Chronocoulometry diffusion coefficients as a measure of cyclodextrin–estradiol complex association

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A B S T R A C T

The formation of inclusion complexes between estradiol and β-cyclodextrin (CD), sulfobutyleter-β-CD (SBCD) and hydroxypropil-β-CD (HPCD) was studied by chronocoulometric and differential pulse voltammetry. In presence of CD there is a decrease of the cathodic peak current with the increase of the amount of CD. This decrease is due to the lower diffusion coefficient (D) of estradiol–cyclodextrin complex compared with the free guest. In absence of CD, an estradiol D value of $8.6 \times 10^{-6} \pm 0.1 \times 10^{-6}$ cm^2 s^{-1} was obtained. The addition of CDs produced a maximum decrease of 20%, 22% and 30% in the diffusion coefficient with CD, SBCD and HPCD, respectively. Using the variation in D, association constants of 132 ± 18, 247 ± 32 and 276 ± 28 M^{-1} for CD, HPCD and SBCD were determined.

Keywords:
Cyclodextrin
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1. Introduction

Cyclodextrins (CDs) are cyclic oligomers of six, seven or eight linked α-D-glucopyranose units, denoted α, β and γ CDs, respectively. These CDs compounds are well known to form inclusion complexes with a variety of organic and inorganic compounds. This ability is based on the capability of CDs to provide a hydrophobic cavity in aqueous solution for both the hydrophobic guest molecule or moieties in the guest molecule [1]. Studies involving inclusion of solutes into CDs are important due to the resulting improvement of aqueous solubility, stability against chemical and photochemical degradation and to the possibility of controlled drug release, which presents many potential applications in drug formulations.

In a previous work [2] we have evaluated the interaction of two estrogen derivatives, estrone and estradiol, with CD by differential pulse voltammetry (DPV) and high performance liquid chromatography (HPLC) determining that estradiol binds to CD with a higher affinity than estrone. As estradiol forms more stable inclusion complex with CD, in the present work we propose to evaluate the affinity of this drug with other CDs derivatives such as sulfobutyleter-β-CD (SBCD) and hydroxypropyl-β-CD (HPCD) as complement of our previous results. In this case, we use the fast and reliable properties of chronocoulometry as main technique to calculate the association constants. The method is based on the diminution observed by us [3] and other authors [4] in the diffusion of the guest when an inclusion complex is formed as compared than that for free guest.

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2. Experimental

2.1. Chemicals

β-CD (CD) was obtained from Calbiochem and was used without prior purification (Fig. 1). Hydroxypropyl-β-CD (HPCD) was supplied by SIGMA. Sulfobutyleter-β-CD (SBCD) [TDS (total degree of substitution) = 6–7; Captisol®] was purchased from CyDex, Inc. 17-β-estradiol (Fig. 1) were supplied by SIGMA. All solvents were HPLC grade and all the other reagents employed were analytical grade. All solutions were prepared with ultrapure water (18.2 MΩ cm) from a Millipore Milli-Q system. All the other reagents employed were analytical grade.

2.2. Differential pulse voltammetry and chronocoulometry

Differential pulse voltammetry (DPV) and chronocoulometry experiments were carried out using a potenciostat/galvanostat model 440A (CH Instruments Inc., USA) attached to a PC computer with proper software for total control of the experiments, data acquisition and treatment. An electrochemical cell with glassy carbon as working electrode, a platinum wire counter electrode, and an Ag/AgCl as reference electrode was used for the measurements.

All the chronocoulometry and voltammetric experiments were obtained after bubbling with N_2 for 10 min in the cell before each run. Temperature was kept constant at 25 ± 0.1 °C in all experiments.

Electrochemical parameters for DPV were selected according previous study [2]: potential scan rate 20 mV s^{-1}, pulse amplitude 50 mV, pulse width 50 ms. Chronocoulometry parameters were:

Initial potential 0 V, final potential 0.7 V, pulse width 0.25 s. Ten different pulse width values between 0.2 and 0.3 s were tested. No changes in diffusion coefficient values were obtained. Standard deviation was ±2% or less.

The area of the electrode was evaluated by measuring a suitable system $Fe(CN)_6^{3-}$, whose diffusion coefficient is known to be 7.6×10^{-6} cm^2/s [5].

3. Results and discussion

3.1. Differential pulse voltammetry study

The electrochemical behavior of estradiol on glassy carbon in aqueous solution has been previously studied [2,6]. Owing to a low solubility of estradiol in aqueous solutions, ethanol should be added to the solution to avoid precipitation of estradiol. A well-defined anodic peak current corresponding to the oxidation of estradiol was obtained at about 0.5 V (vs. Ag/AgCl) in buffer phosphate 0.1 M/ethanol (70/30) at pH 7.4 (solid line,Fig. 2).

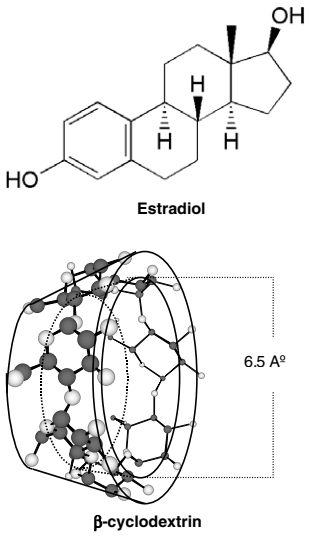

Fig. 1. Molecular structures of estradiol and β-Cyclodextrin.

The electrochemical behavior of estradiol in presence of CD has similar characteristics to those of free estradiol, no new signals were observed. But, a decrease of current intensity with increasing of CD concentration had been observed [2]. Similar behavior is observed upon addition of SBCD and HPCD. No new signals are seen even at higher positive potentials. Other studies performed by ourselves [7] of estradiol using thiolated cyclodextrin modified gold electrode showed an electrochemical response of estradiol at about 50 mV more positive potential than bare electrode. This fact demonstrates that estradiol is electroactive after entering the CD cavity with light difference in the energetic of the electron transfer process.

The dependence of peak current as a function of the concentration of each CD is shown in Fig. 3, being possible to observe a gradual decrease of the current in all the cases. However, the lowest values of current are obtained when SBCD is added to the solution. Besides, the oxidation peak potentials (E_p) are lightly shifted in a positive direction when the CD concentration increases. The shift in the peak potential and the decrease in peak current as the concentration of CD is increased would be the result of fast dissociation and association reactions assuming the formation of a labile complex estradiol–CD due to hydrophobic interactions.

3.2. Determination of the diffusion coefficient of estradiol–CD complexes

As it was described before [4] the observed decrease of the current is mainly due to a smaller diffusion coefficient of the bulk cyclodextrin complex compared to that of free guest, i.e. as the drug is incorporated into the cavity, its diffusion coefficient is reduced due to the larger complex formed. Therefore, a decrease in estradiol diffusion would confirm its incorporation as the CD concentration increases. The diffusion coefficients of estradiol in the absence (D_f) and in presence of CD (D_C) have been determined by

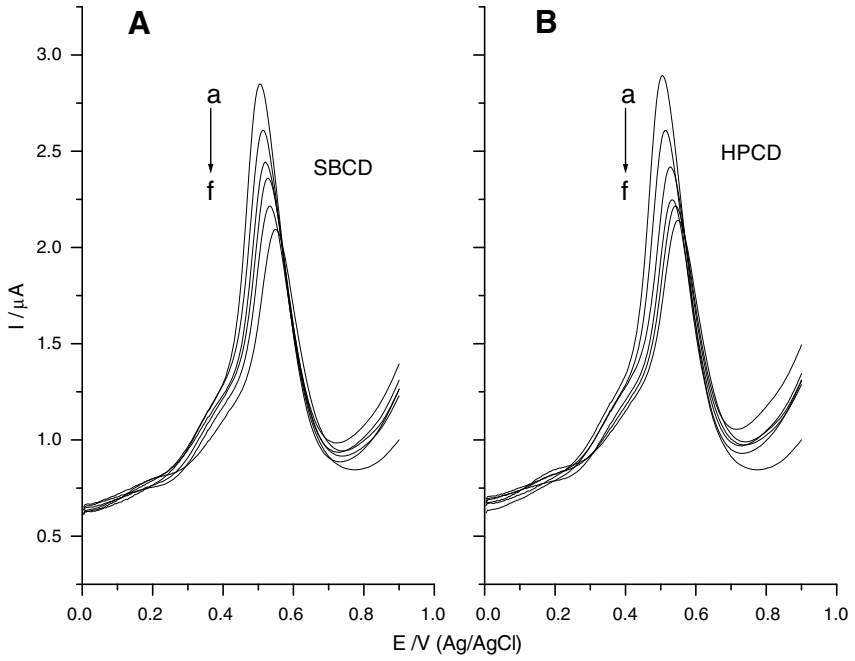

Fig. 2. Differential pulse voltammetry curves for 1×10^{-4} M estradiol in buffer phosphate/ethanol (70/30) at pH 7.0 in the absence and the presence of SBCD (A) and HPCD (B). Potential scan rate 20 mV s^{-1}, pulse amplitude 50 mV, pulse width 50 ms. Curves a–f: 0, 2, 4, 6, 8, 10 mM SBCD or HPCD.

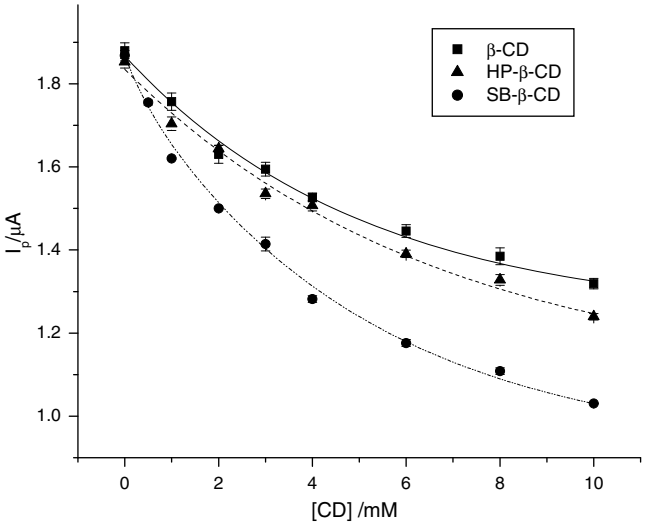

Fig. 3. Current dependence on the concentration of CD, SBCD and HPCD for estradiol in buffer phosphate/ethanol (70/30) at pH 7.0. Current values obtained from DPV measurements.

means of chronocoulometry (CC). According to the theory [8] the chronocoulometric curves obey the following equation:

$$Q = \frac{2nFAC(Dt)^{1/2}}{\pi^{1/2}} + Q_{dl} + Q_{ads} \quad (1)$$

wherein Q is the total coulombs, n is the electron transfer number, F is the Faraday constant (96487 C mol^{-1}); D is the diffusion coefficient (cm^2 s^{-1}), C is the concentration of reactant (mol mL^{-1}), t is time (s), A is the area of the electrode (cm^2), Q_{dl} is the double-layer charge (integration of charging current), Q_{ads} is the faradaic component given by the reduction of adsorbed species. Therefore, from the slope of the linear plots obtained between Q and $t^{1/2}$ the diffusion coefficient can be determined (Anson plot).

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 range of 0–10 mM, no change in solution viscosity could be detected [9]. On the other hand, chronocoulometric experiments are fast enough to maintain equilibrium on the time scale of the experiment.

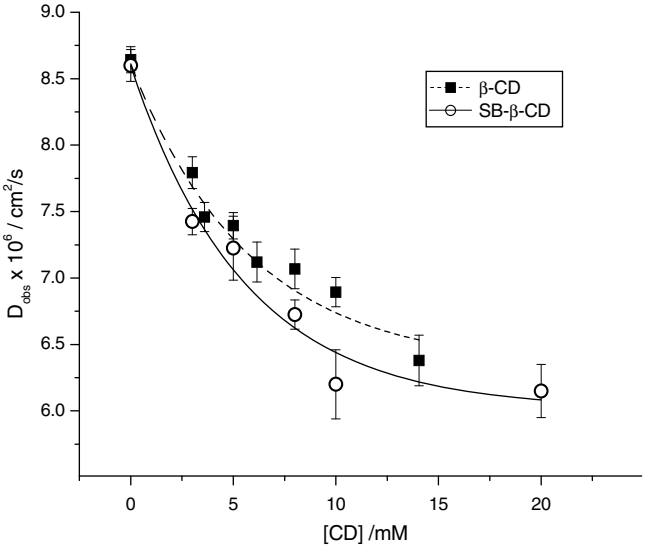

Fig. 4. Changes in the observed diffusion coefficients, D_{obs}, of estradiol in solution of buffer phosphate/ethanol (70/30) at pH 7.0 vs. CDs concentration.

Table 1
Association constant of the inclusion complexes (K_a) of estradiol with CD determined by Cronocoulometry

	K_a/M^{-1}	D_c/cm^2 s^{-1}
CD	132 ± 18	$5.3 \times 10^{-6} \pm 1.0 \times 10^{-7}$
HPCD	247 ± 32	$5.9 \times 10^{-6} \pm 1.2 \times 10^{-7}$
SBCD	276 ± 28	$5.6 \times 10^{-6} \pm 1.1 \times 10^{-7}$

A decrease of the observed diffusion coefficients (D_{obs}) of estradiol-CD mixture in 0.1 M phosphate buffer/ethanol (70/30) determined at different concentrations of each CD was observed. This effect can be seen in Fig. 4 where CD and SBCD are shown. The behavior of HPCD is similar to those and it is no shown to give major clarity to the figure. Special precautions were taken in performing the experiments, providing that the reproducibility of the results obtained was ±2% or less. The concentration of β-CD changed from about 3 mM to 10 mM. In the case of SBCD and HPCD, which are more soluble than CD, the highest concentration studied was 20 mM.

In the case of estradiol in the absence of CD, a diffusion coefficient value of $D = 8.6 \times 10^{-6} \pm 0.1 \times 10^{-6}$ cm^2 s^{-1} was determined. This value is slightly higher than values previously reported in water [10–12]. But, the presence of ethanol in this media could provoke an increase in diffusion coefficient.

3.3. Association constants

According Taraszewska and Piasecki [4], the stability constants of the inclusion complexes 1:1 can be obtained from diffusion coefficients of the free guest molecule, D_f, and the observed diffusion coefficients, D_{obs}, of the guest molecule in presence of different CD concentrations. Using the equation given previously by Osa et al. [13], the association constant and D_c values are obtained from the slope and the intercept of a linear plot of D_{obs} vs. (D_f—D_{obs})/[CD]. The association constant of estradiol with CD, SBCD and HPCD and diffusion coefficient of the complex, D_c, are reported in Table 1. Estradiol with CDs 1:1 inclusion complexes have been reported [14]. Our results show that the lower value of K_a is obtained with β-CD compared to SBCD and HPCD correlated with the current decrease observed in Fig. 3. Therefore, the complexes with SBCD and HPCD are much stronger than with CD. Nevertheless, taking into account the results showed in Fig. 3, a greater difference between K_a of estradiol with SBCD and HPCD would be expected because, according the comparison of SBCD and HPCD with several substrates performed by Zia et al. [15,16], neutral molecules displayed a stronger interaction with SBCD compared to HPCD and, at pH 7.0 estradiol is a non-ionic species (pK_a of estradiol is 10.4 [17]). One explanation could be the calculated K_a represents an average of that it is occurring at all the CD concentrations studied. Then, there is no great difference between HPCD and SBCD at low concentrations. On the other hand, there is a significant difference and reactivity at high concentration, then K_a reflects both facts.

4. Conclusions

From the above results it may be concluded that chronocoulometry is a successful method for the investigation of inclusion complexes. The quantitative parameters, such as diffusion constant and association constant for estradiol and CDs inclusion complexes were obtained from the chronocoulometric experiments.

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