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Disposable terbium (III) salicylate complex imprinted membrane using solid phase surface fluorescence method for fast separation and detection of salicylic acid in pharmaceuticals and human urine

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ABSTRACT

In this work, a simple, low cost, selective and sensitive complex imprinted membrane (CIM) for solid-phase fluorescent detection was developed with terbium (III) salicylate as complex template. Terbium-sensitized luminescence was employed for monitoring salicylic acid (SA) based on the fluorescence enhancement effect of benzoic acid derivatives on lanthanide ion Tb (III). The resulting CIM showed good fluorescent response and high selectivity towards SA with Tb as pivot in protic solvents, while demonstrating better analytical performance than the controlled membranes. The optimized adsorption time was 10 min, indicating rapid kinetics of the imprinted membrane. The linear response of CIM to SA was from 0.20 to 10 mg/L with limit of detection (LOD) of 0.040 mg/L. The prepared CIM was successfully applied to the analysis of salicylic acid in pharmaceuticals and spiked human urine with recoveries of 80.6%–88.1%. The analytical results of the proposed method were in good agreement with those obtained by high performance liquid chromatography (HPLC) method, indicating that the developed membrane has acceptable practicability for fast determination of SA in real samples.

1. Introduction

Molecularly imprinted polymer (MIP) is a functionalized polymeric material with selective binding capability for molecular template [1]. During polymerization process, the template, functional monomer and crosslinker are copolymerized to obtain a polymer network with the template molecule incorporated in the imprinted cavity [2]. Removal of the template molecule will create rigid three-dimensional cavity complementary in size and shape to the target analyte [3]. MIPs show good thermal and chemical stability and have been used in a wide range of fields [4]. MIPs hold advantages over biological recognition elements including low cost, simple preparation, high mechanical stability and convenience for storage [5,6]. Thus MIPs gain broad acceptance as recognition materials for specifically binding target analytes in various samples [7–17]. But there are also some limitations on preparation and application of MIPs, one of which is that the frequently employed hydrogen bond will be interfered by strong polar solvents. As a result, the imprinting effect and selectivity of MIPs may be weakened [18], which has been a significant block on development of MIP. A promising way to solve this problem is to reinforce the interaction between template molecule and functional monomer by adding metal ions to connect them with coordination bond.

The metal ion mediated imprinted polymer was firstly reported in 1984 [19]. In this technique, metal ion acted as an assembled center that regulated the functional monomer and the template molecule around with coordinating interaction, while the following preparation procedure was basically the same as MIP. The composition of metal ion and target molecule can be regarded as a "complex template". Coordination bond has higher strength, specificity and directionality than hydrogen bond, which make it more like a covalent interaction and thus more stable in protic solvents [20]. Due to the characteristics of coordination bond mentioned above, the imprinting effect and selectivity of complex imprinted polymer (CIP) are less affected by polar environments as compared to MIP. By far CIPs are mainly applied as catalyst [21–23], selective separation material [24–26] and recognition element of sensors [27–29].

The features of CIP made it a promising selective binding material in sample preparation [2]. With the help of coordinate effect, CIP could separate analytes selectively from complicated aqueous samples [26]. In recent years, complex imprinted membrane (CIM) has been drawing much attention owing to the advantages of combining CIP with membrane separation technique. CIMs possess features of high selectivity, high specific surface area, accessible imprinted sites, fast mass transfer and short

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binding equilibrium time [30,31]. However, there are still relatively rare reports on this subject. Meanwhile, some applications of CIM were of off-line detection which would lead to more analytical time [32,33]. The development of CIP-based membrane for selective, rapid and on-line analysis is appealing.

Salicylic acid (SA) is widely used as antiseptic, antifungal, keratolytic, analgesic and anti-inflammatory agent in various pharmaceutical preparations [34]. It is also an important active principle of many pharmaceutical products [35]. Besides, SA is a main metabolite of acetylsalicylic acid [36], usually called aspirin (ASP), which is the first drug in the non-steroidal anti-inflammatory class and has been used very widely since 1899 due to its outstanding anti-inflammatory, antipyretic, antiplatelet and analgesic effect [37]. It is therefore significant to develop a fast and selective method to monitor SA in pharmaceutical and biological samples. Several methods have been reported for SA determination, including the "Trinder test" [38], chromatographic methods [39–44], spectrofluorimetry [45,46] and electrochemical analysis [47,48]. But to some extent these works hold disadvantages such as lack of selectivity, time consuming, complicated sample preparation requirement and being laborious or relatively high cost.

One of the most sensitive detection methods for SA is fluorescence. However, many of the fluorescence-based detections of SA would be interfered by substances with similar fluorescent characteristic unless laborious pretreatment was operated. Extra separation technique, such as HPLC, was usually needed for accurate detection, which would lead to more analytical time and cost. Solid-phase fluorescence detection [49,50] is a very convenient and rapid analytical method for direct measurement of analytes on a solid support. There are only limited reports on solid-phase fluorescence-based detection of SA. Some of them offer good sensitivity and fast analysis, but the selectivity of the support employed was expected to be improved [35,51]. The aim of this research was to develop a fast, selective and low cost solid membrane for enrichment and on-line detection of SA. SA reveals good fluorescence enhancement effect on lanthanide ion Tb (III) [52], making terbium-sensitized luminescence based optical monitoring of SA possible. Thus Tb (III) was applied to form complex composition with SA to perform as complex template for preparation of imprinting membrane in this work. The metal Tb (III) bridges the template (analyte) and monomer to form a complex via coordination bond. A glass fiber membrane (GFM), worked as substrate, was functionalized with CIP coating. The solid phase surface emission fluorescence spectra of CIM were measured directly to quantify the complex $Tb(SA)_3$ enriched by the membrane according to the fluorescence intensity at 545 nm attributed to the characteristic emissions of Tb (III) ($^5D_4 \rightarrow {}^7F_5$) [52]. The employment of CIP reduced interferent effect of sample matrix, while real time recording the fluorescence signal could avoid desorption step or off-line detection. The CIM was successfully applied to the analysis of SA in pharmaceuticals and human urine samples. As far as we know, this is the first study on fabricating an optical membrane analytical system by employing CIM as selective binding medium with solid phase surface fluorescence measuring technique. The proposed CIM was endowed with high selectivity, and could recognize SA from complicated matrix.

2. Experimental

2.1. Materials

Glass fiber membrane was from Pall Co. (USA). Salicylic acid and benzoic acid (BA) were analytical grade obtained from Fuchen Chemical Regent Plant (Tianjin, China). 3-hydroxybenzoic acid (3-HBA, 98%), 4-hydroxybenzoic acid (4-HBA, 99%) and aspirin (99%) were supplied by Jingchun Reagent Co. Ltd. (Shanghai, China). Acrylamide (AM) of analytical grade was provided by Xilong Chemical Plant (Guandong, China). Tb_4O_7 (4 N) was from Zhujiang Rare Earth Co. Ltd. (Guangdong, China). 4-vinylpyridine (4-Vpy) was from Sigma-Aldrich (St. Louis, MO, USA). Methacrylic acid (MAA) and azo(bis)-isobutyronitrile (AIBN) were provided by Damao Reagent Plant and both of them are analytical grade (Tianjin, China). Ethylene glycol dimethacrylate (EGDMA) and trimethylolpropane trimethacrylate (TRIM) were from Corel Chemical Plant (Shanghai, China). Other chemicals were of analytical grade. All aqueous solutions were prepared using ultra-pure water obtained from a Millipore purification system in the experiment. The pH value of aqueous solutions was adjusted using hydrochloric acid (HCl) or sodium hydroxide (NaOH) from Damao Regent Plant (Tianjin, China).

2.2. Instruments

Constant temperature shaking bath was from Yiheng Scientific Co. Ltd. (Shanghai, China). A UV3150 spectrophotometer (Shimadzu, Japan) and pH meter (Shanghai Precision Scientific Instruments Co.) were used for UV spectrum and pH measurements, respectively. An S-4300 scanning electron microscope (HITACHI, Japan) was used to investigate the surface morphology of CIP modified membranes. A NICOLET AVATAR 330 Fourier transform infrared (FTIR) spectrometer and a thermal gravity (TG) analyzer (Netzsch-209, Bavaria, Germany) were applied to study the composition and the thermal stability of the coatings, respectively.

The fluorescence spectra were recorded using a RF-5301PC Spectrofluorophotometer (Shimadzu, Japan). CIM was adhered onto the sample disc to record the solid phase surface fluorescence spectra. The fluorescence detections were performed under the following conditions: the slit widths of excitation and emission were both 5 nm, the excitation wavelength was 294 nm with a recording emission range of 220–700 nm, while the fluorescence intensity at 545 nm was chosen for analysis.

2.3. Synthesis of $TbCl_3 \cdot 6H_2O$

50 mL water was heated until boiling under magnetic stirring before 1.5 g Tb_4O_7 was added. Then 1.70 mL concentrated hydrochloric acid was added dropwise and the mixture was stirred and heated until no more Tb_4O_7 could be dissolved. Afterwards, the terbium chloride aqueous solution was filtered. Then the filtrate was heated up to evaporate water and HCl. The solid residue was dehydrated under 90 °C in vacuum to obtain $TbCl_3 \cdot 6H_2O$.

2.4. Preparation of CIM

CIM was prepared by surface modified method with glass fiber membrane as substrate. GFMs were cleaned with ethanol and dried under a stream of nitrogen before use. Fig. 1 presented the preparation and application strategy of CIM. In this work, the high selectivity of CIP and direct solid phase surface fluorescence measurement were combined for determination of target analyte.

CIM was prepared by thermal initiated radical polymerization with GFM as support between two cover glasses under the following polymerization process. 138.0 mg SA, 124.4 mg $TbCl_3 \cdot 6H_2O$ and 0.057 mL MAA were dissolved in 20.0 mL *N*,*N*-dimethyl formamide (DMF). This solution was shaken for 12 h at room temperature to form stable pre-polymer solution before 1.89 mL EGDMA and 37.8 mg AIBN were added. Then the polymerization solutions were mixed thoroughly. A GFM (4.5 cm × 2.0 cm) was soaked in this

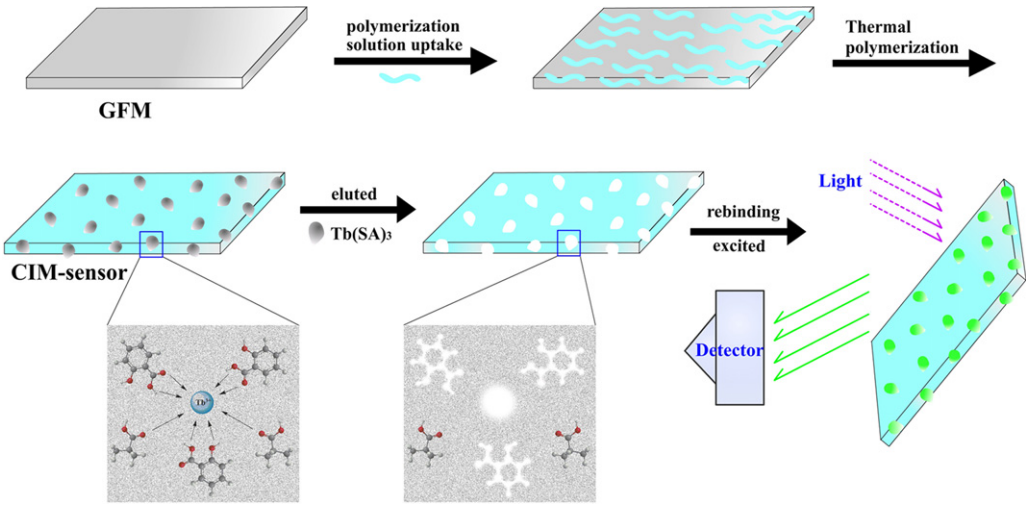

Fig. 1. Schematic fabrication of the preparation and employment of $Tb(SA)_3$ complex imprinted membrane based on solid phase surface fluorescent detection.

polymerization solution for 5 min. Afterwards, the GFM was taken out and deposited between two cover glasses (5.0 cm × 2.4 cm) like a sandwich which was transferred to a weighing bottle. The weighing bottle was deoxygenized with nitrogen for 3 min and sealed before placed in an oven. Successively, radical polymerization was performed thermally at 65 °C for 2 h and then 100 °C for 8 h. Finally the cover glasses were carefully removed. The CIM was washed with methanol/acetic acid (9/1, v/v) and then ethanol to remove the template, residual functional monomer and crosslinker. Molecularly imprinted membrane (MIM) and nonimprinted membrane (NIM) were prepared in the same manner as the CIM without adding $TbCl_3 \cdot 6H_2O$ and both of $TbCl_3 \cdot 6H_2O$ and SA, respectively.

2.5. Fluorescence performance of imprinted membrane

3.00 mL $Tb(SA)_3$ solutions of varying concentrations (count as SA) in ethanol were added to a weighing bottle (40 mm × 25 mm). A piece of membrane of 1.5 cm × 2.0 cm was immersed and incubated under shaking for 10 min. Subsequently, the membrane was taken out and washed with ethanol twice before dried by nitrogen gas. The solid phase fluorescence spectrum of membrane was recorded immediately. The fluorescence response of membrane to SA (I_{SA}) or its analogues ($I_{analogue}$) at each concentration was calculated as the difference between the emission fluorescence intensity at 545 nm after and before recognizing target molecule. After each assay, the disposable membrane was discarded.

2.6. Recognition ability of CIM

The selective recognition ability of CIM for SA was evaluated with BA, 3-HBA, 4-HBA and ASP as structure analogues. Concentration of each single analogue was 2.0 mg/L in ethanol. The selectivity factor (k) of CIM for SA was obtained from fluorescence response according to $k = I_{SA}/I_{analogue}$. Selectivity test was also performed on both MIM and NIM for comparison.

2.7. Determination of salicylic acid in pharmaceuticals and human urine

The analytical performance of the proposed CIM was tested with emplastrum, liniment and human urine samples.

For the analysis of pharmaceuticals, a suitable volume or weight of the samples was dissolved directly in ethanol with sonication and then filtered through a 0.45 μm nylon membrane. The filtrate was transferred to a volumetric flask with terbium chloride ethanol solution. Appropriate dilution was performed in order to make the concentrations of $Tb(SA)_3$ meet the linear range of the proposed method.

Human urine was collected from a healthy, fasting, drug-free volunteer in the morning. 10.0 mL human urine was adjusted to pH 2.0 by 1.0 mol/L HCl and extracted with 20.0 mL dichloromethane each time for three times. The combined extracts were evaporated to dryness under vacuum with a rotary evaporator. The residue was dissolved in 10.0 mL terbium chloride ethanol solution and then filtered by 0.45 μm nylon membrane.

3. Results and discussion

3.1. Characterization studies

3.1.1. FTIR analysis

FTIR spectra of CIM, MIM and NIM were compared in Fig. S1. The absorption bands corresponded to O–H stretching vibration at 3436 cm^{-1}, stretching vibration of C–H bond on MAA at 2987 cm^{-1} and 2955 cm^{-1}, stretching vibration of C=O bonds on carbonyl groups of MAA and EGDMA at 1730 cm^{-1}, stretching vibration of C=C bond at 1660 cm^{-1} and 1462 cm^{-1} and bending vibration of C–H bond on methyl group at 1389 cm^{-1}. The CIM, MIM and NIM showed almost the same profile of major bands because of their similar main compositions.

3.1.2. Morphological characterization

Fig. S2 showed the scanning electron micrographs (SEMs) of GFM, CIM, MIM and NIM under the magnification of 10,000. It was obvious that CIM (Fig. S2(b)) possessed stacked dense and porous surface which was distinct from GFM. This proved that CIP was immobilized on GFM after copolymerization. The pore structures would increase specific surface area and benefit mass transfer of CIM. MIM (Fig. S2(c)) and NIM (Fig. S2(d)) had similar surface structure with CIM.

3.1.3. Binding properties of the imprinted membranes

Lanthanide complexes with aromatic carboxylic acid as ligand are attractive luminescent materials [52]. $Tb(SA)_3$ exhibits strong luminescence corresponding to the intramolecular energy transfer from the coordinated SA to the central terbium ion under excited energy [53]. SA can absorb the excited energy of external light source, and then the energy is efficiently transferred to the

excited state of Tb (III), following with fluorescence emission from the level $^5D_4$ of Tb (III) [54]. This phenomenon was utilized in the analysis of SA or its derivatives in this study.

In order to investigate the $Tb(SA)_3$ binding ability of CIM, the three polymer membranes, CIM, MIM and NIM were immerged in $Tb(SA)_3$ standard solutions for 10 min under shaking. Then the membranes were taken out and solid phase surface fluorescent was measured immediately. The obtained fluorescence spectra of CIM response to a series of $Tb(SA)_3$ solutions from 0.10 to 50 mg/L were shown in Fig. S3. It showed that the fluorescence intensity grew gradually with the increase of $Tb(SA)_3$. For comparison the fluorescence intensities at peak 545 nm of the three membranes were plotted against the concentrations of $Tb(SA)_3$ (Fig. 2). As can be observed, the fluorescence response of CIM to $Tb(SA)_3$ was higher than that of other two membranes at all concentrations. This suggest that CIM has better affinity towards $Tb(SA)_3$ compared with MIM and NIM. The maximum fluorescence response of CIM was 2.3 and 3.2 times as much as that of MIM and NIM respectively, which indicated that the complex imprinting process could enhance detection sensitivity of the CIP-based membrane in polar environment. In the case of MIM, the fluorescence response intensity was closed to that of NIM. This was because the imprinting effect of MIP was based on hydrogen bond between the template molecule and the functional monomer. Solvent with high polarity and intramolecular hydrogen bond of SA would disturb or even destroy the hydrogen bond based interaction, leading to low binding ability of MIM towards SA [37,55].

The effect of the storage time on the repeatability of the CIM was investigated, and results showed that the fluorescence response was not changed with RSD less than 11%, indicating the acceptable duration ability of the membrane.

3.2. Analytical characterization of CIM

3.2.1. Effect of sample solvent

The effect of solvent of $Tb(SA)_3$ on fluorescence response of CIM was assessed with water, DMF, dimethyl sulfoxide (DMSO), acetonitrile, ethanol, dichloromethane and n-hexane. CIM was immerged in standard solutions for 20 min at constant shaking frequency of 100 Hz. The results (Fig. S4) illustrated that ethanol lead to the strongest fluorescence intensity. This might because that CIM recognized complex $Tb(SA)_3$ rather than single SA. Tb (III) and SA can form $Tb(SA)_3$ effectively in ethanol [56], thus CIM had the best affinity towards SA in this solvent. Further test showed that there was no considerable improvement by changing the pH of solution from 1.0 to 12.0. According to these results, ethanol was selected for further experiments.

3.2.2. Effect of shaking frequency

In order to study the effect of shaking frequency, fluorescence response of CIM to $Tb(SA)_3$ at different shaking speeds was investigated. Results in Fig. S5 show that fluorescence intensity of CIM grew along with the shaking frequency resulting from faster mass transfer which caused more $Tb(SA)_3$ bound to the surface of CIM. However, CIM would be damaged when shaking frequency was more than 100 Hz. Therefore, 100 Hz was chosen for the following experiment.

3.2.3. Dynamic response of CIM

To evaluate the dynamic response of CIM, the corresponding fluorescence responses with the variation of adsorption time at different concentrations of $Tb(SA)_3$ were recorded and shown in Fig. 3. As could be seen, fluorescence intensities increased gradually with the increase of bound amount of $Tb(SA)_3$ on membrane along with adsorption time. In the case of 0.20 mg/L, the fluorescence signal tended to be stable within 5 min, while 7 min was enough for 0.50 and 2.0 mg/L. Under concentration of 10 mg/L, the equilibrium time was 10 min. The high specific surface area of the proposed membrane benefits mass transfer, therefore the diffusion of $Tb(SA)_3$ from solution to membrane surface was faster during adsorption process. Meanwhile, the imprinted cavities on CIP film have high affinity towards $Tb(SA)_3$, which also contributes to the rapid equilibrium. In order to ensure high and stable response, 10 min was selected throughout the experiments. This adsorption time was superior to other reported studies based on SA imprinted MIPs, such as MIP particles [57] and MIP membranes [36].

3.2.4. Selectivity study

The chemical structures of and the comparative fluorescence response of CIM, MIM and NIM to SA and its analogues were shown in Fig. 4(a) and (b), respectively. Obviously the response of CIM to SA was much higher than the responses to the other four analogues, indicating that CIM had selective recognition ability towards the target molecule. The selectivity of CIM can be attributed to the recognition sites for $Tb(SA)_3$ immobilized on the CIP film. The selective cavity acted effectively and its

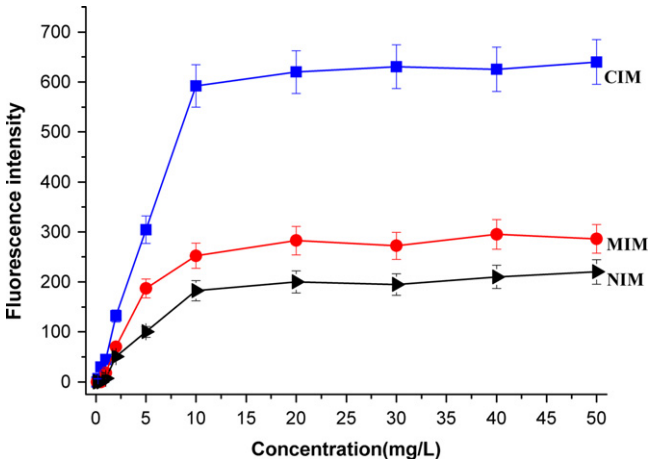

Fig. 2. Fluorescent response curves of CIM, MIM and NIM to $Tb(SA)_3$ under different concentrations.

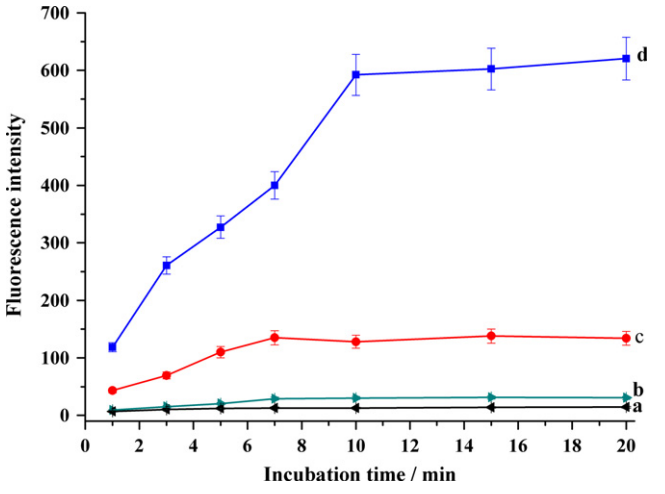

Fig. 3. Dynamic response curves of CIM to $Tb(SA)_3$ under different concentrations. (a) 0.20 mg/L, (b) 0.50 mg/L, (c) 2.0 mg/L, (d) 10 mg/L.

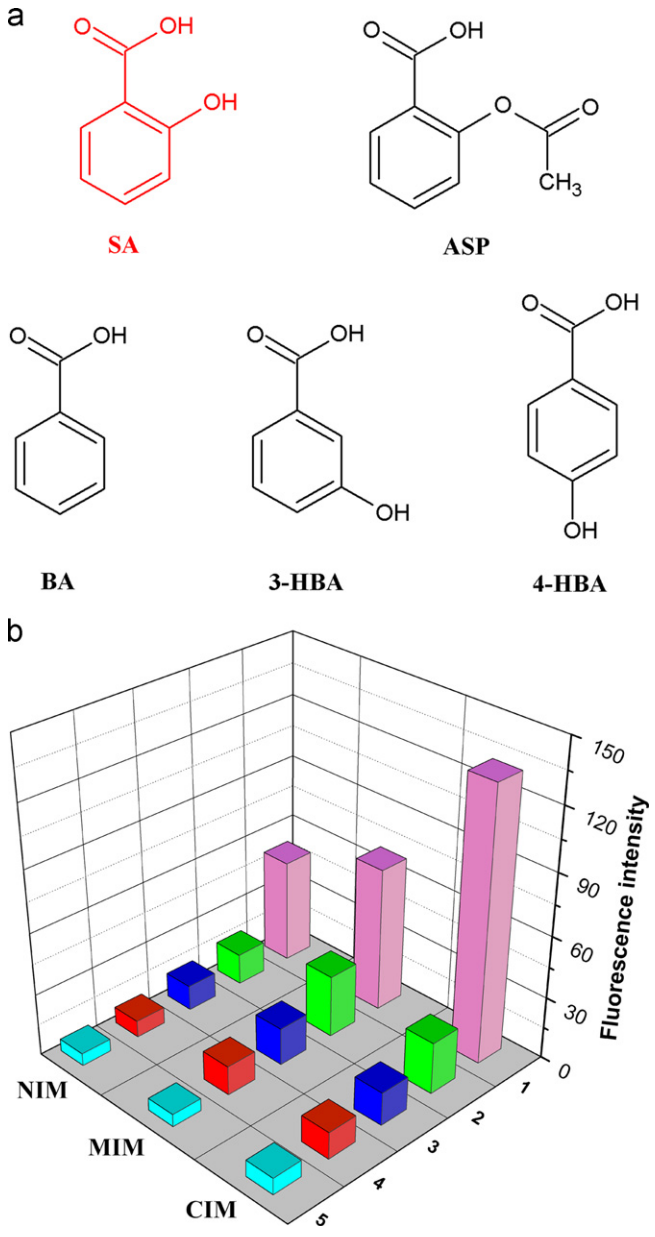

Fig. 4. (a) Molecular structures of SA and its analogues. (b) Fluorescent response of CIM, MIM and NIM to SA and its analogues. (1) SA, (2) ASP, (3) BA, (4) 3-HBA, (5) 4-HBA. Concentration: 2.0 mg/L [$TbCl_3$-ligand], adsorption time: 10 min.

recognition ability to the analogues of SA was related to their structures. ASP is the acetylated form of SA, and BA has one less OH- compared with SA. As a result, the size and shape of their complex with Tb (III) were unmatchable to the recognition sites. 3-HBA and 4-HBA are isomers of SA, but their space structures are different from SA. Consequently the response of CIM to these analogues was relatively lower. The results confirmed that CIM had discrimination and selective binding capability towards target species.

Table 1 illustrates the selectivity factors (k) of CIM and the two control membranes towards analogues of SA in the presence of Tb (III). It can be observed that CIM exhibited better selectivity than MIM and NIM derived from the improvement effect of Tb (III). On the other hand, MIM showed similar selectivity with NIM due to the instability of hydrogen bond in polar environment. The two control membranes adsorbed target analytes mainly through nonspecific interaction.

Table 1
Selectivity factors (k) of CIM, MIM and NIM for SA[a] to its analogues ($n=3$).

Analogue	CIM	RSD/%	MIM	RSD/%	NIM	RSD/%
BA	8.2	7.7	3.9	9.8	4.2	8.6
3-HBA	10.2	8.7	5.0	7.9	6.3	9.4
4-HBA	16.5	7.2	11.7	9.2	8.4	7.7
ASP	5.3	7.1	2.3	8.7	3.4	10.2

[a] Concentration of SA or its analogue is 2.0 mg/L.

3.3. Fast determination of salicylic acid in pharmaceuticals and human urine

Based on the optimized experimental parameters, a calibration curve for determination of SA was established to evaluate the analytical performance of CIM. The proposed membrane showed a linear relationship on response to SA from 0.20 to 10.0 mg/L. The linear regression equation was $Y=55.76X+7.08$ ($R=0.9944$) where Y is the fluorescence intensity and X is the concentration of SA. Higher sensitivity was obtained as compared with the optical fiber sensor based on immobilization of Fe (III) on resin to form purple complex with SA, which showed dynamic response range from 20–500 mg/L [35]. The limit of detection (LOD) was 0.040 mg/L ($S/N=3$), which is comparable with a published report (0.045 mg/L), which combines sequential injection analysis and solid phase Tb-sensitized luminescence as a detection technique [58]. Despite the fact that LOD of this study was higher than a previous work based on solid phase fluorescence [51], the selectivity of the proposed CIM was greatly improved, which facilitate its application to complicated sample matrix. For repeatability test, CIMs of both intra- and inter-batches were investigated with a $Tb(SA)_3$ solution of 1.0 mg/L. The relative standard deviations (RSDs) of 9.1% (same batch, $n=9$) and 11.2% (batch to batch, $n=3$) were obtained.

The proposed solid phase surface fluorescence-based membrane method was applied to the determination of SA in pharmaceuticals and human urine to investigate its reliability. The results were compared with that obtained by high performance liquid chromatography (HPLC) (Table 2). As could be seen, the recoveries of sample analysis were from 80.6% to 88.1% with RSDs ranging from 9.0% to 13.2%. It was shown that the analytical results of the proposed method were in good agreement with those obtained by HPLC method, indicating that the developed membrane has acceptable practicability for fast determination of SA in real samples.

4. Conclusion

This work integrated the high selectivity of CIP and the strong fluorescence property of $Tb(SA)_3$. A CIP coated membrane based on solid phase surface fluorescence measurement was successfully prepared for fast, direct and selective enrichment and detection of SA. Owing to the improvement effect of Tb (III), CIM showed higher sensitivity and better selective recognition ability than the corresponding MIM and NIM in high polar environment. The proposed membrane showed a linear fluorescence respond to SA ranging from 0.20 to 10.0 mg/L with LOD of 0.040 mg/L. The proposed method was applied to real samples with satisfactory results. Our future work will focus on improving the precision of this method. The simple and flexible methodology proposed in this work possessed potential to fabricate a series of membrane separation materials for selective recognition of target analytes. In comparison with the widely employed MIP-based analytical methods, this technique is still in its infancy.

Table 2
Determination of SA in pharmaceuticals and human urine samples (n=3).

Sample	The proposed membrane				HPLC		Nominal
	Found	Spiked	Recovery/%	RSD/%	Found	RSD/%	
Emplastrum	0.66 g/g	0.78 g/g	82.5	13.2	0.70 g/g	5.0%	0.78 g/g
Liniment	41.0 g/L	44.0 g/L	88.1	9.0	41.8 g/L	4.9%	44.0 g/L
Human urine	ND[a]	1.0 mg/L	80.6	11.2	ND		[b]
		5.0 mg/L	81.8	9.5			

[a] Not detected.
[b] No this subject.

However, this approach will gain more and more interest in the near future due to the virtues of CIP.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2012.12.054.

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