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Study of the Inclusion Complexes of Catecholamines with β-Cyclodextrin by Cyclic Voltammetry

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The inclusion complexes of catecholamines (dopamine, DA, and adrenaline, AD) with β-cyclodextrin (β-CD) have been studied by cyclic voltammetry (CV) using glassy carbon electrode (GCE) for expanding the potential window. The variations of peak potential and peak current were observed on cyclic voltammograms, when the electroactive guest molecules, DA and AD are complexed with β-CD. Dissociation constants of cyclodextrin inclusion complexes have been calculated. The experimental results indicate that both DA and AD can form a 1:1 inclusion complexes with β-CD in aqueous solutions. It was observed that the light and oxygen sensitivity of the inclusion complex is much lower than those of the free catecholamines, which can be used to improve their storage and handling, and also widen their applications in pharmaceutical industry. Furthermore, it was found that their electrochemical oxidation in the aqueous solutions could be inhibited by β-CD.

Key words: catecholamines, β-CD, inclusion complex, cyclic voltammetry

Cyclodextrins (CDs) have proved to be useful model systems of inclusion complexes involving host/guest interactions [1–3]. α- and β-CDs may find their use in pharmaceutical industry, especially in reducing bitterness and side effects, and may also be used to increase the solubility of drugs, and thus directly increase the bioavailability of the drugs. Due to inclusion complex formation, their chemical reactivity, diffusion and electrochemical properties are changed [4–9]. Some light- or oxygen-sensitive substances can be stabilized after inclusion by CDs. Dopamine (DA) and adrenaline (AD), one main group of the catecholamines medicines, due to their special chemical structures, are easily auto-oxidized in neutral or acidic medium. Their auto-oxidation, which involves both electron and proton transfer, the free radical and the dimerization processes *etc*. and very strong light and oxygen sensitivity occurs noticeably, especially in basic medium. These processes limit their applications under normal conditions, and also make them inconvenient.

In order to eliminate those inconveniences, as the continuation of our previous works [10–12], we have further investigated the abilities of DA and AD to form inclusion complexes with β-CD, and also their electrochemical oxidation on a glassy carbon electrode (GCE) in the absence and the presence of β-CD. It was observed that the

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light and oxygen sensitivity of the inclusion complexes is much lower than that of the free catecholamines, which can be used to improve their storage and handling, and also widen their applications in pharmaceutical industry. Furthermore, it was found that their electrochemical oxidation in the aqueous solutions could be greatly inhibited in the presence of β-CD.

EXPERIMENTAL

Dopamine (3-hydroxytyramine hydrochloride, 99%), supplied by Acros Organics, adrenaline (Fluka Chemicals, USA), β-CD (GR, Beijing Chemical Reagents Institute, China) were used without further purification. Deionized water containing phosphate buffer solutions (pH 4.80) was used as supporting electrolyte. All other materials were analytical reagents and were used as received. All measurements were carried out in 0.10 mol dm^{-3} phosphate buffer solutions (pH 4.80). All solutions were protected from the light and deaerated by passing a purified stream of nitrogen that had been passed through a 100×5 cm column containing Mn(II) dispersed on vermiculite. Electrochemical measurements were performed using an electrochemical analyzer (Bioanalytical Systems BAS-100B). All experiments were carried out with a three-electrode cell system. The working electrode was a 0.24 cm diameter glassy carbon disk mounted on a Teflon sheath, then polished to a mirror with 0.05 μm alumina and rinsed with double distilled and deionized water in an ultrasonic bath. A large area platinum plate was used as the counter electrode. All potentials are reported *versus* Ag/AgCl in 3 mol dm^{-3} NaCl as a reference electrode. All experiments were performed at room temperature.

RESULTS AND DISCUSSION

Stoichiometric number and the dissociation constant of the inclusion complexes: When an electroactive guest molecule forms an inclusion complex with CDs, it does influence the electrochemistry of the guest molecule. Suppose that the redox equilibria are

$$R - \beta - CD_m \Leftrightarrow R + m\beta - CD \qquad (1) \qquad R - ne \Leftrightarrow O \qquad (2)$$

where $R - CD_m$ denotes the inclusion complex; R the reduced guest; O the oxidized guest. From (1) and its equilibria, using the *Nernst* equation one obtains the following equation, valid for stable complexes

$$\Delta E_{1/2} = E_{1/2(R-\beta-CD_m)} - E_{1/2} = \frac{RT}{2nF} \ln \frac{D_{R-\beta-CD_m}}{D_R} + \frac{mRT}{nF} \ln [\beta\text{-}CD] - \frac{RT}{nF} \ln K_d \qquad (3)$$

where K_d denotes the dissociation constant of the inclusion complex R-β-CD_m; [β-CD], the concentration of β-CD; $D_{R-\beta-CDm}$ and D_R, the diffusion coefficients of the inclusion complex.

In the reversible electrochemical reaction, $E_{1/2} = E_p + C$ (under the condition of constant temperature, C is a constant). So $E_{1/2}$ in (3) can be replaced by E_p . From the linear plot ΔE_p *versus* ln[β-*CD*] one gets *m*, the stoichiometric number of the inclusion complex.

It is known that β-CD mainly forms 1:1 inclusion complexes with small guests [13]. Under such assumption, the following equilibrium occurs

$$R - \beta - CD \Leftrightarrow R + \beta - CD \tag{4}$$

The diffusion coefficient determination: The apparent diffusion coefficient (D_{app}) can be then calculated according to [14]. For the reversible electrochemical process, $I_p^2 \propto D$, and assuming the other parameters constant under experimental conditions, D can be replaced by I_p^2, and then one gets:

$$I_p^2 = \frac{K_d}{[\beta - CD]}(I_{p(R)}^2 - I_p^2) + I_{p(R\text{-}\beta\text{-}CD)}^2 \tag{5}$$

where I_p is the observed current; $I_{p(R)}$ and $I_{p(R\text{-}\beta\text{-}CD)}$, the peak currents of the free guest and the inclusion complex, respectively. Both I_p and $I_{p(R)}$ can be determined from the experiments. The K_d value can be obtained from the slope of the linear plot of I_p^2 *versus* ($I_{p(R)}^2 - I_p^2$)/[β-CD].

The diffusion properties of the free guest in the absence and the presence of β-CD could be investigated by potential-step chronocoulometry (CC). The total charge (Q) can be described by [15]

$$Q = 2nFAC\sqrt{\frac{D_0 t}{\pi}} + Q_{dl} + Q_{ads} \tag{6}$$

where Q_{dl} is the capacitive charge (double layer); and Q_{ads}, the charge of adsorbed reactant. From (6), it can be seen that a plot of Q *versus* $t^{1/2}$ should be linear, and D_0 can be determined from its slope.

The inclusion complexes of dopamine-β-cyclodextrin: It is seen from Fig. 1 that DA in aqueous phosphate buffer solutions (pH 4.80) exhibits a reversible redox process (curve a). Upon addition of β-CD to the system studied, the redox process shows a quasi-reversible behaviour; both oxidation and reduction peak currents of DA are decreased, and also the oxidation peak potential is shifted to positive values (Fig. 1, curve b). This means that DA is more difficult to be oxidized in the presence of β-CD, because DA is more strongly bound in the hydrophobic cavity than its reduced state. A decrease in both oxidation and reduction peak currents were observed, upon

addition of β-CD, due to the smaller diffusion coefficient of cyclodextrin complex compared with that of the free guest DA. The decrease of the oxidation peak currents at different scan rates as a function of the concentration of β-CD is shown in Fig. 2.

The experimental results also show that with increasing scan rate (V), the ratio of the oxidation peak current (I_{pa}) to $V^{1/2}$ decreases from 1.368 to 1.189 (see Table 1). The results mentioned above also indicate that the inclusion complexation process occurs *via* the proposed CE's scheme, in which β-CD includes the guest DA to form

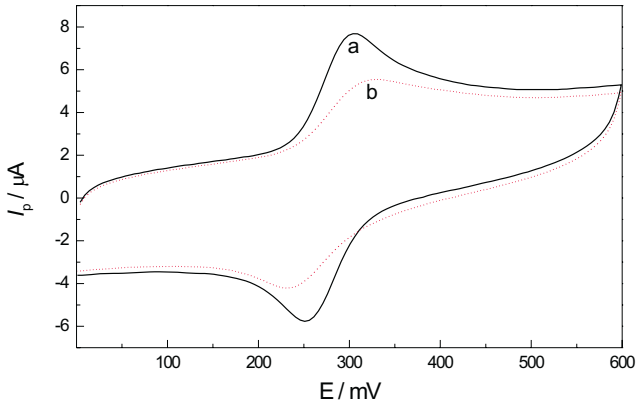

Figure 1. The cyclic voltammograms of DA in absence (a) and presence (b) of β-CD. $c_{DA} = 1\times10^{-4}$ mol dm^{-3}, $c_{\beta\text{-CD}} = 9.6\times10^{-3}$ mol dm^{-3}. Scan rate $V = 50$ mV s^{-1}.

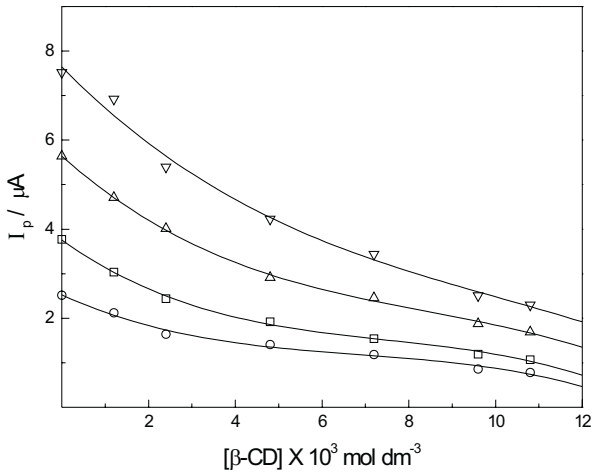

Figure 2. The oxidation peak currents of DA at different scan rates as a function of the total concentration of β-CD. $c_{DA} = 1.0\times10^{-4}$ mol dm^{-3}. (o) for 10, ( ) for 20, (Δ) for 50 and (∇) for 100 mV s^{-1}.

an inclusion complex, DA-β-CD, and then the inclusion complex, DA-β-CD, dissociates to the free guest DA and β-CD followed by the oxidation of the free DA. Due to its reversibility, according to (3) from the slope of the linear plot of ΔE_p *versus* ln[β-CD] one gets the stoichiometric number, $m = 0.96 \approx 1$. According to (5) and from the slope of the linear plot of I_p^2 *versus* $(I_{p(DA)}^2 - I_p^2)/[\beta\text{-}CD]$, the K_d value obtained is equal to 3.56×10^{-3} mol^{-1} dm^3.

Table 1. The relationship between oxidation peak currents (I_{pa}) and scan rate (V).

V/mV s^{-1}	10	20	50	80	100
$I_{pa}/V^{1/2}$/(μA mV$^{-1/2}$ s$^{1/2}$)	1.368	1.310	1.242	1.216	1.189

CDs may be adsorbed on electrodes and changes of peak potentials may be due not only to the inclusion complex formation but also to the inhibition. In our electrochemical studies, however, it was demonstrated that the guest DA forms an inclusion complex with β-CD and adsorption is rather weak. Due to the inclusion complex formation, its diffusion coefficient decreased, and also its oxidation peak current decreased as well. A suitable system, $Fe(CN)_6^{3-/4-}$, whose diffusion coefficient in 0.1 M KCl supporting electrolyte solution is known to be 7.6×10^{-6} cm^2 s^{-1} [16], was employed to evaluate the electrode area (A) by potential-step chronocoulometry (CC). The diffusion coefficients of DA in the absence ($D_f = 2.43\times10^{-6}$ cm^2 s^{-1}) and the presence of 9.6×10^{-3} mol dm^{-3} β-CD ($D_c = 0.90\times10^{-6}$ cm^2 s^{-1}) were determined from the slopes of the corresponding upper lines of the Anson plots shown in Fig. 3. It is known, that β-CD could cause an increase in the solution viscosity with a corresponding decrease in the diffusion coefficients. However, over the concentration range from 0.0 to 1.0×10^{-2} mol dm^{-3}, no changes in solution viscosity could be detected [5]. So the decrease of the diffusion coefficient can be attributed to the complexation of DA with β-CD. From the Anson plot, one can see that the upper lines, which refer to the forward potential-step, show the different slopes with respect to the different diffusion coefficients of the free DA and the inclusion complex, DA-β-CD. While the lower lines have almost the same slope, which means that the oxidation product was free from the cavity of β-CD and diffused away from the electrode surface. The differences of the intercepts of the corresponding lines could be attributed to the influence of β-CD on the capacitance charge. The positive shift of the anodic peak potential also means that after the inclusion complex formation, it requires much more energy to be overcome for the electrochemical oxidation at the electrode surface. Therefore, its oxidation peak potential is shifted in a positive direction.

The inclusion complex of adrenaline with β-CD: According to our electrochemistry results, AD in aqueous phosphate buffer solutions (pH 4.80) exhibited a quasi-reversible electrochemical process. Upon addition of β-CD to the systems studied, the oxidation peak current of AD decreased, and its oxidation peak potential shifted to a positive direction. However, its quasi-reversibility was almost kept in-

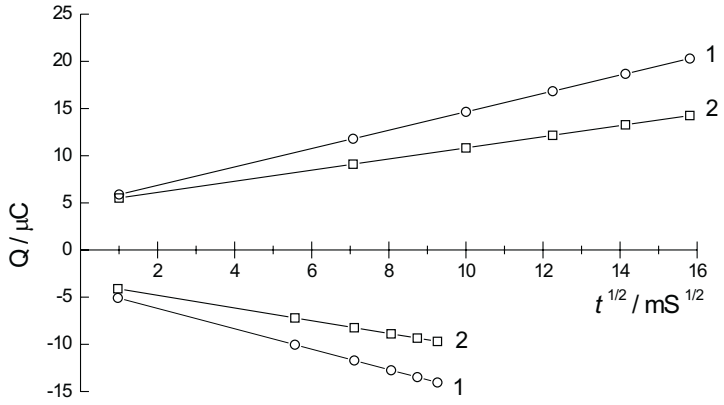

Figure 3. Anson plot for DA in absence (1) and presence (2) of β-CD. τ = 250 ms. $c_{DA} = 1.0\times10^{-4}$ mol dm^{-3}, $c_{\beta\text{-CD}} = 9.6\times10^{-3}$ mol dm^{-3}.

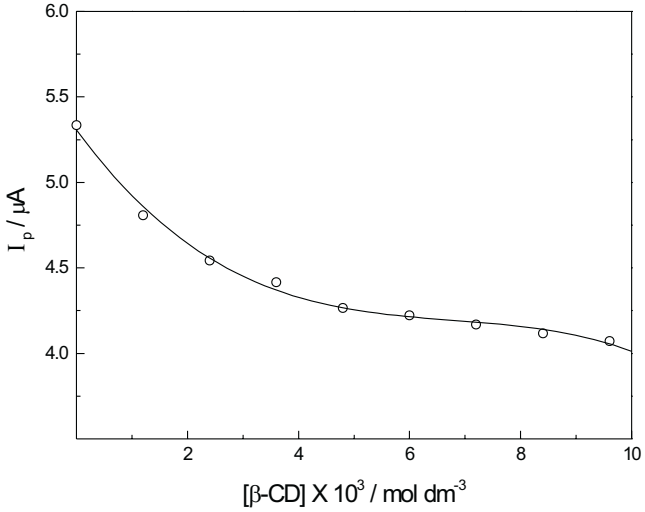

Figure 4. The oxidation peak currents of AD as a function of the total concentration of β-CD. $c_{AD} = 1.0\times10^{-4}$ mol dm^{-3}. Scan rate $V = 50$ mV s^{-1}.

variable (figure omitted). This means, that AD is more difficult to be oxidized in the presence of β-CD; because AD is more strongly bound in the hydrophobic cavity than its reduced state. A decrease in oxidation peak current is also observed upon addition of β-CD, due to the smaller diffusion coefficient of bulk cyclodextrin complex compared with that of the free AD. The decrease of the oxidation peak current as a function of the concentration of β-CD is shown in Fig. 4.

Using the same experimental conditions and procedure, the diffusion coefficients of AD in the absence ($D_f = 1.76\times10^{-6}$ cm^2 s^{-1}) and the presence of 9.6×10^{-3} mol dm^{-3} β-CD ($D_c = 0.95\times10^{-6}$ cm^2 s^{-1}) were obtained from the slopes of the corresponding upper lines of Anson plots. From the reasons mentioned above, the decrease of the diffusion coefficients can be attributed to the complexation between AD and β-CD, and the positive shift of the anodic peak potential also means that after the inclusion complex formation, it requires much more energy to be overcome for the electrochemical oxidation at the electrode surface. Therefore, its oxidation peak potential is shifted in the positive direction.

The experimental results have also shown that, with increasing scan rate (V), the ratio of the oxidation peak current (I_{pa}) to $V^{1/2}$ decreases. The results above mentioned also indicated that the inclusion complexation process occurs *via* a similarly proposed CE's scheme, in which β-CD includes the guest AD to form an inclusion complex, AD-β-CD, and then the inclusion complex, AD-β-CD, dissociates to the guest AD and β-CD followed by the oxidation of the free AD. Using above mentioned method, the K_d value obtained is equal to 2.06×10^{-3} mol^{-1} dm^3.

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