in serum or other body fluids. However, these methods have some drawbacks. For the immunoassay, the antibody is expensive and specificity is not satisfactory. The RIA may be hazardous due to its radioactivity. The HPLC and TLC methods for the polyamine analysis have to be coupled with derivatization experiment.[10, 11] While derivatization processes are time-consuming and interference compounds could be produced. Thus, it is desirable to develop a rapid, sensitive and selective method for the polyamine determination in biological samples.

Molecular imprinting is an effective technique for the preparation of molecularly imprinted polymers (MIPs). The imprinted binding sites in the MIPs can be created by the self-assembly of template molecule and functional monomers, followed by polymerization process.[12, 13] Due to the advantages such as tailor-made selectivity,

chemical stability and ease in preparation, MIPs have attracted great attention in the fields of chemical recognition and separations. They are also used as the alternative to biological receptors.[14] MIP fluorescent sensors have being synthesized and reported in the literature.[15-17] Among these researches, many reporting systems of the sensors relied on the fluorescence of analytes.[18, 19] Some studies utilized electron transfer or formation of a resonance complex between the fluorescent monomer and analytes to switch on fluorophores.[18, 20] These reporting systems are restricted to the analytes with specific structures. MIP fluorescent sensor with broader analyte application is desirable.

In the MIP reparations, β-cyclodextrin (β-CD) is an attractive monomer for organic compound recognition. β-CD has a hydrophobic inner cavity and hydrophilic outer surfaces. It is capable of interacting with a large variety of guest molecules to form inclusion complexes in aqueous environment.[21-24] With this property, β-CD has shown advantage in binding neutral molecules in the aqueous environment such as biological samples. Research of molecular imprinting using cyclodextrin (CD) as monomer has been published in the recognition of molecules such as bilirubin,[25] steroidal,[26, 27] ursolic acid,[28] creatinine[29, 30]and protein.[31] Using fluorophores labeled CD as acceptor for MIP fluorescent sensor have several advantages. It has good selectivity and is suitable for the application in the aqueous solutions. It has broader applications compared with some published work which has certain requirement for the structure of the analytes. We have used dansyl-modified β-cyclodextrin to synthesize the cholesterol imprinted chemosensor. The research demonstrated that flourescent group-modified β-cyclodextrin can be used as building block for the imprinted receptor and attached fluorescent group can be used as the reporter. However, the dansyl-modified β-cyclodextrin MIP in our research exhibited a "Turn-off" response mode with low sensitivity which is the weakness for detection.[32] To construct a MIP flourescent chemosensor with "Turn-on" mode is one of the goals in this research.

As far as we know, there is no research of MIP for polyamines analysis that has been published. This is the first research developed for the spermidine analysis by

using MIP as the acceptor of the fluorescence sensor. In this research, a new fluorescent imprinted chemosensor for the spermidine detection has been developed. A quinoline group derivatized β-CD was synthesized and employed as the functional monomer. The spermidine/β-CD interaction was used to form binding sites of MIP and the quinoline groups are acting as fluorescent reporter. Research has demonstrated that with certain sidearm, the quinoline group can co-exist with included guest molecule in the CD cavities.[33] The inclusion event increases the hydrophobicity of the cavity environment and results in an increase of the fluorescent intensity of the quinoline group.[33] This fluorescence "Turn-on" mechanism was employed in the present research to create a reporting system in the chemosensor. In the research, the sensitivity, selectivity and application of the chemosensor have been studied. The mechanism of imprinting was also investigated by 2D ^{1}H NMR experiment. The result demonstrated that the chemosensor synthesized in this study has rapid and selective response to the binding of the spermidine with a Tune-on mode.

2. Materials and methods

2.1. Materials

The β-cyclodextrin (β-CD) and tosyl chloride were purchased from Guangfu Chemical Co. Ltd. (Tianjin, China). Spermidine, 2-phenethylamine, spermine, 8-hydroxyquinoline and hexamethylene diisocyanate (HMDI) were obtained from Tianjin Heowns Biochemical Technology Co. Ltd (Tianjin, China). *N*, *N*′-dimethylformamide (DMF) and dimethyl sulfoxide (DMSO) were from Kermel Chemical Co. Ltd. (Tianjin, China) and were dried with molecular sieve and distilled under a reduced pressure before use. All other reagents were of analytical grade and used as received. Serum was from Huyu Biochemical Technology Co. Ltd (Shanghai, China) and stored at -20 □ until use.

2.2. Instrumentation

Elemental analyses were carried out with an elemental analyzer (Vario EL CUBE, Germany). A TU-1901 spectrophotometer (Purkinje, China) was used for the UV measurement. ^{1}H NMR experiments were performed on a Mercury Vx-300 spectrometer (Varian, USA). Fluorescence spectra were obtained from a Hitachi-4500 fluorescence spectrometer (Hitachi, Japan). FT-IR spectra were recorded on a Nicolet 6700 FT-IR spectrophotometer (Nicolet, USA).

2.3. Synthesis of 6-O-(p-tosyl)-β-cyclodextrin

The 6-O-(p-tosyl)-β-cyclodextrin (β-CDOTS) was synthesized by the reaction of β-CD and tosyl chloride (Fig. 1) according to the method in the literature.[34] β-CD (11.2 g, 9.9 mmol) was dissolved in 112 mL dry pyridine. Tosyl chloride (3.75 g, 19.7 mmol) was dissolved in 12 mL of dry pyridine and added drop-wise into β-CD solution under nitrogen protection. The reaction was performed under stirring for 7 h at room temperature. After the reaction, the solution was poured into 400 mL of acetone. The white precipitates were collected and washed with acetone. The product: β-CDOTS was purified by three successive re-crystallizations in water and dried in vacuum at 60 □.

^{1}H NMR (300 MHz, D_6-DMSO): δ 2.42 (3H, s, -CH_3), 3.18～3.42 (14H, m, C2-H, C4-H), 3.43～3.74 (28H, m, C3-H, C5-H, C6a, C6b-H), 4.13～4.55 (6H, m, C6-OH), 4.76～4.82 (7H, s, C1-H), 5.58～5.87 (14H, m, C2-OH, C3-OH), 7.40～7.45 (2H, d, J=15 Hz, Ph-H), 7.72～7.77 (2H, d, J=15 Hz, Ph-H). The results of elemental analysis for the product were: C, 43.56; H, 6.18, which agreed with the values of β-CDOTS•3H_2O molecule ($C_{49}H_{76}O_{37}S$•3H_2O). The FT-IR experiment was also carried on to characterize the β-CDOTS (Fig. S1 in the Supporting Information). The results have shown that the tosyl ester was successfully grafted on β-CD.

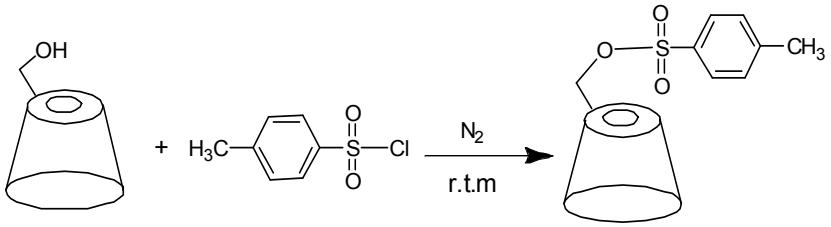


Fig. 1. Synthesis of 6-O-(p-tosyl)-β-CD (β-CDOTS).

2.4. Synthesis of mono [6-O-(8-quinolyl)]-β-cyclodextrin

The mono [6-O-(8-quinolyl)]-β-cyclodextrin (quinolyl-β-CD) was synthesized by the reaction of β-CDOTS and 8-hydroxyquinoline (Fig. 2) according to a literature.[35] In the reaction, β-CDOTS (2 g) and 8-hydroxyquinoline (1 g) were dissolved in 30 mL DMF. After addition of potassium carbonate (0.3 g), the reaction was performed at 90 °C for 20 h under a nitrogen atmosphere and stirring. The yellow precipitates were obtained after removal of the solvents under a reduced pressure at 40 °C. The product was dried at 60 °C under vacuum for 8 h and was purified by recrystallization in water. ^{1}H NMR (DMSO-d_6, TMS): δ 3.2 - 4.3 (m, β-cyclodextrin protons); 7.1 - 9.2 (m, 8-quinolyl protons); Anal. Calcd for $C_{51}H_{75}O_{35}$ N·6H_2O: C, 44.70; H, 6.30; N, 1.02. Found: C, 44.41; H, 5.93; N, 1.08. The FT-IR (Fig. S1 in the Supporting Information) also indicated the successful grafting of the quinolyl group on β-CD.

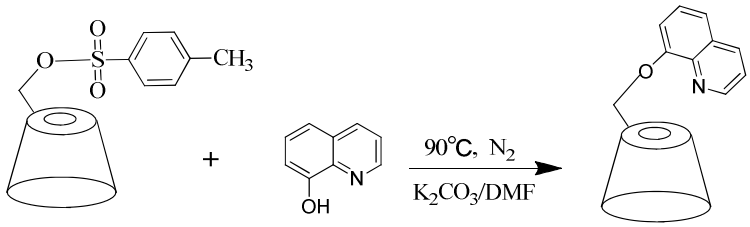


Fig. 2. Synthesis of mono [6-O-(8-quinolyl)]-β-cyclodextrin (quinolyl-β-CD).

2.5. Preparation of spermidine fluorescent imprinted membrane

In the MIP membrane preparation, spermidine was used as the template and quinolyl-β-CD was the functional monomer. HMDI was used as the cross-linker. The quinolyl-β-CD (0.2 g), spermidine (11.5 μL) and HMDI (0.15 mL) were dissolved in 3 mL of dry DMSO under stirring. The pre-polymerization solution was spread onto a clean glass slide (38 mm×20 mm) by the doctor blade coating technique. The MIP membrane was formed on the slide by polymerization at 65 °C for 2 h. The membrane coated slide was washed with acetone, hot water and ethanol consecutively to remove the template molecules and reagent residues under ultrasonication and then dried under vacuum at 40 °C for 2 h. As a control, a non-imprinted membrane was prepared under the same conditions except the template molecules are omitted.

2.6. Fluorescence spectra measurement and binding constant determination

Spermidine standard solutions with different concentrations were prepared with deionized water and stored in refrigerator before use. The membrane coated slide was soaked in spermidine solution for 4 min and was amounted in the specialized solid bracket supplied by Hitachi for the measurement. A 2.5 nm slit and photomultiplier tube voltage of 700 V were employed in the experiment. Fluorescence spectra of the chemosensor were obtained at λ_{ex} of 324 nm and λ_{em} of 411 nm at room temperature. The method of the soaking of the membrane and fluorescence measurement was demonstrated in Fig. 3.

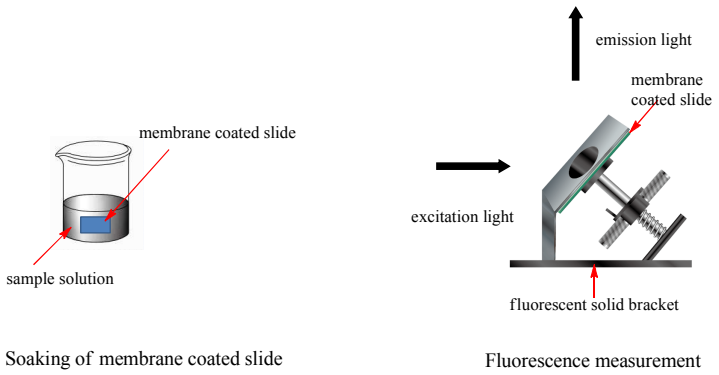


Fig. 3. Schematic representation for the sample soaking and fluorescence measurement.

The binding constant of the spermidine on the imprinted membrane and non-imprinted membrane were determined by modified double reciprocal plot equation:[36]

$$1/\Delta I = (K\,kQ_{com}C_m)^{-1}C_s^{-1} + (kQ_{com}C_m)^{-1} \qquad \text{(Eq. 1)}$$

In the equation, ΔI is the fluorescence intensity difference between the blank and the analyte-bound chemosensor, K is the binding constant, k is the instrumental constant, Q_{com} is the quantum yield of complex, C_m is the concentration of the binding sites of the imprinted membrane, C_s is the concentration of spermidine. The $kQ_{com}C_m$ are constants in the experiment and the product of $kQ_{com}C_m$ can be obtained from the intercept of the plot. The binding constant was calculated by dividing the intercept with the slope of the plot.

2.7. 2D ^{1}H NMR experiment for the determination of quinolyl-β-CD/spermidine complex structure

2D ^{1}H NMR experiments were performed on a Mercury Vx-300 spectrometer (Varian, USA). The sample was prepared by dissolving quinolyl-β-CD and spermidine in d_6-DMSO. The ratio of quinolyl-β-CD/spermidine in solution was the same as that used in the imprinting polymerization. 2D ^{1}H NMR ROESY was performed at a spectral width of 4807 Hz in both dimensions and 512 increments with 64 transients per increment. The mixing time was 240 ms.

2.8. Determination of spermidine in the spiked serum samples

The spermidine standard solution (1×10^{-4} mol·L^{-1}) was prepared in deionized water. Spiked serum samples with different spermidine concentrations were prepared by mixing different volumes (30, 60, 185, 315, 666 μL) of the spermidine standard solution with 1.0 mL serum under vortexing. To remove proteins from the serum samples, 2.0 mL deionized water and 3.0 mL acetonitrile were added into the samples followed by centrifugation at 8000 rpm for 10 min. The supernatant was used for the fluorescence analysis. The sample soaking time and conditions for the fluorescence

measurement were the same as section 2.6.

3. Results and Discussion

3.1. Imprinting and proposed signaling mechanism of the molecularly imprinted fluorescent chemosensor

In the present study, a molecularly imprinted membrane was synthesized as the accepting and reporting system of a fluorescent chemosensor. The artificial receptor was created by molecular imprinting using quinolyl-β-CD as the functional monomer. The synthesis of the spermidine imprinted binding sites and proposed sensing mechanism are demonstrated in the Fig. 4. In the quinolyl-β-CD molecule synthesized in this research, the quinoline group is self-included in the cavity of β-CD before interaction with guest molecules, which has been proved by 2D ^{1}H NMR experiment.[33] We proposed that in the imprinting process, spermidine is encapsulated within the quinolyl-β-CD. The complex structures were fixed in the MIP by polymerization. After the MIP synthesis, the template is removed from the MIP by washing process, which leaves only the quinoline groups in the β-CD cavity. In the rebinding process, spermidine re-enters the β-CD and co-exists with quinoline group in the cavities. The existing of spermidine in β-CD makes the environment surrounding the quinoline group more hydrophobic, which induces an increase of the fluorescence intensity. The fluorescent intensity change of the quinoline group signals the binding event.

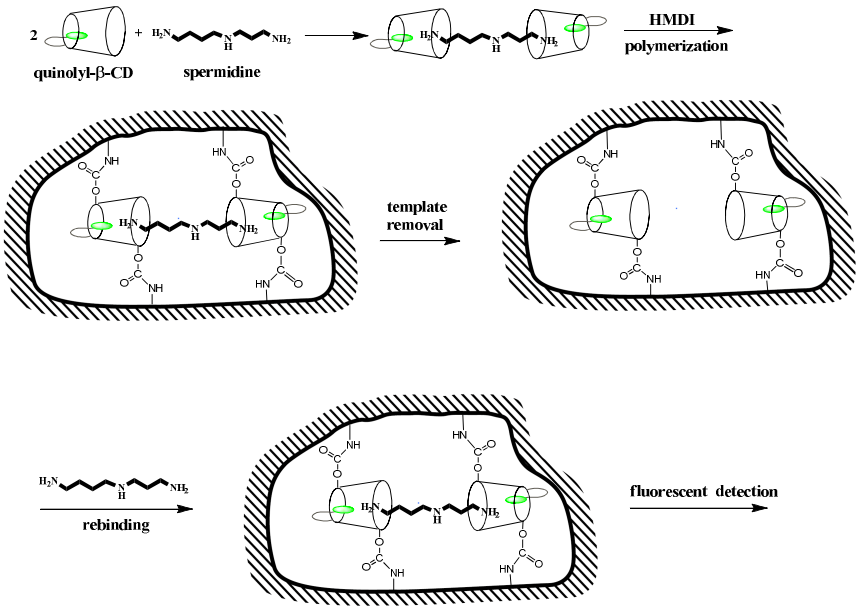


Fig. 4. Schematic demonstration of molecular imprinting for the receptor construction and re-binding of spermidine giving fluorescence signal. = quinoline group.

3.2. Selection of the synthetic condition for imprinted membrane

In the MIP synthesis, the ratio of template/functional monomer/cross-linker has to be selected. To find a proper functional monomer/template ratio, complexation stoichiometry of quinolyl-β-CD/spermidine was studied by the Job's plot method. In the experiment, the ratios of the quinolyl-β-CD and spermidine were changed while the total concentrations of quinolyl-β-CD and spermidine in the solutions were kept constant. The difference of UV absorbance (ΔA) between solutions of mixture and quinolyl-β-CD were measured. The complexation stoichiometric ratio of the quinolyl-β-CD and spermidine was determined through Job's plot (Fig. S2 in the Supporting Information). In the Job's plot, the maximum ΔA appeared when the quinolyl-β-CD/spermidine ratio was 1.5. The quinolyl-β-CD/spermidine ratio of 2:1 was selected in the imprinted membrane synthesis to make the incorporation reaction more completed.

The ratio of 1:6 was selected for the functional monomer/cross-linker, which was

the optimized composition from our previous research experiment.[32]

3.3. The fluorescent response and binding affinity of the imprinted fluorescent chemosensor

The optical response of the spermidine-imprinted chemosensor upon binding of spermidine was studied by the fluorescent experiment. The fluorescence spectra of the chemosensor after soaking in spermidine solutions with different concentrations are shown in the Fig. 5. The fluorescence intensity of the chemosensor increased with the increase of the spermidine concentration. This "Turn on" response mode indicated the cavities of the imprinted β-CD accommodate both the quinoline groups and spermidine molecules. This phenomenon agreed with our proposed binding mechanism (Fig. 4). A linear relation between the negative logarithm of spermidine concentration (–log $C_{spermidine}$) and fluorescence intensity of the chemosensor at $C_{concentration}$ of 5×10^{-7} to 2×10^{-4} mol·L^{-1} has been obtained.

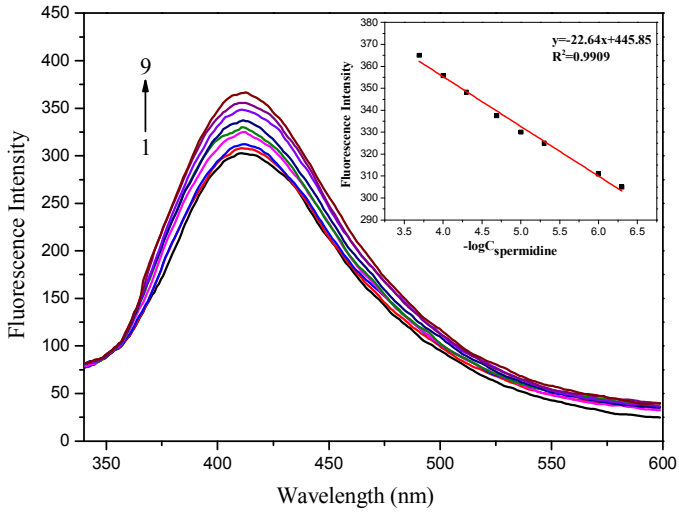


Fig. 5. Fluorescence spectra of imprinted membrane upon soaking of spermidine solutions with different concentrations at room temperature. The concentrations of spermidine were (from 1 to 9) 0, 5×10^{-7}, 1×10^{-6}, 5×10^{-6}, 1×10^{-5}, 2×10^{-5}, 5×10^{-5}, 1×10^{-4}, 2×10^{-4} mol·L^{-1}.

The binding kinetic of the imprinted membrane was determined by the measurement of the response of the imprinted chemosensor for spermidine versus the sample soaking time (Fig. S3 in the Supporting Information). The spermidine H_2O solution with concentration of 1.0×10^{-5} mol·L^{-1} was used in the experiment. The result demonstrated the binding equilibrium was reached at 4 min. The fluorescence measurements were performed after sample soaking for 4 min.

To study the imprinted binding affinity of the imprinted membrane, the binding constant was calculated by modified double reciprocal plot equation (Eq. 1). The plot of ΔI versus spermidine concentrations in the range from 1×10^{-5} to 10×10^{-5} mol·L^{-1} for imprinted and non-imprinted membranes are shown in the Fig. 6. ΔI is the fluorescence intensity difference between the blank and the analyte-bound chemosensor.

The result demonstrated that the imprinted chemosensor has higher fluorescence response and sensitivity than the non-imprinted chemosensor. The data from spermidine concentrations in the range from 1×10^{-5} to 5×10^{-5} mol·L^{-1} were used for the binding constant calculation. The binding constant K_{MIP} of the spermidine on the imprinted membrane is 9.4×10^{4} L·mol^{-1} and it (K_{NIP}) is 4.6×10^{4} L·mol^{-1} for the non-imprinted membrane. The higher binding constant was contributed to the specific binding cavity created in the imprinted process which enhanced the binding affinity for the template. The imprinted factor, calculated by K_{MIP}/K_{NIP} was 2.04, which indicated that good imprinting effect was obtained.

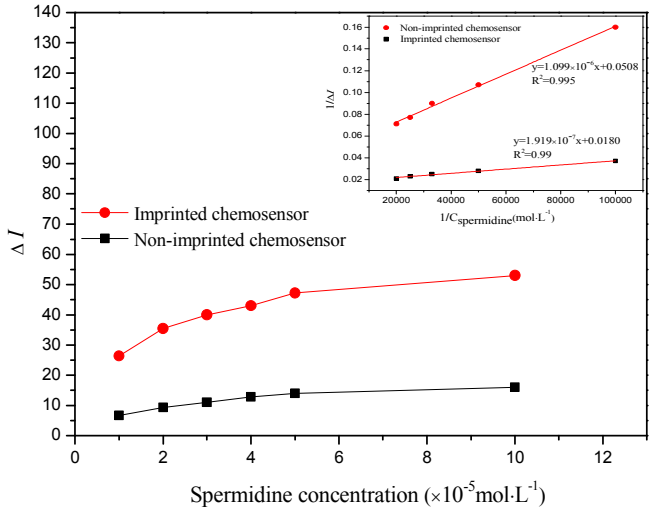


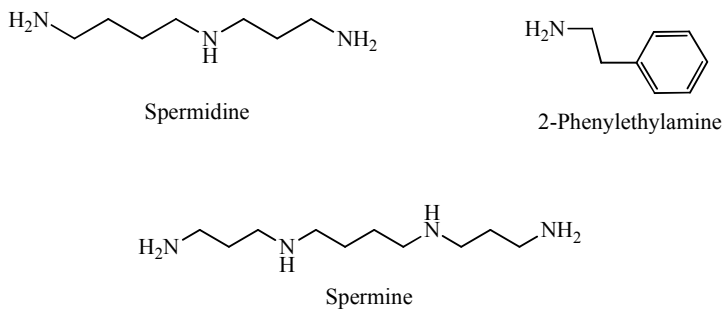
Fig. 6. Sensitivity comparison of imprinted chemosensor with non-imprinted chemosensor in six different concentrations of spermidine (1×10^{-5}, 2×10^{-5}, 3×10^{-5} , 4×10^{-5}, 5×10^{-5} and 10×10^{-5} mol·L^{-1}).

3.4. Selectivity of the spermidine imprinted chemosensor

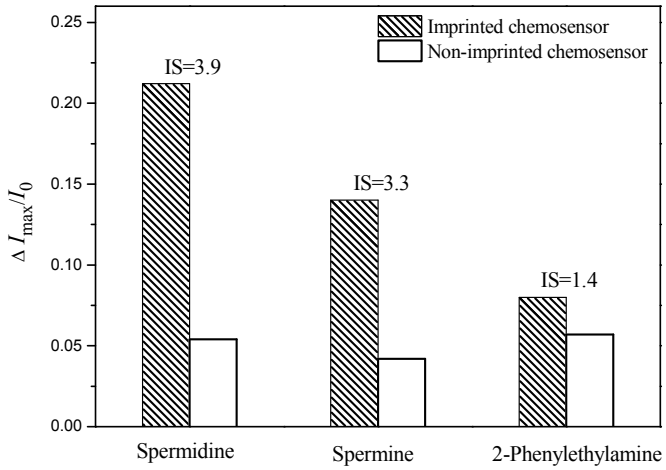
The selectivity of the spermidine imprinted chemosensor was evaluated by comparison of the imprinted membrane sensitivity for spermidine and for its analogs. The structures of the analytes used in the selectivity study are shown in the Fig. 7 (a).

The sensitivity factor of the membrane for spermidine S_f was calculated by $S_f=\Delta I_{max}/I_0$, in which I_0 is the fluorescence intensity of the blank imprinted membrane and ΔI_{max} is the maximum fluorescence intensity difference between the blank and the analyte-bound chemosensor. The sensitivity factors of imprinted chemosensor and non-imprinted chemosensor for three analytes are shown in the Fig. 7 (b). The sensitivity of the imprinted chemosensor for different analytes is in the order of spermidine > spermine > 2-phenylethylamine. While for the non-imprinted chemosensor, the sensitivity is 2-phenylethylamine > spermidine > spermine. The imprinted selectivity (IS) of the MIP calculated by S_f(MIP)/S_f(NIP) are shown in the Fig. 7 (b). The different order of sensitivity between the imprinted and non-imprinted chemosensors proved that the selectivity of the chemosensor comes from the imprinting process. The IS of 3.9 for spermidine demonstrated that good recognition was obtained due to the imprinting process. The selectivity (α) of the chemosensor

was evaluated by the ratio of sensitivity of spermidine to its analogs, $\alpha = S_f(\text{spermidine})/S_f(\text{analog})$. The α(spermidine/2-phenylethylamine) was 2.65 and α(spermidine/spermine) was 1.5 for the imprinted chemosensor, while they were 0.94 and 1.28 for the non-imprinted chemosensor respectively. The results indicated that the imprinted chemosensor has good selectivity for imprinted molecule than its analogs and the selectivity is much better than the non-imprinted chemosensor.



(a)



(b)

Fig. 7. Comparison of fluorescent sensitivity of imprinted and non-imprinted chemosensor. (a) Chemical structures of the analytes used in the selectivity study; (b) fluorescent sensitivity of imprinted and non-imprinted chemosensors for three analytes. The concentration of the analytes was 2×10^{-4} mol·L^{-1}. The excitation wavelength was 324 nm and the emission wavelength was 411 nm.

3.5. The 2D ^{1}H NMR analysis of the quinolyl-β-CD/spermidine complex structure

To explore the imprinting mechanism, we studied the quinolyl-β-CD/spermidine (Fig. 8 (a)) complex structure by 2D ^{1}H NMR experiment. The ROESY spectrum of quinolyl-β-CD/spermidine complex is shown in the Fig. 8 (b). The assignment for the cross-peaks with correlated protons is listed in the Table 1. In the 2D ^{1}H NMR spectrum, ROESY cross-peak A from the correlation between β-CD proton (H_6) and quinoline proton (H_e) was observed (Table 1), which indicated that quinoline is self-included into the β-CD cavity. Meanwhile, as shown in Fig. 8 (b), several cross-peaks between quinolyl-β-CD and spermidine protons appeared in the 2D ^{1}H NMR spectrum, suggesting that the spermidine molecule exists in the cavity of quinolyl-β-CD.

The ROESY cross peaks B, C and D are the correlation signals between the C_1-H (and C_7-H), C_6-H, C_2-H of spermidine and the protons H_3, H_3, H_5 of β-CD, respectively (Table 1). These correlation signals demonstrated the distance between the two protons is within 0.5 nm. This result indicated that spermidine is included into the cavity of quinolyl-β-CD.

The correlation signals between other inner cavity proton H_5 and any quinoline protons are not observed, which excluded the possibility of the quinoline group in the central cavity. All information reveals that the spermidine is included into the β-CD cavity from its secondary side. Fig. 8 (c) shows the possible complex structure of quinolyl-β-CD/spermidine.

For comparison, quinolyl-β-CD structure was also studied by 2D ^{1}H NMR experiment (Fig. S4 in the Supporting Information), ROESY cross-peak A′ between the β-CD proton (H_6) and the quinoline proton (H_e) was also observed, which has similar intensity as peak A in Fig. 8. This phenomenon indicates that the binding of the spermidine does not influence the position of the quinoline group in the cavity of β-CD.

Table 1 2D ^{1}H NMR ROESY cross-peaks between the protons of quinoline group, spermidine and β-cyclodextrin.

Cross peak	Correlated protons		Intensity
A	β-CD, H_6	H_e of quinoline	+ + +
B	β-CD, H_3	C1-H of spermidine	+ + +
		C7-H of spermidine	+ + +
C	β-CD, H_3	C6-H of spermidine	+ +
D	β-CD, H_5	C2-H of spermidine	+

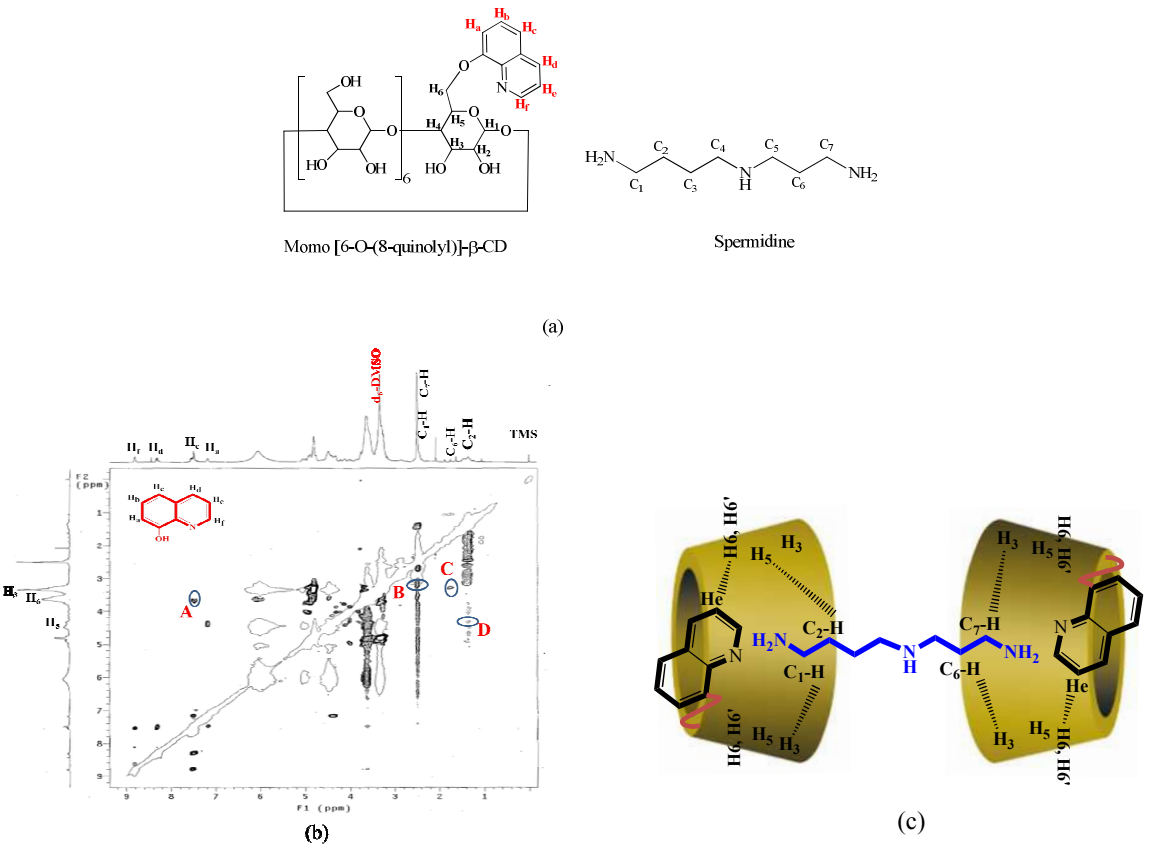


Fig. 8. 2D ^{1}H NMR ROESY analysis of quinolyl-β-CD/spermidine complex. (a) Structures of mono [6-O-(8-quinolyl)]-β-cyclodextrin and spermidine with labelled protons or carbons; (b) 2D ^{1}H NMR ROESY spectrum of quinolyl-β-CD/spermidine complex; (c) proposed quinolyl-β-CD/spermidine complex structure.

3.6. Interference study

To further investigate the selectivity of the chemosensor, interference study was carried out using spermine as the interference component. The spermine has similar structure with spermidine and also exists in the biological fluids. The fluorescence response of mixture solutions with spermidine/spermine molar ratios of 1:1, 1:2, 1:5, 1:10, 1:20, 1:50 and 1:100 were investigated using the MIP chemosensor. In the experiment, the spermidine concentration (1×10^{-6} mol·L^{-1}) was kept constant.

The fluorescence intensity versus different ratios of spermidine/spermine is shown in the Fig. 9. The results indicated that in the spermidine/spermine ratios from 1:1 to 1:50, the increment of the fluorescence intensity was only 1% to 3%. When the ratio of spermine increased to 100:1, the increment of the fluorescence intensity was changed to 5.5%. Because the concentration of spermine in serum is generally lower than spermidine,[8] this situation rarely exist in the real samples. The result of interference study indicated that spermine does not have obvious influence for the spermidine determination.

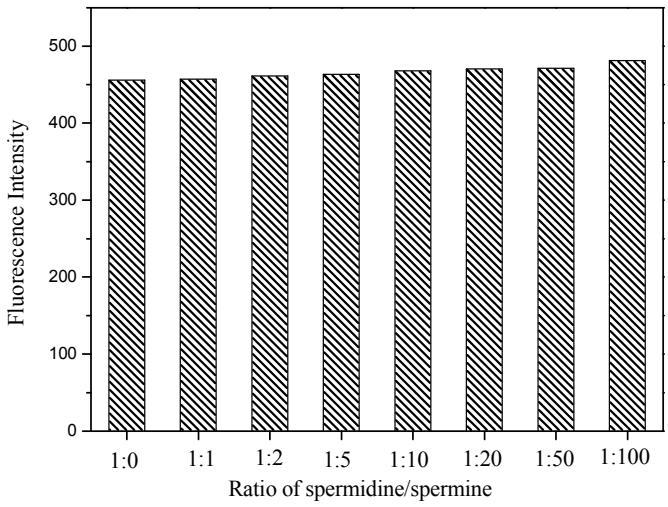


Fig. 9. Fluorescence intensity of imprinted chemosensor in different ratios of spermidine/spermine.

3.7. Determination of spermidine in serum using the imprinted chemosensor

The study of spermidine determination in serum by fluorescence spectroscopy was conducted to investigate the potential application of the imprinted chemosensor in the biological sample analysis. The linearity of the quantification method was determined by spermidine standard solution. A linear relation (y = -9.82x + 266.94) between the fluorescence intensity and –log $C_{spermidine}$ in the spermidine concentration range of 1×10^{-7} to 2×10^{-4} mol·L^{-1} was established (R^2 = 0.9915).

The accuracy and precision of the method were determined by the recovery and RSD from the measurement of the spiked serum samples (Table 2). The spermidine in the blank serum was 1.25×10^{-6} mol·L^{-1} determined by imprinted chemosensor, which closed to the analytical result in the literature.[8, 37] The recoveries measured by the spiked spermidine samples were in the range of 88.0 % - 105.0 %, and the relative standard deviation (RSD%) was less than 5%. The results demonstrated the chemosensor system has good application potential for the detection of spermidine in serum.

Table 2 Determination of spermidine in the spiked serum using imprinted fluorescent chemosensor (n=5).

Spiked Concentration (mol·L^{-1},$\times 10^{-6}$)	Found Concentration (mol·L^{-1},$\times 10^{-6}$)	Recovery (%)	RSD (%)
0.5	0.45	90.0	3.5
1	0.88	88.0	4.3
3	2.81	93.6	4.2
5	5.25	105.0	3.9
10	9.25	92.5	3.9

The comparison of different methods for the spermidine analysis was shown in the

Table 3. Compared with other methods, the method of this work has advantages of short analytical time and simple sample pretreatment (no derivatization requirement). It also has acceptable accuracy and linear range.

Table 3 Comparison of different methods for spermidine analysis in biological samples[a]

Methods	Pretreatment	Time of analysis	Linear range (μ mol·L^{-1})	Accuracy (Recovery, %)	Applied samples
HPLC-UV	derivatization	25 min	0.27-1.6	92-107	serum[8]
LC-MS	derivatization	12 min	0.007-1.7	84-108	serum[37]
Capillary electrophoresis	derivatization	10 min	0.01-1	93.4-102	urine[38]
RIA	-	60-80 samples/day	0.07-0.41	95-107	Serum[39]
Spermidine imprinted Chemosensor	-	4 min	0.1-200	88-105	serum (This work)

[a] "-" represents no derivatization in the pretreatment.

4. Conclusion

A new spermidine imprinted fluorescent chemosensor was prepared by molecular imprinting using quinoline modified-β-cyclodextrin as the functional monomer. The imprinted chemosensor has "Turn-on" response upon the addition of the template molecules, which has better sensitivity. The imprinted fluorescent chemosensor has selectivity to spermidine, which demonstrated that the imprinted cavities have been successfully established. The proposed interaction mechanism between spermidine and quinoline modified-β-cyclodextrin has been proved by 2D ^{1}H NMR experiment. The method using imprinted fluorescence chemosensor has been developed for the detection of spermidine in serum sample with acceptable accuracy and precision. The method also has short analytical time and good selectivity for spermidine, which

demonstrated the chemosensor has application potential for real sample analysis.

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