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Inclusion complexes of estrone and estradiol with β-cyclodextrin: Voltammetric and HPLC studies

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ABSTRACT: The interaction of estrone and estradiol with β-cyclodextrins (βCD) was investigated by differential pulse voltammetry (DPV) and high-performance liquid chromatography (HPLC) in mixed media. The co-solvent influence on the tendency of these estrogens to form inclusion complexes with βCD was examined. Thus, acetonitrile (MeCN) and ethanol (EtOH) were used in a mixed aqueous medium containing phosphate buffer. The association constant of the inclusion complexes (K_a) of estrone and estradiol with βCD were determined in two different media by using both voltammetric and chromatographic techniques. Estradiol was found to bind to βCD with higher affinities than estrone, irrespective of the medium. We have also found a clear influence of the co-solvent on the K_a value, which means a competition of co-solvent molecules with estrogens for binding to the cavity of βCD. Consequently, interaction between βCD and the steroids is weakened when acetonitrile is used. Copyright © 2007 John Wiley & Sons, Ltd.

KEYWORDS: β-cyclodextrin; inclusion complexes; steroids

INTRODUCTION

Cyclodextrins (CDs) are cyclic compounds that have been widely used in the pharmaceutical industry due to their ability to form inclusion complexes. This property has been used to solubilize, stabilize, decrease the volatility of drug molecules, and improve bioavailability.[1] To predict the effects of CDs on these properties, it is important to know the strength of binding of these agents to drugs. Therefore, determination of the association constant (K_a) of cyclodextrin/guest complexes is very important because it accounts for the magnitude of interaction between host and guest molecules.

Several techniques have been used to examine the interaction between CD and different drugs, including spectroscopic,[2] calorimetric,[3] and chromatographic methods.[4–6] Some voltammetric methods have also been used to study the formation of some inclusion complexes of βCD in aqueous[7] and mixed[8] media. Furthermore, a polarographic method has also been used to study α- and βCD interaction with chloronitrobenzenes,[9] and with the supramolecular system, meso-tetrakis (4-*N*-trimethylaminobenzyl) porphyrin, with different CDs.[10]

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In this work, we evaluate the utilization of a voltammetric technique to determine βCD interaction with some steroids of interest such as estrone and estradiol.

Estrone and estradiol are naturally occurring steroid hormones (estrogens) essential for the development and maintenance of female sexual characteristics. Moreover, estrogens are known to be powerful antioxidants independently of their binding to estrogen receptors and hormonal functions.[11] CD-encapsulated steroids are widely used in drug formulation[12,13] resulting in beneficial pharmaceutical effects including increase in water solubility and stability. Studies of steroid/cyclodextrin complexes are interesting from the point of view of drug design.[14] In this process, formulation becomes an important aspect based on the need for appropriate dosage form, stability, solubility, and dissolution characteristics.[15] In spite of the number of publications in this area, there are only about 30 different pharmaceutical products on the market. On this way, investigations of interactions and structure of the complexes are required because it is important for a pharmaceutical formulator to know an optimized cyclodextrin/drug relationship in relation to drug delivery from the formulations. Inclusion complexes of estrogen-related steroids with cyclodextrins have been studied principally by high-performance liquid chromatography (HPLC).[1,5] The stoichiometry and binding constants of the complexes under study have been

established by this technique. Therefore, assuming a 1:1 stoichiometry as it was previously established, we have applied electrochemical techniques to the study of steroid/cyclodextrin inclusion complexes.

To the best of our knowledge, no electrochemical methods have been described for steroid/βCD interaction. On the other hand, according to Bednarek *et al*.,[2] the poor solubility of these systems makes accurate association constant determination difficult to conduct. Considering the poor solubility of steroids in aqueous solutions and the capability of electrochemical techniques to work with diluted solutions, we expect that this novel method will represent an improvement in the study of steroid/cyclodextrin interaction.

The aim of this study was to investigate the feasibility of formation of inclusion complexes between estrone and estradiol with βCD. In addition, we combined electrochemical techniques, that is, differential pulse voltammetry (DPV) with HPLC studies in order to obtain further information about the dynamics of cyclodextrin complexation.

EXPERIMENTAL

Reagents and solutions

βCD was obtained from Calbiochem and was used without prior purification. Estrone and 17-β-estradiol were supplied by SIGMA (Fig. 1). All solvents were of HPLC grade and all the other reagents employed were of analytical grade. All solutions were prepared with ultrapure water (rho = 18.2 MΩ cm) from a Millipore Milli-Q system.

All the 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 the experiments.

Apparatus

Electrochemical experiments were carried out using a totally automated BAS-50 voltammetric analyzer attached to a PC computer with proper software (BAS 50-W version 2.0) for total control of the experiments and data acquisition and treatment.

DPV and Cyclic voltammetry (CV) experiments were carried out using glassy carbon as working electrode. A platinum wire counter electrode and an Ag/AgCl as reference electrode were used for the measurements.

HPLC measurements were carried out by using a Waters assembly equipped with a model 600 Controller pump and a model 996 Photodiode Array Detector. Data acquisition and treatment were performed by means of the Millennium version 2.1 software. As a chromatographic column and a guard column, Symmetry® C18 (3.9×150 mm) and Symmetry® C18 5 μm (3.9×20 mm) were employed, respectively. The injector was a 20 μL Rheodyne valve, model 7125.

Methods

Voltammetric experiments. Current titrations were carried out by keeping constant estrone or estradiol concentrations (1×10^{-4} M) while varying βCD concentrations (0 to 15 mM). Each solution was prepared independently. Two different mixed media were used: MeCN/0.1 M phosphate buffer (35/65) and EtOH/0.1 M phosphate buffer (25/75). All the experiments were performed at pH 5.0. The solutions were shaken thoroughly for 10 min and allowed to reach equilibrium at room temperature. The working electrode was polished between each measurement with 0.3 μm alumina (Buehler). The final polishing was done with 0.05 μm alumina and was followed by a thorough rinse with water.

The current titration equation has been described as follows[16–19] and has been deduced according to references[20–22]

$$\frac{1}{[\beta CD]} = K_a \frac{(1-A)}{1 - I_p/I_{p,0}} - K_a \qquad (1)$$

where K_a is the complex association constant, $I_{p,0}$ and I_p are the peak currents in the absence and in the presence of βCD, respectively. [βCD] is the molar concentration of βCD and A is a constant. This equation can be used if a 1:1 association complex is formed and if CD concentrations are much larger than the total concentration of the drug. The current values were obtained as the average of at least five independent determinations of each solute.

Selection of electrochemical parameters. Differential pulse voltammetry current is dependent on electrochemical parameters, such as scan rate, amplitude, and width of the pulse. The effects of these parameters were studied in order to optimize conditions for the oxidation of estrone and estradiol. The influence of scan rate on oxidation current was investigated. Scan rate values from 5 to 40 mV s^{-1} were tested and the results showed that both oxidation current and background increased with scan rate. However, the ratio of the peak current to the blank, $I_{p,0}/I_B$, reached a maximum using a scan rate of 20 mV s^{-1}, which was adopted for all subsequent experiments.

The amplitude and width of the pulse varied from 30 to 80 mV and from 30 to 80 ms, respectively. These were found not to affect symmetry to a great extent, but the sensitivity of the peak was better using 50 mV pulse amplitude and 50 ms pulse width.

HPLC experiments. An aliquot of solution of each steroid was taken and 20 μL volume was injected into the chromatographic system. A mixture of MeCN/0.05 M phosphate buffer (35/65) at pH 5.0 containing different

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cyclodextrin concentrations was used as mobile phase. The photodiode array (PDA) detector operated at 280 nm for both steroids. Mobile phase flow was kept at 1 mL/min and helium bubbling of 30 mL/min was applied to remove dissolved gases.

The association constant was obtained using:[5]

$$\frac{1}{k'} = [\beta CD]\frac{K_a}{k_0} + \frac{1}{k_0}$$

where, k' and k_0 are the capacity factor with and without CD, respectively. [βCD] is the βCD concentration in the mobile phase, and K_a is the complex association constant.

Capacity factors were calculated as usual using sample retention time (*tr*) and dead time (*tm*). Dead time was measured by injecting 20 μL of methanol at each temperature for each mobile phase.

$$k' = \frac{(tr - tm)}{tm}$$

Capacity factors are based on the average of at least five independent determinations of each solute at each temperature.

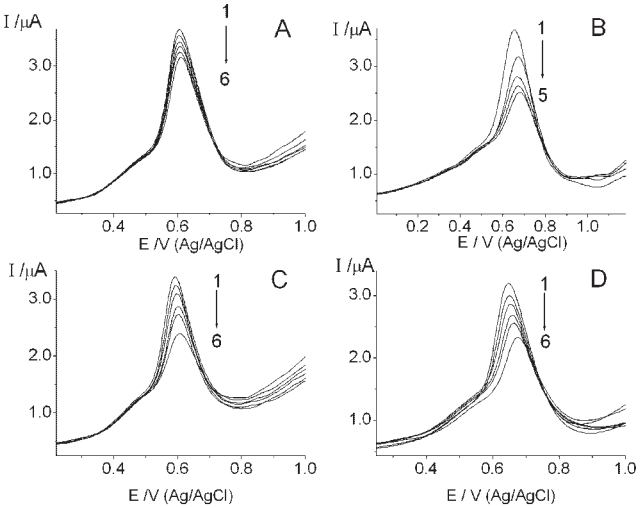

Figure 2. Differential pulse voltammetry curves for 1×10^{-4} M estrone in MeCN/buffer phosphate (A) and EtOH/buffer phosphate (B) and for 1×10^{-4} M estradiol in MeCN/buffer phosphate (C) and EtOH/buffer phosphate (D) in the absence (1) and the presence of (2) 2.5, (3) 4.0, (4) 5.0, (5) 7.5, (6) 10 mm of βCD. Potential scan rate 20 mV s^{-1}, pulse amplitude 50 mV, pulse width 50 ms

RESULTS AND DISCUSSION

Differential pulse voltammetry of 0.1 mM estradiol and estrone solutions in two different mixed media (MeCN/buffer and EtOH/buffer) at pH 5.0 was carried out. As shown in Fig. 2 (lines in the absence of βCD), estradiol and estrone displayed well-resolved voltammetric peaks on glassy carbon electrode. The peak potential is slightly more positive for estrone than for estradiol (604 and 592 mV in MeCN/buffer; 659 and 648 mV in EtOH/buffer, respectively). According to a previous study[23] related to electrochemical oxidation of estradiol, it is possible to affirm that the electrode process involves a two-electron transfer producing a phenoxonium ion. This phenoxonium ion forms a ketone by a further chemical reaction. We have also obtained cyclic voltammograms of estradiol and estrone in mixed media. Both compounds produced an irreversible wave on glassy carbon electrode at sweep rates between 0.1 and 3 V/s (Fig. 3). As expected, the current peak in the absence of βCD, $I_{P,0}$, increased with scan rate (v).[24] Furthermore, the plot between log $I_{P,0}$ *versus* log v (inset Fig. 3) was linear and showed slope values of 0.82 and 0.65 for estradiol and estrone, respectively. When a diffusion process takes place, a slope of 0.5 is obtained and a slope of 1 is obtained for an adsorption process. Intermediate values of the slope suggest a 'mixed' diffusion–adsorption process.[25] Thus, the electrochemical process has a mixed control for these steroids.

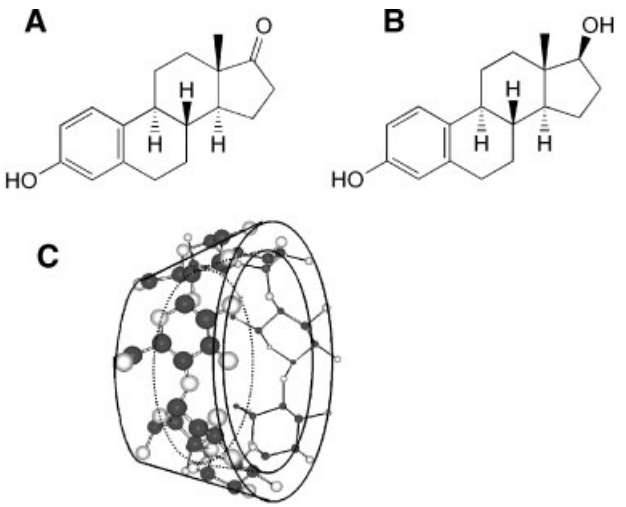

Figure 1. Molecular structures of estrone (A), estradiol (B), and β-cyclodextrin (C)

The effect of βCD concentration on the DPV response of estrone and estradiol is shown in Fig. 2. A decrease of current intensity with increasing βCD concentration is observed. This effect is due to the decrease in the apparent diffusion coefficient of estrogen, when it is complexed with βCD. Both, guest and host are in equilibrium with the complex. However, cyclodextrin has no redox processes. Therefore the current response is the result of the oxidation of both the free estradiol and the complexed estradiol molecule. In order to confirm that formation and dissociation of the inclusion complex are fast enough to maintain equilibrium on the time scale of the experiment, we have repeated the experiments at different pulse widths. We obtained the ratio of oxidation peak current to its value in the absence of βCD, $I_{p,0}$ as a function of pulse width. This ratio was measured at different βCD concentrations (2, 5, 7.5, and 10 mM). Two of these different concentrations are shown in Fig. 4.

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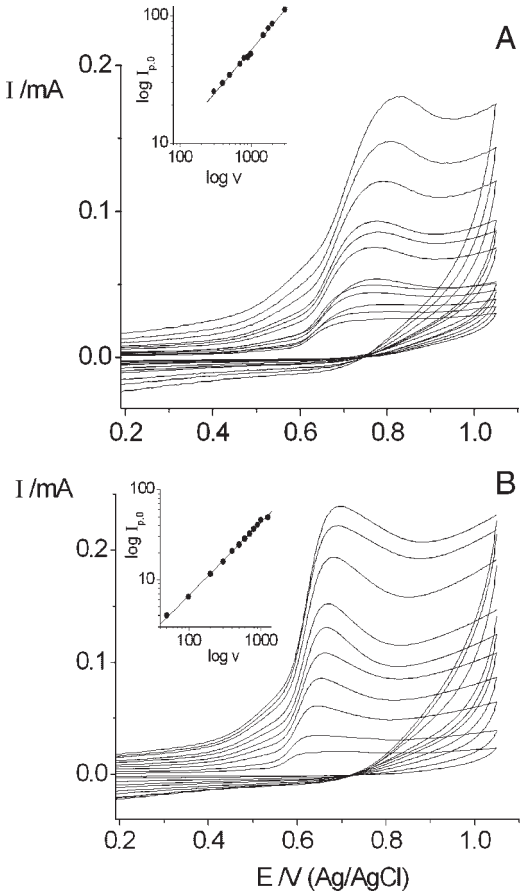

Figure 3. Cyclic voltammograms of estrone (A) and estradiol (B) in MeCN/buffer phosphate at different sweep rates. Inset: Dependence log of the peak current on sweep rate for each set of experiments

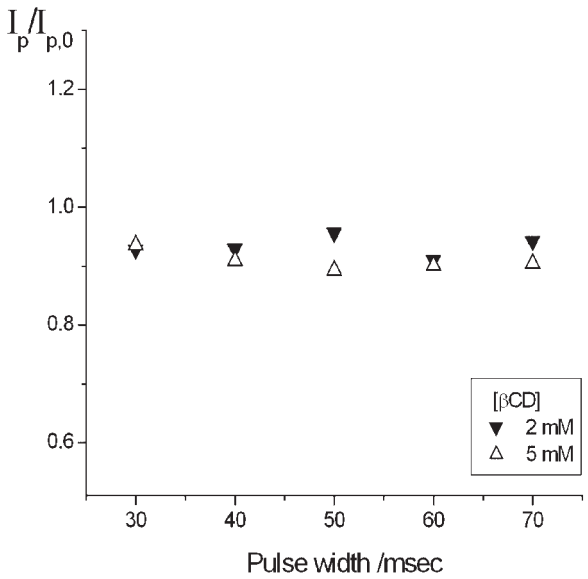

Figure 4. Ratio of oxidation peak current in the presence of βCD (I_p) to oxidation peak current in the absence of βCD ($I_{p,0}$) as a function of pulse width at 2 and 5 mm of βCD

Similar results were obtained for all βCD concentrations. We can see that there is no influence of the pulse width, showing that the system remains close to equilibrium at the scan rates tested. In other words, this equilibrium is not disturbed by the electrode process. With regards to oxidation peak potential, there is no change with the increase in βCD concentration. Considering that Sadlej-Sosnowska[26] has described that these steroids can form inclusion complexes with βCD at a stoichiometric ratio 1:1, we have assumed the same ratio for this study. As can be seen in Fig. 5, there is a lower decrease in current intensity when βCD is interacting with estrone as compared with estradiol, revealing a difference in the interaction strength of the two complexes. Using the equation for current titration previously described (Eqn 1 in Experimental), the complex association constant (K_a) for estrone/βCD and estradiol/βCD can be obtained. According to the above Eqn 1, linear plots between $[CD]^{-1}$ and $(1\text{-}I_p/I_{p,0})^{-1}$ were obtained confirming the existence of a 1:1 type complex (Fig. 6). Association constant values (K_a) for estrone and estradiol in both media are shown in Table 1. The different resulting K_a values suggest that inclusion possibly takes place through the penetration of the D-ring of these steroids into the cavity of the βCD. In spite of the special precautions in performing the experiments, the reproducibility of the results obtained was higher than 10% as can be seen in Table 1. As is often observed with solid electrodes, the results depend upon the treatment of the electrode surface. For glassy carbon electrodes, a common method involves polishing with alumina as it was previously described in Experimental. Thus, an acceptable degree of reproducibility can be obtained (about 5%).[9] However, the reproducibility in our case might be related to 'mixed' diffusion–adsorption process of these steroids on glassy carbon.

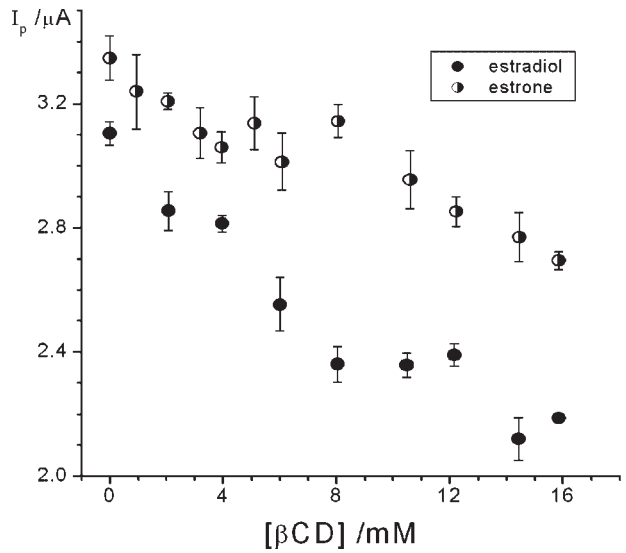

Figure 5. Current dependence on the concentration of the βCD for estradiol (●) and estrone (◑) in MeCN/buffer phosphate. Current values obtained from DPV measurements

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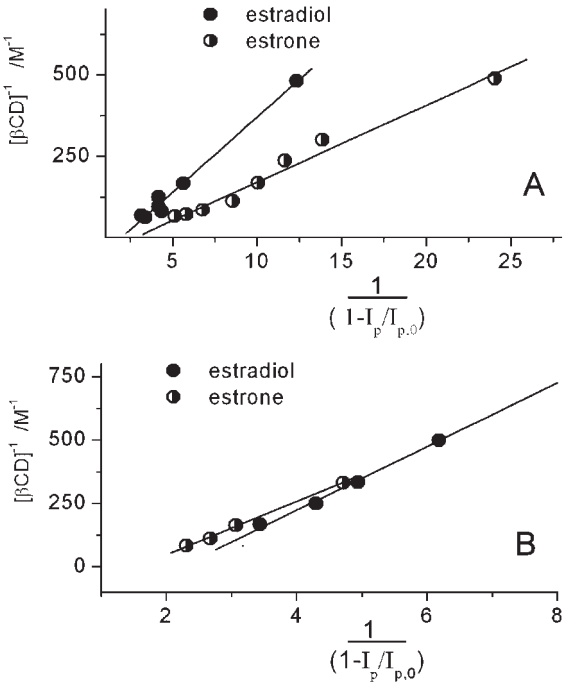

Figure 6. Plot of 1/[CD] *versus* $1/(1-I_p/I_{p,0})$ for estradiol (●) and estrone (◑) in MeCN/buffer phosphate (A) and EtOH/buffer phosphate (B). Current values obtained from DPV measurements

Moreover, according to the above results, it is clear that there is a co-solvent effect on the interaction of these estrogens with the cavity of cyclodextrin. In spite of the fact that the constants reported in the literature for the association of βCD to the co-solvent molecules are negligibly small (K_a of acetonitrile with βCD[27] is $6.0\,M^{-1}$, whereas K_a of ethanol with βCD[28,29] is $0.94\,M^{-1}$), the difference observed can be explained by the encapsulation of the co-solvent molecule by the cyclodextrin, in competition with the CD/steroid complexes formation.

The inclusion of substrates into the cyclodextrin cavity has been attributed to several binding forces including van der Waals forces, hydrophobic effect, hydrogen bonding, macrocycle relaxation, and the release of energetic water molecules from the cavity.[30,31] Actually, some other factors are involved in the formation of the inclusion complexes, such as temperature, solvent polarity, and ion strength. The presence of charged species, molecular conformations, and guest orientation can also be considered as critical for the strength of the complex stability.

Table 1. Association constant of the inclusion complexes (K_a) of estradiol and estrone with βCD determined by DPV

	K_a/M^{-1}	
	MeCN/buffer 35/65	EtOH/buffer 25/75
Estradiol	112 ± 14	200 ± 32
Estrone	64 ± 8	138 ± 20

The effect of a co-solvent has been studied by some authors. According to Junquera *et al.*,[32] when the association constant between the shortest alcohols and βCD are, at least, a couple of orders of magnitude lower than the association constant of CD/guest complex, the interaction with the co-solvent can be neglected. Thus, the effects observed in the presence of different alcohols used as co-solvent can be attributed only to a change in the solvophobic characteristics of the medium, which affects the affinity of an apolar drug in binding cyclodextrin. However, in the presence of other organic solvents, the higher the hydrophobic character of the medium due to the organic co-solvent, the lower the association to the apolar drug into the CD cavity. On the other hand, Al Omari *et al.*[33] have reported that the utilization of different buffers produces changes in the solubility of cyclodextrin and/or guest compound, affecting the K_a values.

Our voltammetric results are in accordance with Sadlej-Sosnowska[34] who found similar tendencies for the study of the interaction between CD and steroids using MeOH-H_20 (45:55) and MeCN-H_20 (30:70) as solvents. The values of K_a are higher in the first medium than in the second. Those results were interpreted as being a result of the more positive enthalpy change connected with the removal of co-solvent molecules from the CD cavity, and thus the different competition of MeOH and MeCN with the solute for binding to CD. The determination of interactions in alcoholic media is important since in the same cases, addition of ethanol enhances percutaneous absorption. An example of this is an anti-inflammatory drug such as sodium diclofenac.[35]

With the aim to compare the K_a values obtained by our voltammetric approach in similar conditions, HPLC experiments using MeCN/buffer were also performed. Thus, βCD was introduced in the mobile phase as described in Experimental. The retention times observed for each steroid were shorter than those observed in the absence of βCD. Chromatographic responses for estrone are shown in Fig. 7 (similar results are obtained for estradiol). Indeed, this indicates that there is some interaction degree between βCD and the steroids. Using solute retention time and dead time, k' values were determined for all CD concentrations at different temperatures. Linear plots of $1/k'$ *versus* [βCD], with a correlation coefficient higher than 0.998, were obtained in all cases (Fig. 8). According to Ravelet *et al.*,[6] this kind of behavior is obtained for inclusion complexes with 1:1 stoichiometry.

Furthermore, we have obtained K_a at different temperatures, as shown in Table 2. As expected, K_a decreased with increasing temperature. Our results are in accordance with Sadlej-Sosnowska[34] that reported K_a values of 191 and $92\,M^{-1}$ for estradiol and estrone, respectively, in MeCN:H_2O (30:70) at 30 °C. However,

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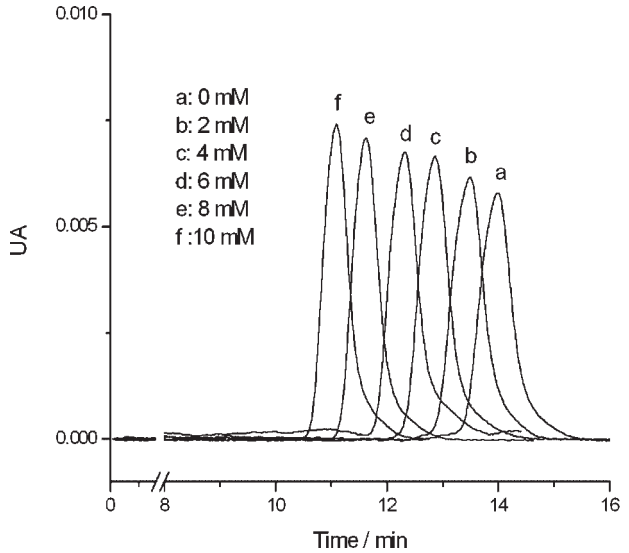

Figure 7. Chromatograms of estrone with different concentrations of βCD in mobile phase: (a) 0, (b) 2, (c) 4, (d) 6, (e) 8, and (f) 10 mM. Column, Symmetry C18; Flow at 1 mL/min; detection, UV 280 nm

K_a voltammetric values for estrone and estradiol with βCD differed from the K_a values as determined using HPLC technique. In fact, K_a values for steroid/βCD obtained from voltammetric measurements are lower than the association constant determined by HPLC

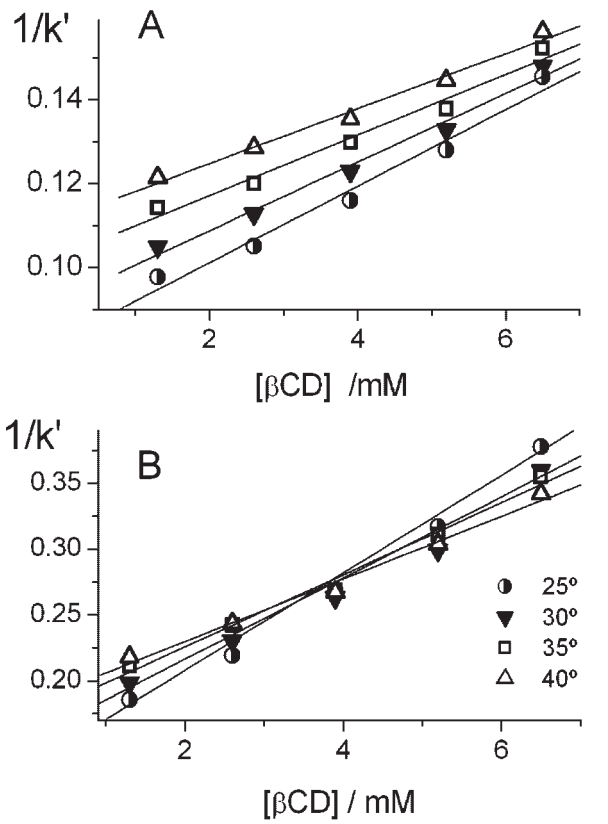

Figure 8. Plot 1/k' against [βCD] of estrone (A) and estradiol (B) at 25° (◑), 30° (▼), 35° (□), and 40° (△). Chromatographic conditions are the same as in Fig. 7

Table 2. Association constant of the inclusion complexes (K_a) of estradiol and estrone with βCD in MeCN/buffer 35/65 at different temperatures determined by HPLC

	K_a/M^{-1}			
	25°	30°	35°	40°
Estradiol	267 ± 6	194 ± 4	158 ± 4	126 ± 3
Estrone	89 ± 2	88 ± 2	76 ± 2	75 ± 2

experiments. In the case of estradiol, the difference between voltammetric and chromatographic values is substantially higher than in the case of estrone. Some factors can explain the observed results. It has been established by Rozou *et al.*[36–38] that chromatographic methods can be affected by other components such as multiple equilibriums. On the other hand, in the case of the great difference observed for estradiol, this is probably due to the effect of adsorption affecting voltammetric measurements (i.e., dlog I_P/dlog v values are 0.82 and 0.65 for estradiol and estrone, respectively). Consequently, the voltammetric method developed in the present study is recommended for diffusion controlled processes and it seems to be not adequate when 'mixed' diffusion–adsorption process takes place. However, this method was quite fast and gave a good approach to the difference of the binding strength of estrone and estradiol with βCD. Further work is in progress in order to quantify the impact of the adsorption process in Eqn 1.

CONCLUSIONS

The voltammetric interaction between βCD and the steroids, estradiol and estrone, produced a peak current decrease when βCD concentration was increased. We have used these changes and we have applied a voltammetric method to quantify the interaction. From the voltammetric results, it may be concluded that βCD forms inclusion complexes with the steroids estradiol and estrone. These complexes obeyed a 1:1 type stoichiometry and the resulting association constants were 112 and 64 M^{-1} for estradiol and estrone, respectively, in MeCN:Buffer (35:65) at 25 °C.

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REFERENCES

1. Rajewski RA, Stella VJ. *J. Pharm. Sci.* 1996; **85**: 1142–1169. DOI: 10.1021/js960075u.
2. Bednarek E, Bocian W, Poznanski J, Sitkowski J, Sadlej-Sosnowska N, Kozerski L. *Perkin Trans.* 2002; **2**: 999–1004. DOI: 10.1039/b110435g.

3. Li N, Xhang YH, Wu YN, Xiong XL, Zhang YH. *J. Pharm. Biomed. Anal.* 2005; **39**: 824–829. DOI: 10.1016/j.jpba.2005.05.011.
4. Sadlej-Sosnowska N. *J. Pharm. Biomed. Anal.* 1995; **13**: 701–704. DOI: 10.1016/0731-7085(95)01322-C.
5. Flood K, Reynolds E, Snow N. *J. Chromatogr. A*. 2000; **903**: 49–65. DOI: 10.1016/S0021-9673(00)00867-0.
6. Ravelet C, Geze A, Villet A, Grosset C, Ravel A, Wouessidjewe D, Peyrin E. *J. Pharm. Biomed. Anal.* 2002; **29**: 425–430. DOI: 10.1016/S0731-7085(02)00088-2.
7. Matsue T, Evans DH, Osa T, Kobayashi N. *J. Am. Chem. Soc.* 1985; **107**: 3411–3417. DOI: 10.1021/ja00298a002.
8. Matsue T, Akiba U, Suzufri K, Osa T. *Denki Kagaku.* 1985; **53**: 508–509 (in English).
9. Taraszewska J, Piasecki AK. *J. Electroanal. Chem.* 1987; **226**: 137–146. DOI: 10.1016/0022-0728(87)80039-6.
10. Wang XP, Pan JH, Li WH, Zhang Y. *Talanta.* 2001; **54**: 805–810. DOI: 10.1016/S0039-9140(00)00661-5.
11. Badeau M, Adlercreutz H, Kaihovaara P, Tikkanen MJ. *J. Steroid Biochem.* 2005; **96**: 271–278. DOI:10.1016/j.jsbmb.2005.04.034.
12. Fromming H, Szeitli J. In *Cyclodextrins in the pharmacy. Topics in inclusion science*, Vol. 5. Davies JED (ed.). Kluwer Akademic Publishers: The Netherlands, 1994; 151–193.
13. Stella VJ, Rajewski RA. *Pharm. Res.* 1997; **14**: 556–567. DOI: 10.1023/A:1012136608249.
14. Uekama K. *Chem. Pharm. Bull.* 2004; **52**: 900–915.
15. Loftsson T, Jarho P, Másson M, Järvinen T. *Expert Opin. Drug Deliv.* 2005; **2**: 335–351. DOI: 10.1517/17425247.2.1.335.
16. Zhao GC, Zhu JJ, Zhang JJ, Chen HY. *Anal. Chim. Acta* 1999; **394**: 337–344. DOI: 10.1016/S0003-2670(99)00292-5.
17. Ibrahim MS, Shehatta IS, Al-Nayeli AA. *J. Pharm. Biomed. Anal.* 2002; **28**: 217–225. DOI: 10.1016/S0731-7085(01)00624-0.
18. Yañez C, Nuñez-Vergara LJ, Squella JA. *Electroanal.* 2003; **15**: 1771–1777. DOI: 10.1002/elan.200302720.
19. Ureta-Zañartu MS, Yañez C, Gutierrez C. *J. Electroanal. Chem.* 2004; **569**: 275–286. DOI: 10.1016/j.jelechem.2004.03.011.
20. Kelly JM, Lyons MEG, van der Putten WJM. In *Electrochemistry, Sensors and Analysis, Analytical Chemistry Symposium series*, vol. 25. Smyth MR, Vos JG (eds). Elsevier: Amsterdam, 1986; 205.
21. Carter MT, Rodriguez M, Bard AJ. *J. Am. Chem. Soc.* 1989; **111**: 8901–8911. DOI: 10.1021/ja00206a020.
22. Feng Q, Li N, Jiang Y. *Anal. Chim. Acta.* 1997; **344**: 97–104. DOI: 10.1016/S0003-2670(97)00008-1.
23. Ngundi MM, Sadik OA, Yamaguchi T, Suye S. *Electrochem. Commun.* 2003; **5**: 61–67. DOI: 10.1016/S1388-2481(02)00538-6.
24. Bard AJ, Faulkner LR. *Electrochemical Methods, Fundamentals and Applications*. John Wiley and Sons, Inc.: New York, 1980; 218–219.
25. Gosser DK Jr. *Cyclic Voltammetry, Simulation and Analysis of Reaction Mechanisms*. VCH Publishers, Inc.: New York, 1993; 42–43.
26. Sadlej-Sosnowska N. *J. Incl. Phenom. Macro.* 1997; **27**: 31–40. DOI: 10.1023/A:1007964116987.
27. Taraszewska J. *J. Incl. Phenom. Macro.* 1991; **10**: 69–78. DOI: 10.1007/BF01041641.
28. Tee OS, Gadosy TA, Giorgi JB. *Can. J. Chem.* 1996; **74**: 736–744.
29. Tee OS, Gadosy TA, Giorgi JB. *J. Chem. Soc. Perkin Trans.* **2**: 1993; 1705–1706. DOI: 10.1039/P29930001705.
30. Rekharsky MV, Inoue Y. *Chem. Rev.* 1998; **98**: 1875–1917. DOI: 10.1021/cr9700179.
31. Liu L, Guo QX. *J. Incl. Phenom. Macro.* 2002; **42**: 1–14. DOI: 10.1023/A:1014520830813.
32. Junquera E, Ruiz D, Aicart E. *J. Colloid Interf. Sci.* 1999; **216**: 154–160. DOI: 10.1006/jcis.1999.6290.
33. Al Omari MM, Zughul MB, Davies JED, Badwan AA. *J. Incl. Phenom. Macro.* 2006; **5**: 159–164. DOI: 10.1007/s10847-006-9242-7.
34. Sadlej-Sosnowska N. *J. Chromatogr. A.* 1996; **728**: 89–95. DOI: 10.1016/0021-9673(95)01050-5.
35. Berner B, Mazzenga GC, Otte JH, Steffens RJ, Ebert CD. *J. Pharm. Sci.* 1989; **78**: 314–318.
36. Rozou S, Antoniadou-Vyza E. *J. Pharm. Biomed. Anal.* 1998; **18**: 899–905. DOI: 10.1016/S0731-7085(98)00227-1.
37. Rozou S, Antoniadou-Vyza E. *J. Chromatogr. A.* 2004; **1041**: 187–193. DOI: 10.1016/j.chroma.2004.05.002.
38. Rozou S, Michaleas S, Antoniadou-Vyza E. *J. Chromatogr. A.* 2005; **1087**: 86–94. DOI: 10.1016/j.chroma.2005.02.039.