Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Available online at www.sciencedirect.com
SCIENCE DIRECT®
Analytica Chimica Acta 513 (2004) 385–392
ANALYTICA CHIMICA ACTA
www.elsevier.com/locate/aca

Optical sensor for berberine utilizing its intrinsic fluorescence enhanced by the formation of inclusion complex with butylated-β-cyclodextrin

Yu Yang [a], Xin Yang [b], Chen-Xu Jiao [a], Hai-Feng Yang [a], Zhi-Min Liu [a], Guo-Li Shen [a], Ru-Qin Yu [a,*]

[a] *State Key Laboratory of Chemo/Biosensing and Chemometrics, College of Chemistry and Chemical Engineering, Hunan University, Changsha 410082, PR China*
[b] *Beijing Institute of Pharmacology and Toxicology, Beijing 100850, China*

Received 3 September 2003; received in revised form 31 December 2003; accepted 16 January 2004

Abstract

An optical sensor for berberine, the basic ingredient of the widely used traditional Chinese medicine *Coptis Chinensis*, based on its intrinsic fluorescence enhanced by butylated-β-cyclodextrin (HDB-β-CD) immobilized in plasticized poly(vinyl chloride) (PVC) membrane has been developed. The drastic enhancement of fluorescence intensity of berberine was attributed to the formation of an inclusion complex between HDB-β-CD and berberine, which has been utilized as the basis of the fabrication of a berberine-sensitive fluorescence sensor. The proposed sensor was quite distinct from those fluorescent sensors for berberine reported so far which relied upon quenching the fluorescence of the sensing reagent immobilized on membrane by berberine. The response mechanism of optode membrane was discussed in detail from the view of molecular dynamics and the optimum steric configuration of the inclusion complex was presented by molecular dynamics simulation. The analytical performance characteristics of the proposed berberine-sensitive sensor were investigated. The sensor can be applied to the quantification of berberine with a linear range covering from 4.0×10^{-7} to 2.0×10^{-5} mol l^{-1} with a detection limit of 8.0×10^{-8} mol l^{-1}. The sensor exhibits excellent reproducibility, reversibility and selectivity. The recommended method was successfully used for the determination of berberine in pharmaceutical preparations.

Keywords: Optical sensor; Butylated-β-cyclodextrin; Berberine; Molecular dynamics

1. Introduction

Fluorescent sensors are advantageous to analyze and detect various organic and biological molecules due to their high sensitivity and selectivity [1–4]. Meanwhile, supramolecular transducing systems have attracted enormous research interest in the development of chemical sensors in recent years [5]. A variety of receptor molecules, such as cyclodextrin (CD) [6,7], crown ethers [8], calixarenes [9], and porphyrin [10], as typical host compounds, can selectively recognize organic species. Among them, the naturally occurring chiral α-, β- and γ-cyclodextrin are well known as simple model receptors which have the remarkable property of selectively including organic, inorganic and biological molecules in their toroidal cavity to form stable host-guest inclusion complexes [11]. The formation of an inclusion complex is often able to effect enhancements or perturbations of the photophysical and photochemical properties of included guest molecules [12]. Several weak intermolecular force between host and guest, such as dipole–dipole, hydrophobic, electrostatic, van der Waals, and hydrogen-bonding interaction, cooperatively contribute to the molecular recognition process [13].

Berberine, a quaternary ammonium salt, is the basic active ingredient of a widely used traditional Chinese medicine *Coptis Chinensis* with antibacterial and anticonvulsant activities commonly used for the treatment of diseases such as bacillary dysentery, lobar pneumonia and pertussis. Particularly, berberine has been used in Chinese hospitals for the auxiliary treatment of severe acute respiratory syndrome (SARS) to reduce its mortality rate. Some analytical methods have been developed for the quantification of berber-

* Corresponding author. Tel.: +86-731-882-2577; fax: +86-731-882-2782.
E-mail address: rqyu@hnu.net.cn (R.-Q. Yu).

0003-2670/$ – see front matter © 2004 Published by Elsevier B.V.
doi:10.1016/j.aca.2004.01.022

ine. These methods include the use of liquid chromatography [14,15], capillary electrophoresis [16], electrochemical analysis [17–19], fluorophotometry [20–22], chemiluminescence [23]. Among the fluorescence methods reported so far for berberine assay, the sensors or analytical methods designed were all based on quenching the fluorescence of the sensing reagent immobilized on membrane by berberine. Actually, berberine possesses its unique intrinsic fluorescence property as manifested in the use of berberine as a probe for fluorescence assay of DNA and RNA [24,25]. It is very interesting to explore the potential of utilizing the intrinsic fluorescence property of berberine for its assay. Berberine only manifests very weak fluorescence property in aqueous solutions without addition of sensitizing agents such as DNA or RNA. Though the role of DNA or RNA in sensitizing the fluorescence of berberine has not been discussed in the literatures [24,25], it seems that DNA or RNA provides a protective microenvironment which would decrease the opportunity of berberine colliding with molecules existed in the bulk solution. In the search of an appropriate environment suitable for the intense enhancement of fluorescence intensity of berberine, we observed that the addition of β-CD could enhance the fluorescence intensity of berberine in aqueous solutions more remarkably comparing with DNA or RNA. The possible reason is that β-CD can provide a more protective hydrophobic microenvironment to accommodate berberine than DNA or RNA. The enhanced fluorescence sensitivity obtainable by the use of β-CD is, unfortunately, still insufficient for designing a practically usable berberine assay methodology. To our knowledge, no fluorescent sensor for berberine based on its characteristic fluorescence enhancement was reported in the literature.

In this paper, we tried to synthesize a lipophilic cyclodextrin, heptakis (2,6-di-*o*-*n*-butyl)-β-cyclodextrin (HDB-β-CD) and used it as sensing element in a hydrophobic membrane of poly(vinyl chloride) (PVC) matrix. It turned out that the fluorescence intensity of berberine in the membrane drastically enhanced which was obviously associated with the formation of an inclusion complex between HDB-β-CD and berberine. This has been utilized as the basis of the preparation of berberine-sensitive fluorescent sensor. This paper reports the preparation and analytical performance characteristics of the proposed berberine-sensitive sensor. The sensor can be used for the determination of berberine with a linear range covering from 4.0×10^{-7} to 2.0×10^{-5} mol l^{-1} with a detection limit of 8.0×10^{-8} mol l^{-1}. Also, the response mechanism of the optode membrane was investigated. Furthermore, molecular dynamics computation and computer simulation technique were combined to predict the steric configuration of the inclusion complex. An optimum conformation of inclusion complex was presented, which would serve as an aid for better understanding the recognition mechanism controlled by the simultaneous operation of several weak interactions and the factors governing inclusion phenomena of guest molecules by host CDs.

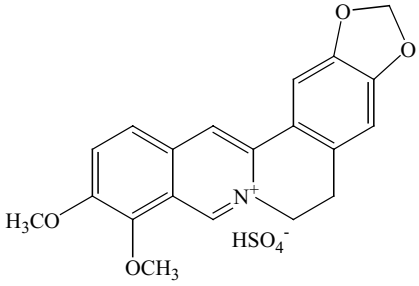

Fig. 1. The chemical structure of berberine hemisulfate salt.

2. Experimental

2.1. Reagents

High molecular weight PVC, diisooctyl sebacate (DOS) and tetrahydrofuran (THF) were purchased from Shanghai Chemical Reagents (Shanghai). β-Cyclodextrin (β-CD) was provided by Beijing Aoboxing Biochemical Reagents (Beijing). HDB-β-CD was prepared and purified by method reported by Bates et al. [26]. Berberine hemisulfate salt (see Fig. 1) was obtained from ICN Biomedicals (Aurora, OH, USA). All reagents were of analytical reagent grade. Doubly distilled water was used throughout.

2.2. Apparatus

All fluorescence measurements were performed with a Hitachi F-4500 fluorescence spectrometer. Excitation and emission slits were set at 5.0 and 10.0 nm, respectively. All experiments were carried out at 20 °C. A laboratory-made flow cell (shown in Fig. 2) was used for the berberine-sensing measurements.

2.3. Membrane fabrication

The optode membrane solution was prepared by dissolving a mixture of 8.0 mg of HDB-β-CD, 80 mg of PVC,

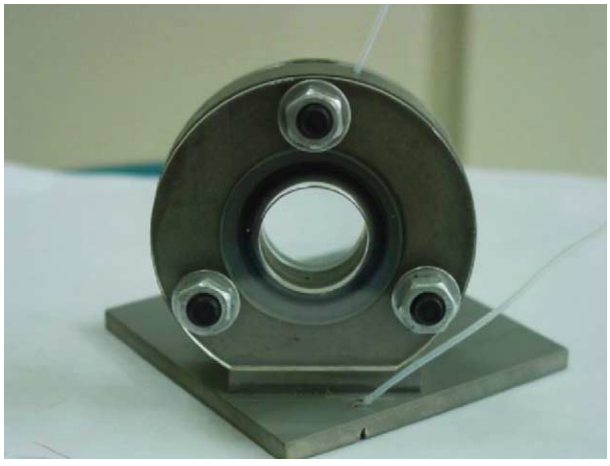

Fig. 2. Picture of the flow cell.

160 mg of DOS in 2.0 ml of freshly distilled THF. A circular 35 mm diameter quartz plate was fixed on the end of an aluminum alloy rod and then rotated at a frequency of 600 rpm. With a syringe, 0.2 ml of the membrane solution was injected on the center of the plate. After spinning for 5.0 s, a membrane of approximately 4.0 μm thickness was then coated onto the quartz plate and dried in ambient air for 30 min prior to use [27]. The concentration of HDB-β-CD in PVC membrane was about 2.0×10^{-3} mol l^{-1}.

2.4. Measurement procedure

The prepared PVC membrane was installed in a home-made flow cell with about 3.4 ml volume capacity. The cell was mounted into the fluorescence spectrometer in a fixed position to ensure the detection of fluorescence emission intensity without interference from the excitation light source [28–30]. The fluorescence intensity was measured at the maximal excitation wavelength of 355 nm and the maximal emission wavelength of 513 nm. The berberine solution was introduced into the flow cell by a peristaltic pump. After each measurement, the membrane was washed with phosphate buffer solution (pH 3.0) until its fluorescence intensity reached the initial blank value.

A standard stock solution of 1.0×10^{-3} mol l^{-1} berberine was prepared. The working solutions were obtained by serial dilutions of this stock solution with phosphate buffer solution (pH = 12.0).

Sample solutions were prepared by directly dissolving an appropriate amount of tablets containing berberine in distilled water without any pretreatment and then diluting to predefined volume. The resulting solution was filtered and the filtrate was collected with the original portion discarded and used for analytical determination.

3. Results and discussion

3.1. The fluorescence spectrum of the sensing membrane

Fig. 3 shows the fluorescence excitation and emission spectra of the sensing membrane exposed to the solution containing different concentration of berberine. Berberine itself exhibits very weak fluorescence emission in aqueous solution and PVC membrane without HDB-β-CD. (The recorded signal almost coincides with the abscissa not shown in Fig. 3.) Noticeable fluorescence excitation and emission peaks of berberine appear in the presence of HDB-β-CD, which is attributed to the formation of inclusion complex between HDB-β-CD and berberine. Moreover, the fluorescence intensity of optode membrane is gradually enhanced with increasing berberine concentration, which has been used as the basis of the berberine sensor design. The degree of fluorescence enhancement of berberine was determined with the fluorescence sensitivity factor f ($f = (F - F_0)/F_0$. F and F_0 are fluorescence intensities of berberine in the presence and absence of HDB-β-CD, respectively.). The fluorescence sensitivity factor in this paper was 22.

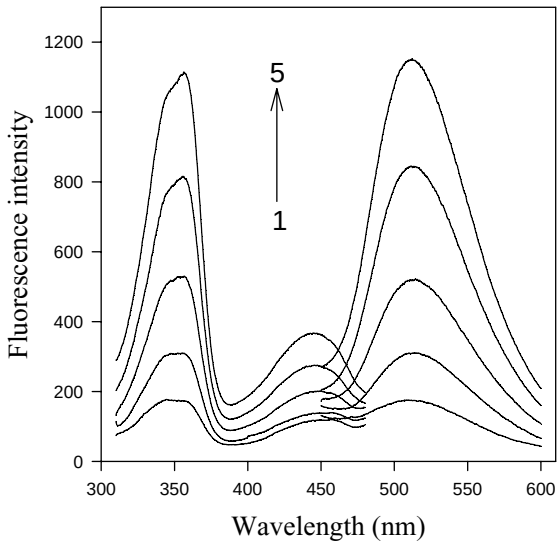

Fig. 3. The fluorescence excitation and emission spectra of optode membrane in the presence of different concentration of berberine. The concentration of berberine: (1) 4.0×10^{-7} mol l^{-1}; (2) 8.0×10^{-7} mol l^{-1}; (3) 2.0×10^{-6} mol l^{-1}; (4) 4.0×10^{-6} mol l^{-1}; (5) 6.0×10^{-6} mol l^{-1}.

In order to confirm the existence of inclusion phenomenon between HDB-β-CD and berberine, the titration experiments with the HDB-β-CD in solution have been performed. As can be seen from Fig. 4, berberine itself exhibited very weak fluorescence emission in aqueous solution. Addition of HDB-β-CD to an aqueous solution of berberine resulted in a remarkable variety of berberine fluorescence signal. The fluorescence intensity of berberine was gradually enhanced

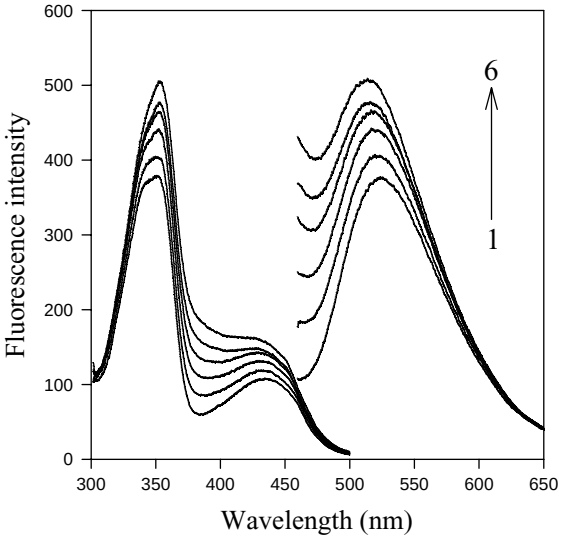

Fig. 4. The fluorescence excitation and emission spectra of berberine (4.0×10^{-6} mol l^{-1}) in the presence of different concentration of HDB-β-CD.The concentration of HDB-β-CD: (1) 1.0×10^{-3} mol l^{-1}; (2) 2.0×10^{-3} mol l^{-1}; (3) 3.0×10^{-3} mol l^{-1}; (4) 4.0×10^{-3} mol l^{-1}; (5) 5.0×10^{-3} mol l^{-1}; (6) 6.0×10^{-3} mol l^{-1}.

with increasing HDB-β-CD concentration. Moreover, the maximum excitation wavelengths of berberine shifted towards longer wavelengths, while the maximum emission wavelengths shifted towards shorter wavelengths. These results provided another proof for the formation of an inclusion complex of berberine with HDB-β-CD.

3.2. Effect of pH

The effect of pH values on the fluorescence intensity of optode membrane was examined with the concentration of berberine fixed at 4.0×10^{-6} mol l^{-1}. Berberine is a quaternary ammonium salt, so its fluorescence emission intensity should be irrelative with pH. This is really the case when berberine reacts with β-CD in aqueous phase (as shown in Fig. 5). On the organic membrane phase, the situation seems to be much more complicated. Apparently, from the fluorescence intensity curve recorded with HDB-β-CD containing membrane phase after contacting with berberine solution of different pH for 30 min, with the increase of pH values, the fluorescence intensity of optode membrane increases and inclines to level off after pH 12.0. The difference in two systems seems to be related with the fact that for the organic membrane phase, a process of the transfer of berberine from aqueous phase into the organic membrane phase is involved. The inclusion equilibrium of berberine and β-CD in aqueous solution was a fast homogeneous reaction for a few minutes. While, The inclusion equilibrium of berberine in aqueous solution and HDB-β-CD in membrane phase was a very slow heterogeneous reaction, since berberine in aqueous solution need to diffuse to permeate into organic membrane phase to contact with HDB-β-CD. Also, under pH ≥ 11.0, the hydroxyl groups of HDB-β-CD would deprotonate, and then hydrophobic and electrostatic force would jointly contribute to the inclusion interaction between HDB-β-CD and berberine, which would result in the increase of extraction efficiency of berberine by HDB-β-CD in optode membrane. Experimental observations showed under pH ≥ 12.0, the fluorescence intensity reached the maximum value within about 5 min which was far less than the time required under lower pH values. The change of fluorescence intensity under lower pH values was a slow process that the increase of fluorescence intensity was quite noticeable during the first 30 min and then slowed down and could not reach the maximum value even after several hours. From the point of view of sensitivity and response speed of sensor, pH 12.0 was chosen as optimum experimental condition where the maximum fluorescence intensity could be reached after 5 min contact of the membrane with berberine solution.

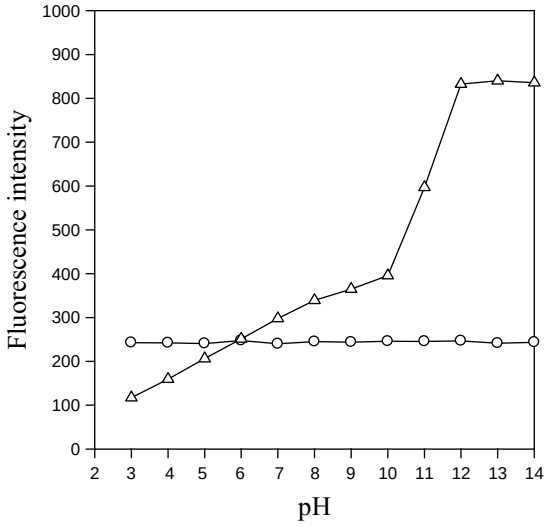

Fig. 5. Effect of pH on the fluorescence intensity in two phases of: (△) HDB-β-CD optode membrane recorded after contacting berberine solution (4.0×10^{-6} mol l^{-1}) of different pH for 30 min (see the text for details); (○) aqueous solution containing β-CD (3.0×10^{-3} mol l^{-1}) and berberine (4.0×10^{-6} mol l^{-1}) recorded instantly after solution preparation.

3.3. Theoretical principle of optode membrane determination

According to the references [31,32], the theoretical principle of optode membrane determination was proposed.

If the inclusion equilibrium between berberine in the aqueous solution (aq) and HDB-β-CD in the plasticized PVC membrane phase (org) will form a *m*:*n* complex, the overall equilibrium can be represented as follows:

$$m\mathrm{A}_{(\mathrm{aq})} \overset{k_\mathrm{d}}{\Leftrightarrow} m\mathrm{A}_{(\mathrm{org})} \tag{1}$$

$$m\mathrm{A}_{(\mathrm{org})} + n\mathrm{B}_{(\mathrm{org})} \overset{\beta}{\Leftrightarrow} A_m B_{n(\mathrm{org})} \tag{2}$$

i.e.

$$m\mathrm{A}_{(\mathrm{aq})} + n\mathrm{B}_{(\mathrm{org})} \overset{K}{\Leftrightarrow} A_m B_{n(\mathrm{org})} \tag{3}$$

where A represents berberine, B denotes HDB-β-CD. When the difference between the activity and concentration is neglected for simplification, the corresponding equilibrium constant K can be expressed by the law of mass action:

$$K = k_\mathrm{d} \times \beta = \frac{[\mathrm{A}_m\mathrm{B}_n]_{(\mathrm{org})}}{[\mathrm{A}]^m_{(\mathrm{aq})}[\mathrm{B}]^n_{(\mathrm{org})}} \tag{4}$$

where k_d is the distribution coefficient of berberine between the organic and aqueous phases, β is complex formation constant, respectively, and $[\mathrm{A}_m\mathrm{B}_n]$, [A], [B] are the concentrations of respective species.

When the concentration of berberine and HDB-β-CD in membrane are low, the observed fluorescence intensity of the membrane is a sum of several contributions.

$$F_0 = K_\mathrm{B} C_{\mathrm{B}(\mathrm{org})} \tag{5}$$

$$F = K_\mathrm{A}[\mathrm{A}]_{(\mathrm{org})} + K_\mathrm{B}[\mathrm{B}]_{(\mathrm{org})} + K_{\mathrm{A}_m\mathrm{B}_n}[A_m B_n]_{(\mathrm{org})} \tag{6}$$

where F_0 is the fluorescence intensity of the sensing membrane when it is applied to blank solution, F the fluorescence intensity of the sensing membrane when it is applied to berberine solution, $C_{\mathrm{B}(\mathrm{org})}$ the total concentration of

HDB-β-CD in the membrane, and the K_i values represent the individual proportion constants and include instrumental parameters as well as the quantum efficiency of the complex species.

To indicate the degree of association between HDB-β-CD and berberine in the optode membrane, a response parameter, α, needs to be introduced and defined as follows:

$$\alpha = \frac{C_{B(org)} - [B]_{(org)}}{C_{B(org)}} = \frac{n[A_mB_n]_{(org)}}{C_{B(org)}} \quad (7)$$

It can be derived from Eqs. (5) to (7) that:

$$\alpha = \frac{F - F_0}{F_1 - F_0} \quad (8)$$

where F_1 is the fluorescence intensity at membrane saturation.

Combining Eqs. (4) and (7), one can obtain the following quantitative equation:

$$\frac{\alpha^n}{1-\alpha} = \frac{1}{nKC_B^{n-1}[A]^m} \quad (9)$$

The relationship between α and [A] as expressed by Eq. (9) is the basis of the quantitative determination of berberine using this optode membrane.

3.4. Response characteristics of the optical chemical sensor

The reproducibility and reversibility of the optode membrane in the determination of berberine were evaluated by repetitively exposing the optode membrane to different concentration berberine solution and phosphate buffer solution (pH 12.0). The standard deviations of different sample solutions were found to be 2.95 (6.0×10^{-7} mol l^{-1}), 3.40 (2.0×10^{-6} mol l^{-1}), and 4.02 (1.0×10^{-5} mol l^{-1}). The standard deviation of blank solution was 2.03 for 12 determinations.

The berberine complexed with HDB-β-CD could be eluted out of the CD cavity quickly and completely, which demonstrated the excellent reproducibility and reversibility of the sensor.

As for the response time of the optode membrane, it depended on the thickness of membrane. In order to obtain relatively thin membrane, the spin-on device was used, which allowed the production of very thin, homogeneous and reproducible PVC-based membrane. Besides, the response time also depended on the berberine concentration. It was noted that the time required to reach the equilibrium prolonged with increasing berberine concentration, the stable fluorescence intensity was obtained over a period of 5 min.

For the optode membrane in contact with a 8.0×10^{-7} mol l^{-1} berberine solution, the fluorescence intensity at 513 nm was recorded over a period of 8 h. The fluorescence intensity was recorded with a 30 min interval, a standard deviation of 2.33 was obtained. After a series of 200 times measurements of berberine solution, the fluorescence intensity value of the sensor did not change.

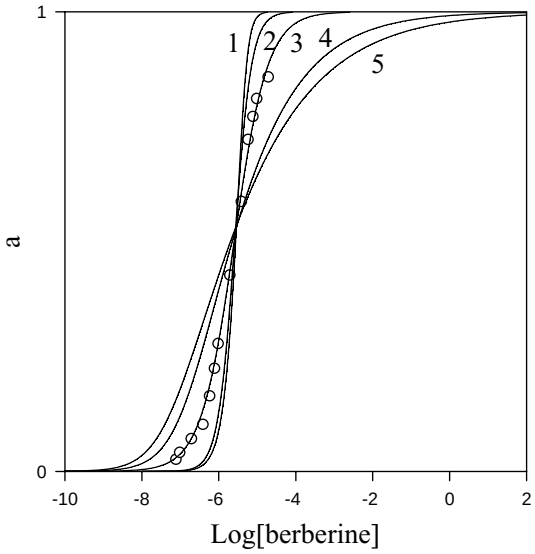

Fig. 6. Relative fluorescence intensity α as a function of log[berberine]. The curves fitting the experimental data were calculated from Eq. (4). (1) $m{:}n = 4:1$, $K = 6.0 \times 10^9$; (2) $m{:}n = 2:1$, $K = 1.5 \times 10^{11}$; (3) $m{:}n = 1:1$, $K = 4.0 \times 10^5$; (4) $m{:}n = 1:3$, $K = 6.0 \times 10^{11}$; (5) $m{:}n = 1:4$, $K = 1.0 \times 10^{15}$. (○) Data points experimentally obtained.

From Eq. (9), it can be seen that when the stoichiometric ratio of complex changes, the relative fluorescence intensity value α with the various concentration of berberine exhibits different functional relationships. According to Eq. (9), the experimental data were fitted by altering the ratio of m to n and the equilibrium constant K. Fig. 6 shows the fitted curve for the determination of berberine. The curve of the 1:1 complex ratio and $K = 4.0 \times 10^5$ l mol^{-1} was in agreement with the experimental data, which can serve as the calibration curve. A linear range for berberine covered from 4.0×10^{-7} to 2.0×10^{-5} mol l^{-1}. The detection limit, defined according to 3σ concept, was 8.0×10^{-8} mol l^{-1}.

3.5. Selectivity

Some ions and species commonly existing in biological samples were chosen for the study on selectivity of the berberine 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 1.0×10^{-6} mol l^{-1} berberine. The results presented in Table 1 reveal that following species caused no interference when existed in specified molar excesses of 1000 times for Na^+, K^+, NH_4^+, Cl^-, CH_3COO^-, SO_4^{2-}, NO_2^-, citrate, carbonate, ascorbic acid, salicylate, saccharose, glucose, lactose, maltose, fructose, 100 times for Ca^{2+}, 10 times for Mg^{2+}.

3.6. The response mechanism of the optode membrane

Berberine itself exhibits very weak fluorescence emission in aqueous solutions. However, its fluorescence intensity

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

Interferent	Concentration[a] ($mol\,l^{-1}$)	Fluorescence intensity ($\Delta F = F_i - F_{i0}$)[b]	Relative error (%) ($\Delta F/F_{i0}$) × 100
Na^+	1.0×10^{-3}	3.7	0.81
K^+	1.0×10^{-3}	5.1	1.11
NH_4^+	1.0×10^{-3}	−2.1	−0.46
Ca^{2+}	1.0×10^{-4}	−4.7	−1.02
Mg^{2+}	1.0×10^{-5}	2.9	0.63
Cl^-	1.0×10^{-3}	4.0	0.87
SO_4^{2-}	1.0×10^{-3}	4.8	1.05
CH_3COO^-	1.0×10^{-3}	5.9	1.28
NO_2^-	1.0×10^{-3}	−1.2	−0.26
Citrate	1.0×10^{-3}	−2.3	−0.50
Carbonate	1.0×10^{-3}	1.2	0.26
Ascorbic acid	1.0×10^{-3}	3.4	0.74
Salicylate	1.0×10^{-3}	−2.4	−0.52
Saccharose	1.0×10^{-3}	5.5	1.20
Glucose	1.0×10^{-3}	7.2	1.57
Lactose	1.0×10^{-3}	−4.1	−0.89
Maltose	1.0×10^{-3}	−4.6	−1.00
Fructose	1.0×10^{-3}	−5.7	−1.24

[a] The concentration of berberine was fixed at $1.0 \times 10^{-6}\,mol\,l^{-1}$ (pH 12.0).

[b] F_i and F_{i0} are the fluorescence intensities of the optode membrane contacting with $1.0 \times 10^{-6}\,mol\,l^{-1}$ berberine solution with and without the addition of the interferent ($F_{i0} = 459.3$).

was notably enhanced when berberine molecule entered the optode membrane containing HDB-β-CD. The fluorescence enhancement can be interpreted as due to the formation of an inclusion complex between HDB-β-CD and berberine. HDB-β-CD possesses the hydrophobic cavity identical with that of β-CD. Besides, as HDB-β-CD was obtained by replacing the hydrogen atoms of the C-6-primary and C-2-secondary hydroxyl groups on parent β-CD with butyl groups, this substantially increased the nonpolarity and hydrophobicity of the exterior shell surrounding the interior cavity of HDB-β-CD. A much more hydrophobic and protective microenvironment for berberine was formulated shielding the excited state of berberine from water molecules and other species presented in bulk aqueous solution. An additional factor favorable for fluorescence enhancement is that the substitution of hydrogen atoms by butyl groups would cause the deformation of the cavity and decrease the degree of freedom in the motion of the berberine species entrapped in it. Moreover, the unsubstituted hydroxyl groups on HDB-β-CD would deprotonate under strong alkaline conditions and an extra electrostatic force produced between HDB-β-CD and berberine, which made berberine more accessible to HDB-β-CD molecule and ready to enter into its cavity. Therefore, a better protection was obtained leading to a greater enhancement of the fluorescence intensity of berberine.

According to CS Chem3D Pro version5.0 developed by CambridgeSoft Corporation, the molecule motion of berberine entering into the cavity of HDB-β-CD was simulated with the help of molecular dynamics using MM2 method based on Newton's equations of motion for simulating the movement of atoms involved. The simulation was initiated at a starting geometry which was chosen as the location of berberine molecule outside the HDB-β-CD cavity. The single point energy calculation was performed from the starting geometry. Then the coordinates for the atoms involved were changed with a step size of 2 Å, and another single point energy calculation was performed to determine the energy of that new conformation. Following the next incremental change, the energy was again determined and the procedure continued covering the whole process when the berberine molecule move from the wide side of HDB-β-CD to enter into the cavity till it went out from the narrow side. For β-CD, the narrow side refers to the side of torus formed by the oligosaccharide ring where the primary hydroxyl groups of the glucose resides are located, while the wide side refers to the side where the secondary hydroxyl groups are located. For HDB-β-CD, all primary hydroxyl groups and the C-2-secondary hydroxyl groups of the parent β-CD were replaced by butyl groups, and the discrimination of narrow and wide sides remained unchanged. As can be seen from Fig. 7, when the guest molecule berberine entered into the cavity from the wide side of HDB-β-CD, the total energy of system dropped dramatically. One could imagine if the berberine molecule attempted to enter the cavity from the narrow side, it needed to overcome a huge energy barrier which practically forbade such a move. From the view of system stability, it was obviously favorable for berberine to enter into the cavity from the wide side of HDB-β-CD. The stereoconformation of inclusion complex corresponding to the lowest energy of system is shown in Fig. 8.

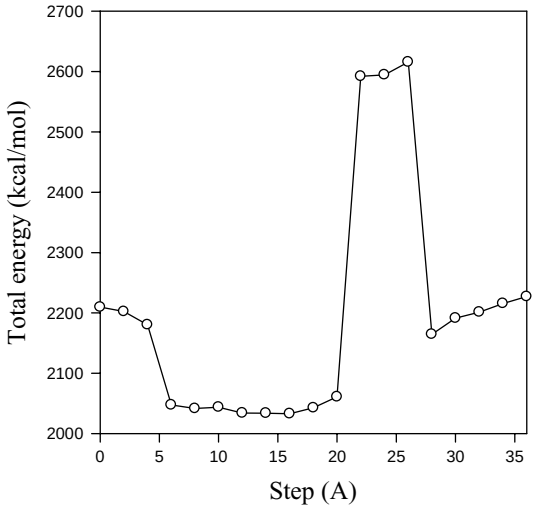

Fig. 7. The curve of the change of system total energy calculated by molecular dynamics simulation. The calculation was initiated at a starting geometry (step 0) as the location of berberine molecule outside the HDB-β-CD cavity. The increment of step from 0 to 36 Å represents the movement of berberine entering the wide side of HDB-β-CD through the cavity and exiting from the narrow side.

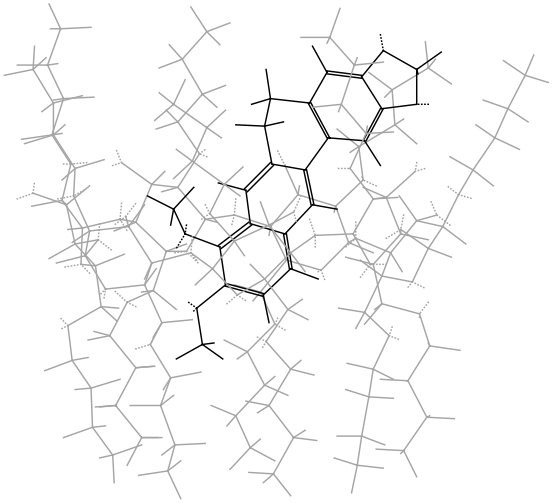

Fig. 8. The optimum configuration of HDB-β-CD-berberine inclusion complex.

Table 2
Determination of berberine in pharmaceutical preparations

Sample	Berberine $mg^{-1}\,g^{-1}$ of tablet			
	Proposed method		Pharmacopoeia method [33]	
	Mean ± S.D.[a,b]	R.S.D.[c] (%)	Mean ± S.D.[a,b]	R.S.D.[c] (%)
1	177.80 ± 1.65	0.93	177.60 ± 1.35	0.76
2	246.19 ± 2.43	0.99	246.40 ± 2.08	0.84
3	319.14 ± 2.57	0.81	319.33 ± 2.00	0.63

[a] Mean of six determinations.
[b] S.D.: standard deviation.
[c] R.S.D.: relative standard deviation.

3.7. Analytical application

The proposed sensor was applied to the determination of berberine in pharmaceutical tablets. An appropriate amount of sample solution was transferred into a volumetric flask and diluted with phosphate buffer solution of pH 12.0 and then measured by the use of the berberine sensor. Results showed that the concentrations of berberine determined by the proposed sensor were in agreement with those obtained by the pharmacopoeia method [33] and the relative standard deviations were less than 1.00% (as shown in Table 2). Furthermore, the proposed optical sensor was testified in the recovery studies of berberine in urine samples. Varying amounts of berberine were added to the diluted (50-fold) urine samples (Table 3). The results suggested that the sensor could be used for the determination of berberine with satisfactory recoveries of 97.80–103.00%.

Table 3
Recovery studies in urine samples

Added ($mol\,l^{-1}$)	Found ($mol\,l^{-1}$)	Recovery (%)
5.00×10^{-7}	4.89×10^{-7}	97.80
3.00×10^{-6}	3.04×10^{-6}	101.33
1.00×10^{-5}	1.03×10^{-5}	103.00

4. Conclusions

An optical sensor for berberine based on its intrinsic fluorescence enhanced with a lipophilic β-cyclodextrin has been proposed. Under the optimum conditions, a detection limit of $8.0 \times 10^{-8}\,mol\,l^{-1}$ berberine was obtained. The sensing mechanism of the optode membrane was based on the formation of an inclusion complex between HDB-β-CD and berberine. The formation and optimum stereoconformation of the inclusion complex was investigated by combining molecular dynamics computation and computer simulation. The method for the determination of real sample was simple, precise and sensitive.

Acknowledgements

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

References

[1] J. Lin, Q.S. Hu, M.H. Xu, L. Pu, J. Am. Chem. Soc. 124 (2002) 2088.
[2] H. Takakusa, K. Kikuchi, Y. Urano, S. Sakamoto, J. Am. Chem. Soc. 124 (2002) 1653.
[3] S. Mizukami, T. Nagano, Y. Urano, A. Odani, J. Am. Chem. Soc. 124 (2002) 3920.
[4] C. Peter, M. Meusel, F. Grawe, Fresenius J. Anal. Chem. 371 (2001) 120.
[5] J. Bugler, J.F.J. Engbersen, D.N. Reinhoudt, J. Org. Chem. 63 (1998) 5339.
[6] T. Tanabe, K. Touma, K. Hamasaki, A. Ueno, Anal. Chem. 73 (2001) 3126.
[7] A.J. Tong, A. Yamauchi, T. Hayashita, Z.Y. Zhang, Anal. Chem. 73 (2001) 1530.
[8] R.T. Bronson, J.S. Bradshaw, P.B. Savage, J. Org. Chem. 66 (2001) 4752.
[9] Y. Liu, C.C. You, S.Z. Kang, C. Wang, Eur. J. Org. Chem. 4 (2002) 607.
[10] G.R. Deviprasad, B. Keshavan, J. Chem. Soc. Perkin Trans. I 19 (1998) 3133.
[11] J. Szijtli, T. Osa, Comprehensive Supramolecular Chemistry, Pergamon, Oxford, 1996.
[12] K.A. Connors, Chem. Rev. 97 (1997) 1325.
[13] M.V. Rekharsky, Y. Inoue, Chem. Rev. 98 (1998) 1875.
[14] K. Suto, S. Kakinuma, Y. Ito, J. Chromatogr. A 786 (1997) 371.
[15] S.J. Lin, H.H. Tseng, K.C. Wen, T.T. Suen, J. Chromatogr. A 730 (1996) 17.
[16] Y.R. Chen, K.C. Wen, G.R. Her, J. Chromatogr. A 866 (2000) 273.
[17] X.B. Zhang, C.C. Guo, S.H. Chen, G.L. Shen, R.Q. Yu, Fresenius J. Anal Chem. 369 (2001) 422.
[18] W.L. Xing, X.W. He, Anal. Chim. Acta 349 (1997) 283.

[19] Y.M. Liu, S.J. Sheu, J. Chromatogr. 639 (1993) 323.
[20] X.B. Zhang, Z.Z. Li, C.C. Guo, S.H. Chen, G.L. Shen, R.Q. Yu, Anal. Chim. Acta 439 (2001) 65.
[21] W.H. Liu, Y. Wang, J.H. Tang, G.L. Shen, R.Q. Yu, Talanta 46 (1998) 679.
[22] Y. Wang, W.H. Liu, K.M. Wang, G.L. Shen, R.Q. Yu, Fresenius J. Anal. Chem. 360 (1998) 702.
[23] Z.H. Song, T.Z. Zhao, L. Wang, Z. Xiao, Bioorg. Med. Chem. 9 (2001) 1701.
[24] W.Y. Li, Z.H. Lu, Microchem. J. 60 (1998) 84.
[25] G.Q. Gong, Z.X. Zong, Y.M. Song, Spectrochim. Acta Part A 55 (1999) 1903.
[26] P.S. Bates, D. Parker, A.F. Patti, J. Chem. Soc. Perkin Trans. II 4 (1994) 657.
[27] K. Seiler, W. Simon, Anal. Chim. Acta 266 (1992) 73.
[28] W.H. Chan, R.H. Yang, K.M. Wang, Anal. Chim. Acta 444 (2001) 261.
[29] R.H. Yang, K.M. Wang, D. Xiao, X.H. Yang, H.M. Li, Anal. Chim. Acta 404 (2000) 205.
[30] Y. Wang, K.M. Wang, W.H. Liu, G.L. Shen, R.Q. Yu, Analyst 122 (1997) 69.
[31] R.H. Yang, K.M. Wang, D. Xiao, X.H. Yang, L.D. Zhang, Microchem. J. 64 (2000) 213.
[32] R.H. Yang, K.M. Wang, D. Xiao, X.H. Yang, Fresenius J Anal. Chem. 367 (2000) 429.
[33] Sanitation Faculty, Pharmacopoeia of the people's Republic of China, Part III, Sanitation Press, Beijing, 1990, p. 358 (in Chinese).