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Analytica Chimica Acta 389 (1999) 291–298

ANALYTICA CHIMICA ACTA

Supramolecular inclusion complex formation and application of β-cyclodextrin with heteroanthracene ring cationic dyes

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Received 17 April 1998; received in revised form 4 January 1999; accepted 25 January 1999

Abstract

β-Cyclodextrin forms 1:1 inclusion complexes with methylene blue, azure A, toluidine blue, resorcinol blue, neutral red, safranine T, indigo carmine and acridine orange in aqueous media. The formation constants are determined by differential pulse polarography and spectrophotometry. The supramolecular interaction in the inclusion complexes can be employed to immobilize dyes on an electrode. This gives high sensitivity and stable electrochemical behavior for H_2O_2 detection at the mmol l^{-1} level by means of the supramolecular interaction between β-CD and dye molecules.

Keywords: Supramolecular inclusion complex; Formation constant; β-Cyclodextrin; Heteroanthracene ring cation dye

1. Introduction

β-Cyclodextrin (β-CD) with its peculiar ''interior hydrophobic, exterior hydrophilic'' structure, forms a 1:1 or 1:2 inclusion complex with a guest molecule, so that the physical, chemical and biochemical characters of the guest molecule are modified [1,2]. The inclusion complex has been used widely in pharmaceutical [3], food [4], environmental protection analyses [5], analysis of organic and inorganic materials [6], and enzyme modeling [7].

Fundamental to such investigations is an accurate determination of the formation constants of these complexes. Current methods for the measurement of the inclusion constants include UV-Vis spectrophotometry [8,9] and spectrofluorimetry [7,10], as well as electrochemical methods. Considerable attention has been given to the electrochemical evaluation of the cyclodextrin complexes of electroactive species. Electrochemical techniques such as cyclic voltammetry [11–14], polarography [15,16], conductivity [17], and pH determination by potentiometry [18], are particular useful in the study of guest molecules which lack a chromophore for spectrophotometric measurement but which has a suitable electrophore. Electrochemical measurements of the formation constants of β-CD inclusion complexes have been reviewed [12].

The inclusion complex formed between the heteroanthracene ring cationic dye methylene blue (MB) and β-CD, was observed by Cramer early in 1953 [19], in which the redox potential increased by 0.043 and 0.048 V, respectively, at pH 7.0 and 8.0. However, the change in potential of MB was not been utilized in the determination of its inclusion constant until its

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PII: S0003-2670(99)00134-8

formation constant was measured using spectrophotometry in 1985 [9]. In addition to MB, the formation constant of the MB analog, azure A (AA) included by β-CD was determined using spectrofluorimetry in 1974 [20], and that of acridine orange (AO) was measured using spectrophotometry in 1994 [21]. It has also been observed that the fluorescence intensity of AA was increased three times when included by β-CD [20]. However, the inclusion of MB with β-CD is responsible for the increase of the light intensity [22].

In the present work, differential pulse polarography with its extremely high sensitivity is reported for the determination of formation constants (K_s) with MB, AA, toluidine blue (TB), resorcinol blue (RB), neutral red (NR), safranine T (ST), indigo carmine (IC) and AO as the guest of β-CD, which acted as the host. The formation constants were also determined by the Benesi–Hildebrand method from spectrophotometric measurements for comparative purposes. The supramolecular interaction between dyes and β-CD was employed to immobilize the mediator dye on a biosensor. The immobilized mediator has a certain mobility in the cavity of β-CD, besides its stability in the matrix. This provided a configuration for a H_2O_2 sensor with considerable sensitivity and useable lifetime.

2. Experimental

2.1. Material

β-CD was used as received. All dyes were purchased from Beijing Chemicals and recrystallized twice before use. The aqueous solutions of β-CD and dyes were prepared using water and phosphate buffer solution (pH 7.0, μ=0.1) except for Britton–Robinson (B–R) buffers in ethanol solutions.

The dye solutions were stirred for 1 h at room temperature so that the aggregated dye came to equilibrium with its monomer before they were made up to a 1.0×10^{-3} mol l^{-1} solution. Hydrogen peroxide solution was freshly prepared by dilution of a 1.0×10^{-3} mol l^{-1} stock solution. The cross-linked polymer of β-CD and epoxychloropropane was synthesized according to the literature [23], and milled to a fine resin (140 mesh). B–R buffer solution was applied at pH 7.0. All aqueous solutions were prepared in double distilled water.

2.2. Apparatus

Polarographic data were obtained with a PARC polarographic analyzer, Model 174 (Princeton Applied Research Corporation, Princeton, NJ), equipped with a Model 303 static mercury drop electrode, which was used as the working electrode in the formation constant determination, and a glassy carbon electrode which was applied in the configuration of the H_2O_2 sensor. The reference electrode was a standard saturated calomel electrode, and the auxiliary electrode was platinum wire. Spectral measurements were made on a Shimadzu UV-240 spectrophotometer with 10 mm pathlength silica fused curvettes (Tokyo).

2.3. Methods

2.3.1. Differential pulse polarographic determination of formation constants of inclusion compounds

Samples contained 0.1 mol l^{-1} KCl, a certain concentration of dye solution and various concentrations of β-CD (0.5×10^{-3}–2.5×10^{-3} mol l^{-1}). Differential pulse polarograms were measured after the oxygen in solution was purged by nitrogen gas for 5 min. The measurements were carried out at a constant temperature (293±2 K).

2.3.2. Spectral measurement of formation constants of inclusion compounds

The concentrations of dyes and β-CD were the same as those used in the differential pulse polarographic method. Electronic absorption spectra were measured in the range 400–700 nm. The absorption spectra of dyes changed with the concentration of β-CD. The inclusion formation constants were calculated by the Benesi–Hildebrand [8] formula at the wavelength with the maximum ΔA (the difference between the absorbances of dyes in the presence and absence of β-CD). The continuous variation method was applied to determine the mole ratio of the inclusion complexes.

3. Results and discussion

3.1. The inclusion phenomena of dyes with β-CD

The inclusion of cationic dyes (MB, AA, TB, RB, NR, ST, IC and AO) with β-CD was observed using

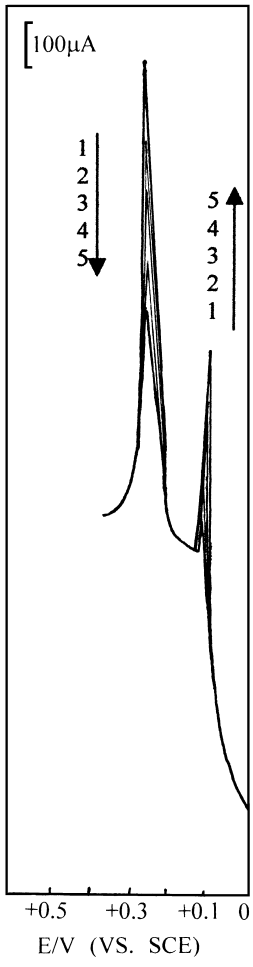

Fig. 1. Differential pulse polarogram of MB (2.62×10^{-5} mol l^{-1}) at various concentration of β-CD (1: 0.0×10^{-3}; 2: 0.5×10^{-3}; 3: 1.0×10^{-3}; 4: 1.5×10^{-3}; 5. 2.0×10^{-3} mol l^{-1}). Scan rate: 5 mV s^{-1}.

differential pulse polarograms and electron absorption spectra, as shown in Fig. 1. The peak potential of MB shifted from +0.006 to +0.049 V at pH 7.0, in accordance with the results in the literature [19]. The peak with a formal potential of +0.332 V increased slightly to +0.334 V, while its current decreased. The peak currents of the inclusion complexes are less than those of the dyes owing to the smaller diffusion coefficient of the bulky cyclodextrin concentration, as a function of the β-CD concentration, as shown in Fig. 1, but the peak at +0.16 V was higher. The changes both in potential and current suggested the formation of an inclusion complex. The similar shift of the peak potential as well as the change of the peak current was observed in the cases of other cationic dyes included by β-CD. The peak potential with its formal potential at approximately 0.30 V displaced positively 15 mV with AA, positively 18 mV with TB, negatively 12 mV with RB, negatively 11 mV with NR, positively 11 mV with ST, negatively 13 mV with IC and positively 16 mV with AO as the guest molecule.

The electronic absorption spectra of the solutions are shown in Fig. 2, in the presence and absence of β-CD. Changes in the electronic spectra of the guest molecule by inclusion with β-CD were observed. The wavelength of maximum absorption of the dyes apparently shifted to a longer or shorter wavelength, while the absorbance at the maximum wavelength increased or decreased, which suggested an inclusion phenomenon. The original absorption maxima of MB at 605 and 660 nm slightly shifted to a longer wavelength (by 10 and 5 nm, respectively). The absorption of the monomer at 660 nm increased while that of the dimer at 605 nm decreased gradually, and disappeared eventually, with the addition of β-CD. The spectral change showed that it was the monomer not the dimer of MB that was included by β-CD, so that β-CD forms a 1:1 complex with MB. It was reported in the literature that, in addition to MB [9], AA [20] and AO [21] form 1:1 complexes with β-CD. The mole ratio of the selected dyes to β-CD in the inclusion complex was calculated by the continuous variation method, which indicated 1:1 complex formation of all of these dyes with β-CD.

3.2. *Formation constants measurement of cationic dyes and β-CD*

The changes of potential and current in cyclic voltammetric curves were usually used in the electrochemical measurement of inclusion constants. In this work, the shift of voltage in the cyclic voltammograms is <20 mV, so it is difficult to determine the constant accurately by means of the voltammetric method. Moreover, the reactivation of the solid electrode after measurement is difficult owing to the strong absorption of dyes on the electrode, which affects the repeatability of the measurements and then limits their application. Pulse polarographic methods with their increased sensitivity (usually by two orders of

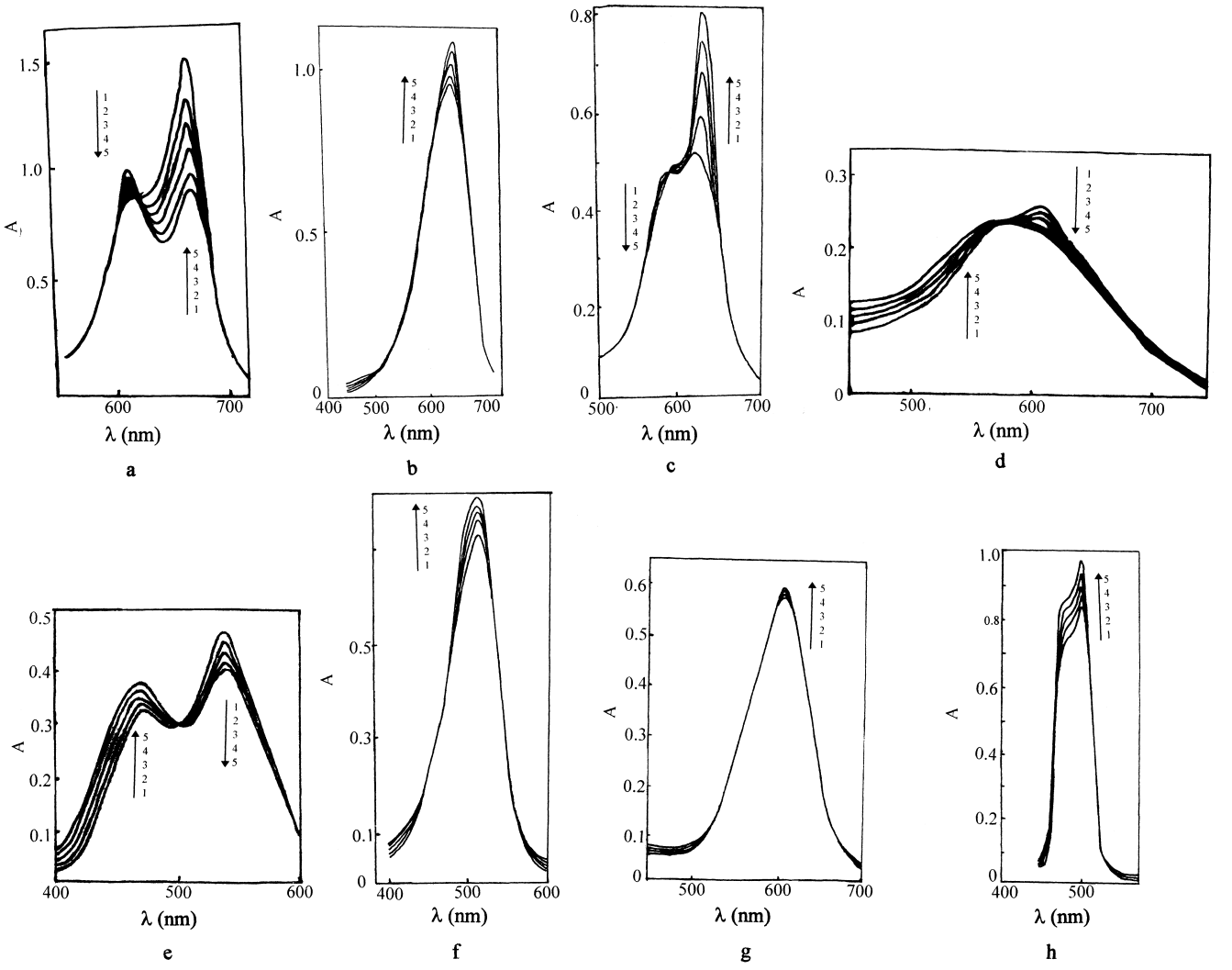

Fig. 2. Absorption spectra of MB (a: 2.62×10^{-3} mol l^{-1}), AA (b: 1.00×10^{-4} mol l^{-1}), TB (c: 1.10×10^{-4} mol l^{-1}), RB (d: 5.02×10^{-5} mol l^{-1}), NR (e: 5.22×10^{-4} mol l^{-1}), ST (f: 2.28×10^{-5} mol l^{-1}), IC (g: 2.59×10^{-5} mol l^{-1}) and AO (h: 2.27×10^{-5} mol l^{-1}) at various concentration of β-CD (1: 0.0×10^{-3}; 2: 0.5×10^{-3}; 3: 1.0×10^{-3}; 4: 1.5×10^{-3}; 5: 2.0×10^{-3} mol l^{-1}).

magnitude) and good repeatability has been applied to evaluate the dissociation constants of selected α-CD and β-CD–benzaldehyde inclusion complexes [16]. The results were compared with those obtained by spectrophotometric measurement. However, the actual concentration of β-CD in the experimental process [16] was similar to that of the guest molecule. This does not satisfy the conditions for the Benesi–Hildebrand formula. In the present experiment, differential pulse polarography was applied in the measurement of formation constants, because its sensitivity is 1 to 2 orders of magnitude greater than normal pulse polarography, due to its higher resolution.

The equation that could be applied for the determination of the formation constants of the 1:1 complex was deduced from the work of Choi et al. [16]. It is assumed that the oxidized state of the guest molecule forms the 1:1 inclusion complex with β-CD, so the electrochemical reduction goes in two stages:

$$\mathrm{O{-}\beta\text{-}CD} \Leftrightarrow \mathrm{O} + \beta\text{-CD} \quad (1)$$

$$\mathrm{O} + \mathrm{ne} \Leftrightarrow \mathrm{R} \quad (2)$$

where O and R are the oxidized and reduced state of the guest molecule. The diffusion coefficient of the guest molecule is given by Eq. (3) when the concentration of β-CD is in great excess (usually [CD]>20 [Guest molecule]).

$$D = \frac{D_{\mathrm{O}} - D_{\mathrm{O\text{-}CD}}([\mathrm{CD}]/K_{\mathrm{d}})}{1 + ([\mathrm{CD}]/K_{\mathrm{d}})}, \quad (3)$$

where D is the formal diffusion coefficient and D_{O} and $D_{\mathrm{O\text{-}CD}}$ are the diffusion coefficients of the guest molecule and complex, respectively. The peak current i_{p} in differential pulse polarography is expressed as Eq. (4):

$$i_{\mathrm{p}} = \frac{nFAD^{1/2}C}{\pi^{1/2}(\tau - \tau')^{1/2}} \times \frac{(1-\sigma)}{(1+\sigma)}, \quad (4)$$

where σ=exp($-nF/RT\ \Delta E/2$), which is constant over a certain pulse range, $\tau{-}\tau'$ is the pulse maintenance time, and F, A and C are the Faraday constant, the area of the electrode and the concentration of the guest molecule, respectively. In the case of constant guest concentration C, $i_{\mathrm{p}} \propto D^{1/2}$. Eq. (3) can be rewritten as Eq. (5), when D is replaced by i_{p}^2:

$$i_{\mathrm{p}}^2 = K_{\mathrm{d}}(i_{\mathrm{p_0}}^2 - i_{\mathrm{p}}^2)/(CD) - i_{\mathrm{p_{O\text{-}CD}}}^2, \quad (5)$$

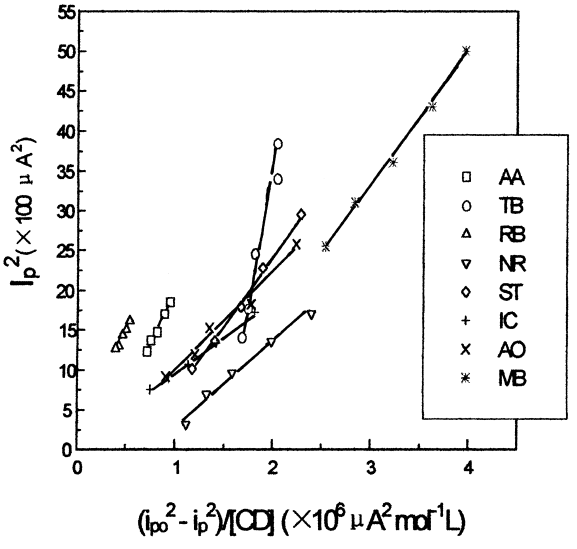

Fig. 3. The plot of $(i_{\mathrm{p_0}}^2 - i_{\mathrm{p}}^2)/(CD)$ vs. i_{p}^2. The straight lines follow Eq. (5).

where $i_{\mathrm{p_0}}$ and $i_{\mathrm{p_{O\text{-}CD}}}$ are the peak currents of the guest molecule and complex, respectively.

Thus from a i_{p}^2 vs. $(i_{\mathrm{p_0}}^2 - i_{\mathrm{p}}^2)/[\beta - \mathrm{CD}]$ plot, an approximate straight line was obtained, as shown in Fig. 3. From its intercept, the value of K_{d} can be calculated (K_{s}=1/K_{d}). The values of the formation constants, K_{s}, with eight cationic dyes as the guest of β-CD are given in Table 1. The results from spectrophotometric measurement are also given in Table 1, corresponding well with the results from differential pulse polarographic measurement. However, it can be seen from the data in Table 1 that the values of K_{s} from electrochemical measurement were generally lower than those from the spectrophotometric method. This is also reported in the literature [16]. It is also shown in Table 1 that the relationship i_{p}^2 vs. $(i_{\mathrm{p_0}}^2 - i_{\mathrm{p}}^2)/[\beta - \mathrm{CD}]$ was generally more linear than that of $(C_0 \times C_{\mathrm{CD}})/\Delta A$ vs. $C_{\mathrm{CD}}/\Delta\epsilon$. This fact is probably associated with the greater level of variation of i_{p} due to its extremely high sensitivity, compared to the variation of absorbance in the formation constants determination. For the same reason, some substances, such as MB, as the guest of β-CD, could be measured by differential pulse polarography but not by the spectrophotometric method in much lower concentration than β-CD. Moreover, in the case where no absorbance difference (ΔA) with β-CD present or absent in the dye solutions is observed

Table 1
Formation constants of inclusion complexes and linear correlation of the measurement methods

Guest	Differential pulse polarography		Spectrophotometric method		Literature values K_s (mol l^{-1})	Reference
	K_s (mol l^{-1})	Linear correlation (n=5)	K_s (mol l^{-1})	Linear correlation (n=5)		
MB	621	0.997	714	0.992	814	[9]
AA	375	0.994	410	0.96	110	[20]
TB	142	0.999	193	0.996		
RB	456	0.990	497	0.87		
NR	952	0.994	984	0.92		
ST	585	0.998	616	0.994		
IC	1072	0.993	–	–		
AO	825	0.994	926	0.97	251	[21]

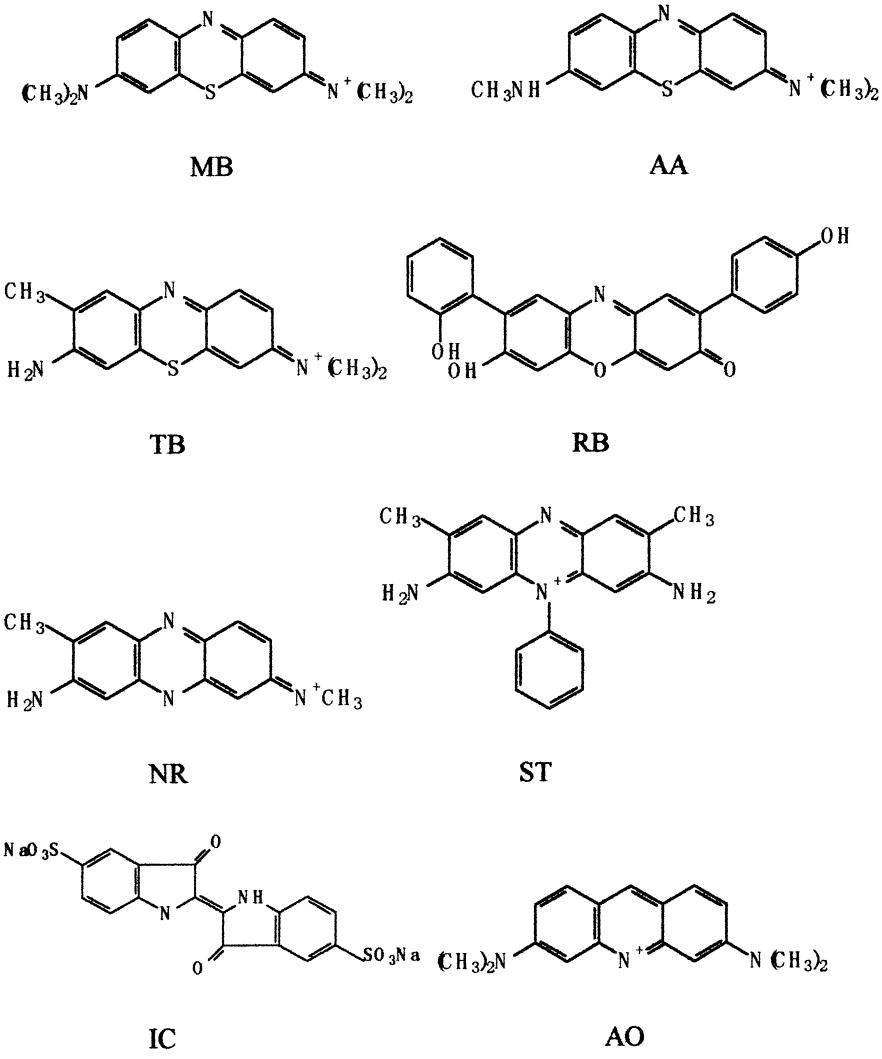

Fig. 4. The chemical structures of the heteroanthracene ring cationic dyes studied.

(such as IC), the electrochemical methods might be advantageous.

The chemical structures of the dyes studied are shown in Fig. 4. The size of the guest molecule and its hydrophobic character are decisive factors in the formation of stable inclusion compounds.

Multiple methyl groups in the cationic dyes are advantageous for hydrophobicity, but increase the space requirement. Thus a cooperative mechanism involving hydrophobic and steric effects is necessary to describe the influence of substituted groups in the β-CD/heteroanthracene ring cationic dye system. NR and IC give the least space hindrance, so have the greatest K_s value, as shown in Table 1. There are dimethylamino and hydrophilic amine groups in the TB structure, which result in a lower K_s value. RB is too large to pass through the small cavity, so it could not be included by β-CD. However, it could be included partly by the inclusion interaction between the phenol-substituted group and the β-CD cavity.

3.3. Electrochemical behavior of H_2O_2 sensor

Heteroanthracene ring cation dyes such as MB, NR, AA, TB, RB have been employed as electron transfer shuttles between biomolecules and inert electrode surfaces [24–27]. They can be immobilized on the basic electrode by chemisorption, covalent attachment, electropolymerizing, ion exchange, etc. This work uses the supramolecular interaction of β-CD and a mediator molecule. A novel mediated H_2O_2 sensor can be constructed with a cationic dye mediator incorporated in the β-CD polymers immobilized on a glassy carbon electrode surface. The cyclic voltammograms of the MB-mediated H_2O_2 sensor in the presence or absence of H_2O_2 are shown in Fig. 5. Only the immobilized dye on the H_2O_2 sensor contributes to the electrochemical response. The plots of peak current vs. square root of scan rate ($i_p/v^{1/2}$) were linear in the oxidation and reduction of dye incorporated in the β-CD cross-linked polymer, which indicates that the electrode reaction might be diffusion controlled. When H_2O_2 was added to the solution, the voltammetric behavior changed dramatically with a sharp increase of the reduction current and an almost complete disappearance of the oxidation current, showing that a catalytic reaction occurred at the H_2O_2 sensor. This demonstrated that MB incorporated in the β-CD polymer could effectively shuttle electrons between the enzyme's redox center and the base electrode. It was also shown that the sensor could be used eight times, and retain 80% of the response at the beginning of the experimental period after 20 days. In addition to MB, AA, TB, RB, NR and ST showed the similar electronic behavior when employed to construct a H_2O_2 sensor.

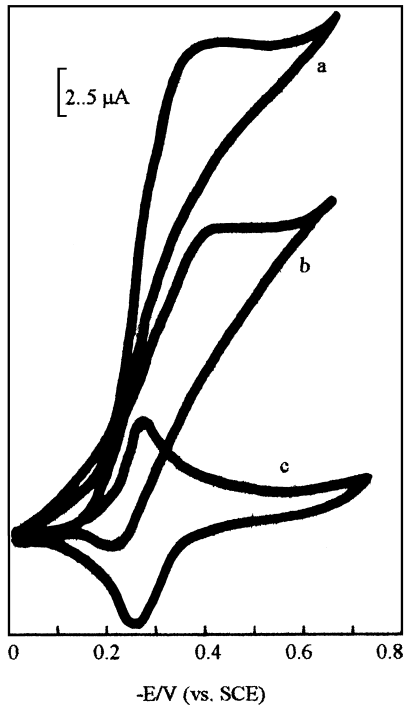

Fig. 5. Response of the H_2O_2 sensors based on immobilized MB: (a) in the absence of H_2O_2; (b) in the presence of 1.5 mM H_2O_2; (c) in the presence of 3.0 mM H_2O_2 at pH 7.0.

Acknowledgements

The authors thank the National Natural Foundation Commission of China for financial support.

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