Journal of Electroanalytical Chemistry 661 (2011) 179–185

Contents lists available at SciVerse ScienceDirect

Journal of Electroanalytical Chemistry

journal homepage: www.elsevier.com/locate/jelechem

A spectrophotometric and NMR study on the formation of an inclusion complex between dopamine and a sulfonated cyclodextrin host

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ARTICLE INFO

Article history:
Received 2 May 2011
Received in revised form 20 July 2011
Accepted 25 July 2011
Available online 9 August 2011

Keywords:
Inclusion complex
β-cyclodextrin
Dopamine

ABSTRACT

The formation of an inclusion complex between the sulfonated β-cyclodextrin (Sβ-CD) with dopamine (DA) was confirmed using UV–vis measurements and NMR methods. It was also established that a 1:1 complex was formed between the Sβ-CD and the DA, where the inclusion occurred predominantly through the aromatic ring of the DA and the hydrophobic CD cavity. The results also suggest that a change in the anion, of supporting electrolyte, had an influence on the DA-Sβ-CD complexation while no change was observed for the cation.

1. Introduction

Cyclodextrins (CD) are macrocyclic oligosaccharides composed of α-D-glucopyranoside units. The three common members are α-, β- and γ-CD, which have 6, 7, and 8 repeating glucopyranoside units, respectively. These units are orientated in a cyclic manner giving the typical conical or truncated cone structure with a relatively hydrophobic interior and a hydrophilic exterior [1]. This structure gives cyclodextrins their unique ability to form host–guest inclusion complexes with a wide range of suitably sized guest molecules [2–8]. In these complexes, the guest molecule is held within the cavity of the cyclodextrin host system. Penetration of the guest molecule into the cavity may be complete or, alternatively, only part of the guest molecule may fit within the cavity.

Formation of the host–guest complex can be easily monitored using techniques such as UV–visible spectroscopy (UV–vis), nuclear magnetic resonance (NMR), fluorescence spectroscopy, IR spectroscopy, electrochemical approaches and solubility measurements [9–11], as the physiochemical properties of the guest molecule caged within the host cavity is very different to that of the free guest.

Recently, charged cyclodextrins have received a lot of attention as chiral selectors in capillary electrophoresis [12–14]. It has been suggested that the anionic CDs provide maximum separation by migrating in opposite directions to the analyte [15]. In particular, sulfated and highly sulfated β-CDs have been used with some success [12–15]. A number of studies have been carried out on the characterization of these CDs. For example, Amini et al. [16] reported that sulfation occurs predominantly at the C-2 and C-6 positions, while, Chen et al. [17] confirmed nearly complete sulfation at the C-6 position of the primary hydroxyl groups and partial sulfation at the C-2 secondary hydroxyl groups. However, no substitution, or sulfation, occurred at the C-3 positions.

Although sulfonated cyclodextrins have been used in capillary electrophoresis, there have been very few studies devoted to the formation of inclusion complexes between sulfonated β-CD and guest molecules, or indeed between guest molecules and any anionic β-CD. One example is a study by Rajewski et al. [18,19] who investigated the role of charge by comparing the binding of neutral and charged cyclodextrins with neutral and charged guests. They found that the negatively charged sulfobutyl ether β-CD had a higher binding affinity than neutral β-CD for neutral guest species. In the case of papaverine, a protonated species, the binding constants were evaluated as 10 and 570, in the presence of the neutral β-CD and the negatively charged β-CD, respectively. It was concluded that the charge on the cyclodextrin gave a further site of interaction for the protonated guest molecule.

In this paper we investigate the formation of an inclusion complex between sulfonated β-CD and dopamine, a member of the catecholamine family. Catecholamines have a wide variety of biological functions ranging from hormones (adrenaline, noradrenaline), neurotransmitters (dopamine), aminoacids (tyrosine), melanin precursors (dopa) to therapeutic agents. Dopamine is also an interesting guest as it is protonated at near-neutral pH, providing a positively charged guest molecule. There are no reports, to the best of our knowledge, on the formation of inclusion complexes between DA and an anionic β-CD. UV–visible spectroscopy and ^{1}H NMR

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doi:10.1016/j.jelechem.2011.07.041

measurements were used to study the complexation process and to obtain a measure of the stability of the host–guest complex, while cyclic voltammetry was used to probe the influence of the supporting solution.

2. Experimental

Dopamine hydrochloric salt and sulfonated β-cyclodextrin sodium salt were purchased from Sigma–Aldrich and were used as received. The degree of sulfation was reported as 7–11 mol of sulfonated groups per mol of β-cyclodextrin (β-CD). All other reagents were of analytical grade and were obtained from either Sigma–Aldrich or Riedel de-Haen and were used as received. The glassy carbon (GC) (4 mm in diameter) was supplied by Goodfellow.

Spectrophotometric studies were carried out with a Cary 50 UV–visible spectrometer. The DA guest was kept at a constant concentration of 5.00×10^{-4} mol dm^{-3} in a citrate–phosphate buffer, while the concentration of the sulfonated β-CD host was varied over the range of 5.65×10^{-4} to 2.00×10^{-2} mol dm^{-3}. The UV absorption spectrum of each sample was obtained and the data were analysed at 280 nm (λ_{max} of DA). The citrate–phosphate buffer was formed by mixing 62.1 mL of 0.2 mol dm^{-3} Na_2HPO_4 and 37.9 mL of 0.1 mol dm^{-3} $C_6H_8O_7$ to give a pH of 6.0. Other pH values were obtained by adjusting the ratio of Na_2HPO_4 to $C_6H_8O_7$. In the Job's analysis, two stock solutions of 1.0×10^{-4} mol dm^{-3} DA and 1.0×10^{-4} mol dm^{-3} Sβ-CD were prepared and then mixed to give DA mole fractions varying from 0.0 to 1.0 in increments of 0.1. All experiments were carried out at least three times and the results presented are the average of all experiments.

^{1}H-NMR experiments were performed on a Bruker 300 MHz NMR spectrometer at 293 K in D_20 (>99.92% isotopic purity and purchased from Apollo Scientific). ^{1}H NMR peak protons were reported in ppm. A 0.1 mol dm^{-3} KCl was used to buffer the ionic strength, as the sulfonated β-CD is highly charged and ionised. Varying amounts of sulfonated β-CD dissolved in 0.1 mol dm^{-3} KCl/D_2O were added to a 5.00×10^{-4} mol dm^{-3} DA stock solution, made up in 0.1 mol dm^{-3} KCl/D_2O, in order to generate final sulfonated β-CD concentrations ranging from 1.00×10^{-4} to 2.50×10^{-3} mol dm^{-3}. The samples were allowed to equilibrate for 60 min before acquiring the ^{1}H NMR spectra. This is somewhat different to the method used in the literature where the ligand is added to the CD and variations in the proton resonances of the CD are followed. However, the NMR spectrum of the Sβ-CD is too complex (with 7–11 sulfonated groups, giving different isomers with overlapping and poorly defined signals). Accordingly, the aromatic region of the DA guest molecule was monitored on varying the concentration of the Sβ-CD to follow the formation of the inclusion complex.

Cyclic voltammetry was carried out using a Solartron Model SI 1285 potentiostat. All measurements were made at room temperature in a standard three-electrode cell with a glassy carbon (GC) electrode as the working electrode, saturated calomel electrode (SCE) as the reference electrode and a high surface area platinum wire as the counter electrode. The GC electrodes were encased into a larger insulating Teflon sheath and set in place using a non-conducting epoxy resin. Electrical contact was achieved using a copper wire. The cyclic voltammograms were recorded at 50 mV s^{-1} in the potential interval of −250–800 mV vs. SCE. The DA concentration was maintained fixed at 5.00×10^{-4} mol dm^{-3} in the supporting electrolyte at a constant pH, while the concentration of the sulfonated β-CD host was varied over the range of 3.12×10^{-4} to 2.00×10^{-2} mol dm^{-3}. Different supporting electrolytes were used and these included 0.2 mol dm^{-3} NaCl, 0.2 mol dm^{-3} KCl, 0.2 mol dm^{-3} $CaCl_2$, 0.2 mol dm^{-3} NH_4Cl, 0.2 mol dm^{-3} Na_2SO_4, 0.2 mol dm^{-3} Na_2HPO_4 and 0.2 mol dm^{-3} $Na_2H(C_3H_5O(COO)_3)$. All solutions were adjusted to a constant pH of 5.0. A citrate–phosphate buffer was also used.

3. Results and discussion

3.1. UV spectrophotometric approach

Dopamine absorbs in the UV region with an absorption maximum at 280 nm, while the sulfonated β-CD has little or no absorbance at this wavelength, making it easy to follow the formation of the DA-sulfonated β-CD using a spectrophotometric approach.

The absorption spectra of DA (λ_{max} = 280 nm) in the absence and presence of varying concentrations of sulfonated β-CD are overlaid in Fig. 1. It can be seen that increasing the concentrations of sulfonated β-CD gives rise to an overall decrease in band intensity at 280 nm, or a hypochromic effect. This hypochromic effect is shown more clearly in Fig. 2, which plots the absorbance value at 280 nm against the concentration of the sulfonated β-CD present in the sample solution. A simultaneous bathochromic shift is evident with increasing concentrations of sulfonated β-CD. This red shift of the spectral band to longer wavelengths, or bathochromic effect, has been reported previously with other guest molecules and has been explained in terms of a change in the environment of the molecule as it is included within the cavity of the CD [20–22]. However, it is important to state that this method, although normally used in most of the reported UV studies, is only correct when the effect caused by the presence of the cyclodextrin is just the increase in the absorbance of a given peak, without any wavelength shift. This is the case of the spectra reported herein. An isosbestic point at 282 nm is also clearly evident in Fig. 1, indicating the presence of two chemically different DA species, one free and the other complexed. As shown in Fig. 2, the absorbance becomes smaller with increasing concentrations of sulfonated β-CD, reaching a near constant value at concentrations close to 0.01 mol dm^{-3} suggesting that the DA is completely included within the cavity when a large excess of the sulfonated β-CD is present in solution. A similar observation was made by Yanez et al. [23] for the complexation of nicardipine with β-CD. These changes in the absorption spectrum of DA upon addition of the sulfonated β-CD are consistent with the formation of an inclusion complex between DA and the sulfonated β-CD.

In order to obtain information on the stoichiometry of the inclusion complex, a Job's plot was generated using the UV data. The absorbance spectra of different solutions of DA and sulfonated β-CD, where the mole fraction of DA was varied from 0.0 to 1.0 in increments of 0.1, were recorded. The change in the absorbance at 280 nm relative to that of an equal concentration of free DA

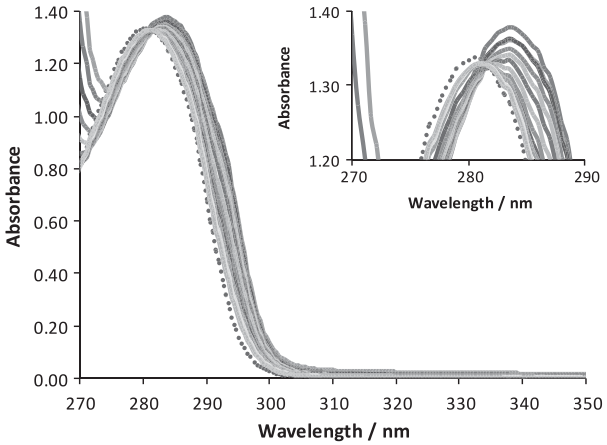


Fig. 1. UV spectra of 5.00×10^{-4} mol dm^{-3}DA in the absence (- - - - -) and presence of varying amounts of sulfonated β-CD from 5.65×10^{-4} to 2.00×10^{-2} mol dm^{-3} (——) in a citrate–phosphate buffer, pH = 6.0. Inset highlights the280 nm wavelength of interest.

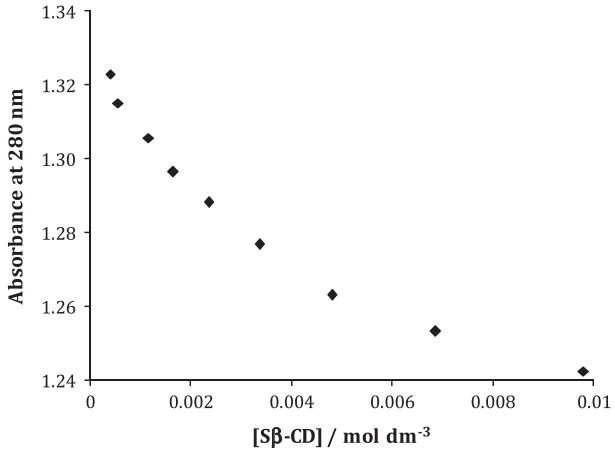


Fig. 2. Absorbance of 5.0×10^{-4} mol dm^{-3} DA recorded at 280 nm as a function of the concentration of sulfonated β-CD in a citrate–phosphate buffer, pH = 6.0.

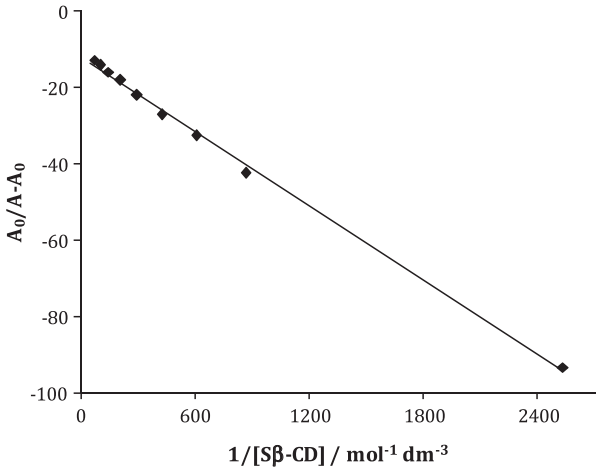


Fig. 4. Plot of $(A_0/A_0 - A)$ as a function of (1/[Sβ-CD]) for data recorded with 5.0×10^{-4} mol dm^{-3} DA in the presence of sulfonated β-CD in a citrate–phosphate buffer, pH = 6.0.

was computed as $\Delta A = A_{DA} - A_{DA\text{-}\beta\text{-}CD}$ and this was then used to generate the Job's plot. A typical Job's plot is presented in Fig. 3. It is clear that the maximum absorbance is obtained at a mole fraction of 0.5, indicating a 1:1 stoichiometric ratio for the inclusion complex, i.e., one DA molecule included in one sulfonated β-CD host.

The association constant for the inclusion complex was evaluated using the Heildebrand–Benesi relationship provided in Eq. (1), where A_0 and A are the absorbencies of the free guest and the complex, respectively, and ε_G and ε_{H-G} are the absorption, or extinction coefficients of the guest and the complex, respectively.

$$\frac{A_0}{A - A_0} = \frac{\varepsilon_G}{\varepsilon_{H-G} - \varepsilon_G} + \frac{\varepsilon_G}{\varepsilon_{H-G} - \varepsilon_G} \times \frac{1}{K_f[\mathrm{CD}]} \quad (1)$$

In Fig. 4, a Heildebrand–Benesi plot is presented, giving a linear relationship with a correlation coefficient of 0.997. From the intercept and slope, a K_f value of 336.92 ± 24.83 was computed, indicating a weak inclusion complex.

3.2. NMR study

^{1}H NMR spectroscopy is particularly useful as it gives direct and detailed information on the dynamics of the system and on the individual nuclei which are involved in forming the inclusion complex. However, in this analysis, the NMR study was used only to validate the UV–Vis measurements and to give some indications

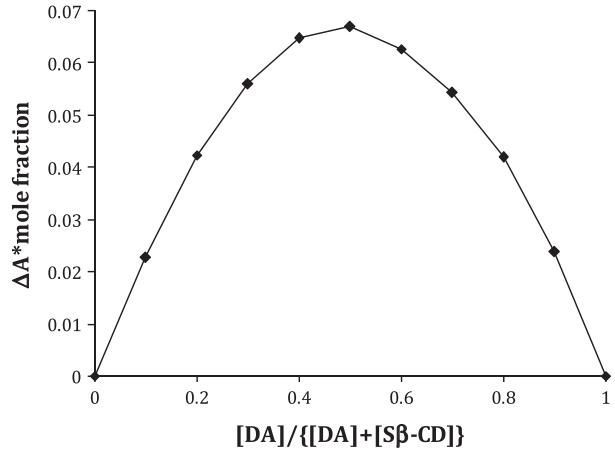


Fig. 3. Job's plot curve generated from UV data recorded for DA with various amounts of sulfonated β-CD in a citrate–phosphate buffer, pH = 6.0.

of a probable geometry of the inclusion complex. Due to the complex ^{1}H NMR spectrum of the Sβ-CD with overlapping signals, only the chemical shifts of the aromatic DA protons could be monitored. Moreover, meaningful two-dimensional correlations, such as NOESY, which involve an analysis of the protons from the Sβ-CD could not be extracted.

The spectrum of the DA in a deuterated solvent was first measured, small aliquots of Sβ-CD were then added and the spectrum was recorded. In all these experiments, the concentration of the DA guest was kept constant and the concentration of the sulfonated β-CD host was varied. Fig. 5a shows the aromatic region of the ^{1}H NMR spectra of 5.0×10^{-3} mol dm^{-3} DA and a mixture of 5.0×10^{-3} DA and 0.02 mol dm^{-3} sulfonated β-CD. The letters shown on the plot represent the aromatic protons depicted in Fig. 5b, and serve to illustrate the chemical shift of the individual protons in the presence of the sulfonated β-CD. It is evident from Fig. 5a that the chemical shift of the a–H proton in DA is significant, with $\Delta\delta$ at 0.106 ppm. There is less of a chemical shift in the b–H proton, $\Delta\delta$ = 0.017 ppm, while the chemical shift of the c–H is negligible, with $\Delta\delta$ at 0.002 ppm, indicating that it remains outside the CD cavity. This upfield, or low frequency shift, of the aromatic a–H protons on the DA molecule indicates a shielding effect, which is probably due to the increase in the electron density inside the cavity from the non-bonding electron pairs of the glycosidic oxygen bridges [24]. This is clear evidence that the aromatic ring of the DA molecule penetrates the cavity of the sulfonated β-CD.

The formation, or association, constant for the inclusion complex was evaluated using the NMR data and a non-linear least square analysis using the relationships, described as follows [25].

$$\delta = \delta_h - \frac{\Delta\delta}{2}\left(b - \sqrt{b^2 - 4R}\right) \quad (2)$$

where

$$b = 1 + R + \frac{1}{(K[\mathrm{CD}])}$$

Here, δ is the observed chemical shift of the protons, δ_h is the chemical shift observed in the presence of the sulfonated β-CD and R is the mole fraction. In Fig. 6 a plot of $\Delta\delta$ of the a–H and b–H protons of DA as a function of the sulfonated β-CD molar ratio is presented. Using a non-linear curve fitting method, the K_f value was determined as 384.5 ± 164.8 and 394.39 ± 163.8 for the a–H and b–H

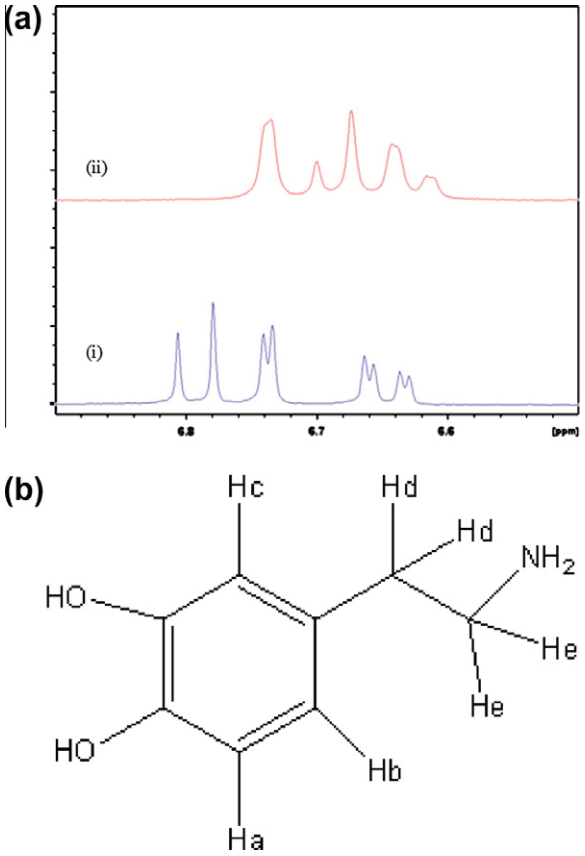


Fig. 5. (a) ^{1}H NMR spectra of the DA aromatic region in D_2O and 0.1 mol dm^{-3} KCl for (i) 5×10^{-4} mol dm^{-3} DA, (ii) 5×10^{-4} mol dm^{-3} DA and 0.02 mol dm^{-3} sulfonated β-CD. (b) Labeled protons in DA molecule.

protons, respectively. This is in good agreement with the value of 336.92 ± 24.83 obtained with the spectrophotometric approach.

A schematic of the probable inclusion complex is presented in Fig. 7, which shows the c–H proton and the protonated amine group outside the cavity and the a–H proton residing deep within the cavity. It is highly probable that the protonated amine group is bound electrostatically by the anionic sulfonated groups on the rim of the cavity. Indeed, Bratu et al. [26] observed that the methylene groups of fenbufen remained outside the cavity of neutral β-CD and the fenbufen molecule entered from the larger side or the second-

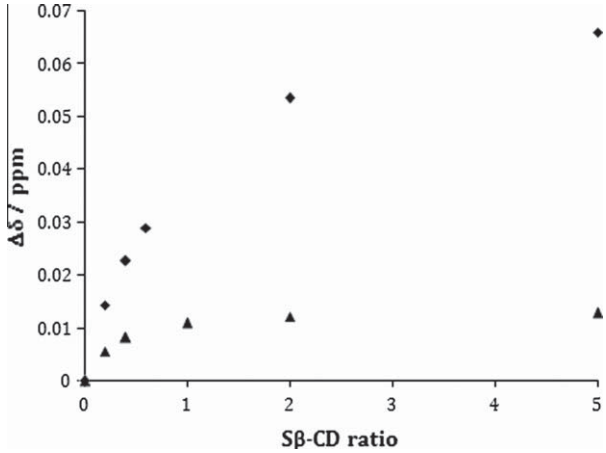


Fig. 6. Plot of chemical shift in a–H (♦) and b–H (▲) protons of DA as a function of the mole fraction of sulfonated β-CD in the solution.

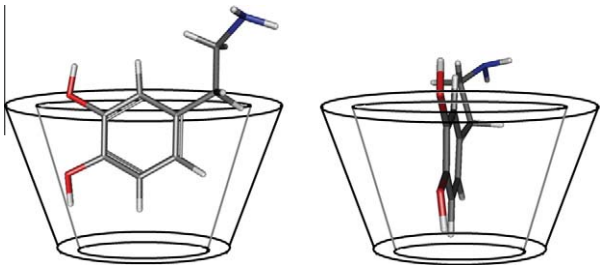


Fig. 7. A schematic representation of the inclusion complex between DA and Sβ-CD.

ary opening of the β-CD ring. Also Chao et al. [27] demonstrated, using NMR that the aromatic ring of caffeic acid, a molecule with similarities to DA, lay inside the β-CD cavity, while the more polar groups remained outside the cavity. This schematic represents one possible arrangement, and without any detailed information on the proximity of the protons of the DA and the Sβ-CD, it is impossible to draw any further conclusions on the geometry of the inclusion complex.

3.3. Influence of pH, cations and anions on formation of the inclusion complex

As the guest and host molecules are both charged species, the pH and composition of the supporting solution was varied to determine if this would influence the inclusion of DA within the cavity of the sulfonated β-CD. The pH of the citrate–phosphate buffer was adjusted from 6.0 to 3.0 and UV spectroscopy was used to follow the formation of the inclusion complex. On analysis of the data, a plot similar to that obtained in Fig. 4 was obtained, giving a K_f value of 452.68 ± 12.45. This is somewhat higher than the value obtained at a pH of 6.0 and may be connected to the level of protonation of the DA molecule. The ratio of DA in the neutral and protonated states can be obtained by considering the Henderson Hasselbalch equation, given below in Eq. (3), where HA^+ represents the protonated DA and A indicates the neutral form ($HA^+ \rightleftharpoons H^+ + A$).

$$pH = pK_a + \log\frac{[A]}{[HA^+]} \tag{3}$$

This relationship can be arranged to give Eq. (4), providing the ratio of the neutral to the protonated DA, in terms of the pH and p*K*a value of DA, which is 8.9.

$$10^{pH-pKa} = \frac{[A]}{[HA^+]} \tag{4}$$

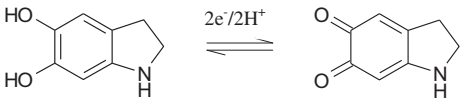
Using this relationship, the ratio of protonated DA to the neutral DA is computed as 7×10^5 and 7×10^3 at pH values of 3.0 and 6.0, respectively, indicating a slightly higher proportion of neutral DA molecules at pH 6.0 compared to pH 3.0, which may be connected to the increase in the K_f value as the pH is varied from 6.0 to 3.0. It was not possible to increase the pH of the DA above 8.9 to generate higher amounts of the neutral DA, as DA is oxidised under these conditions. However, when the pH was reduced further to 1.4 with a 0.1 mol dm^{-3} sulfuric acid solution, a reduction in the K_f value to 261.87 ± 28.61 was found. This suggests that the nature of the supporting solution has an influence on the formation of the inclusion complex with the sulfuric acid inhibiting the complexation.

Accordingly, a set of experiments was carried out to establish if the sulfate and citrate anions had any role to play in the complexation of DA with the sulfonated β-CD. In addition, the influence of cations was investigated by using chloride salts of sodium, ammonium, potassium and calcium. The ammonium cation was

selected as the NH_4^+ can be related to the protonated DA and may compete with the electrostatic interactions between protonated DA and the sulfonated groups on the β-CD. As DA is electroactive and easily oxidized, these studies were carried out using voltammetry. Cyclic voltammograms were recorded at a glassy carbon electrode in 5.00×10^{-4} mol dm^{-3} DA in the absence and presence of 0.02 mol dm^{-3} sulfonated β-CD in each supporting electrolyte. The pH was maintained constant at a pH of 5.0. In the case of the influence of the cations, the anion was kept constant; only chloride salts were used. Fig. 8 shows typical voltammograms for the oxidation of DA in each supporting electrolyte; 0.2 mol dm^{-3} solutions of NaCl, KCl, $CaCl_2$ and NH_4Cl. The data shown correspond to the 5th cycle of the voltammogram. Oxidation of DA is observed with a peak potential, E_p^A, in the vicinity of 520 mV vs. SCE, which corresponds to the oxidation of the protonated DA to the dopamine-quinone. Upon reversal of the potential, a cathodic peak, E_p^C, close to 200 mV vs. SCE is observed, corresponding to the reduction of dopamine-quinone back to DA. This is consistent with the well-known quasi-reversible electrochemistry of DA.

On addition of an excess of the sulfonated β-CD, a considerable reduction in the peak oxidation current for the oxidation of DA was observed. The ratio of the peak oxidation current recorded in the absence and presence of the sulfonated β-CD, $i^A_{p\,(DA)}/i^A_{p\,(DA+S\beta\text{-}CD)}$, was calculated as 1.61, 1.59, 1.59 and 1.61 for the NaCl, KCl, $CaCl_2$ and NH_4Cl electrolytes, respectively, indicating little effect of the cations. These significant reductions in the oxidation current are consistent with the formation of the inclusion complex. As the sulfonated β-CD is large and bulky it will give rise to a lowering in the diffusion coefficient of the included DA compared to the free DA molecule. Indeed, this has been shown in several other works with a variety of electroactive guests and bulky cyclodextrins [28,29]. There is a corresponding small increase in the half-wave oxidation potential, with the greatest shift being observed in the NH_4^+-containing electrolyte. Again, these potential shifts are indicative of an inclusion complex, where it becomes more difficult to oxidize the DA molecule confined within the sulfonated β-CD cavity. However, the different cations seem to exert little effect on the formation of the inclusion complex and there is no evidence for a competitive electrostatic interaction between the NH_4^+ and protonated DA for the anionic sulfonated groups.

On closer inspection of Fig. 8, it is clear that a shoulder peak is evident at approximately 0.0 mV SCE in the voltammograms recorded in the absence of the sulfonated β-CD. This can be attributed to the redox reactions of the leucodopaminechrome/dopaminochrome couple, Eq. (5), which is formed through the cyclization reaction of dopamine-quinone [30,31]. However, this reduction peak is absent in the presence of the sulfonated β-CD, which indicates that the cyclization reaction of the quinone, generated through the oxidation of DA, is inhibited when the DA is included in the cyclodextrin cavity.



(5)

It is clear from Fig. 8, that the cations have little effect on the formation of an inclusion complex between DA and the sulfonated β-CD. Indeed, they appear to exert more influence on the

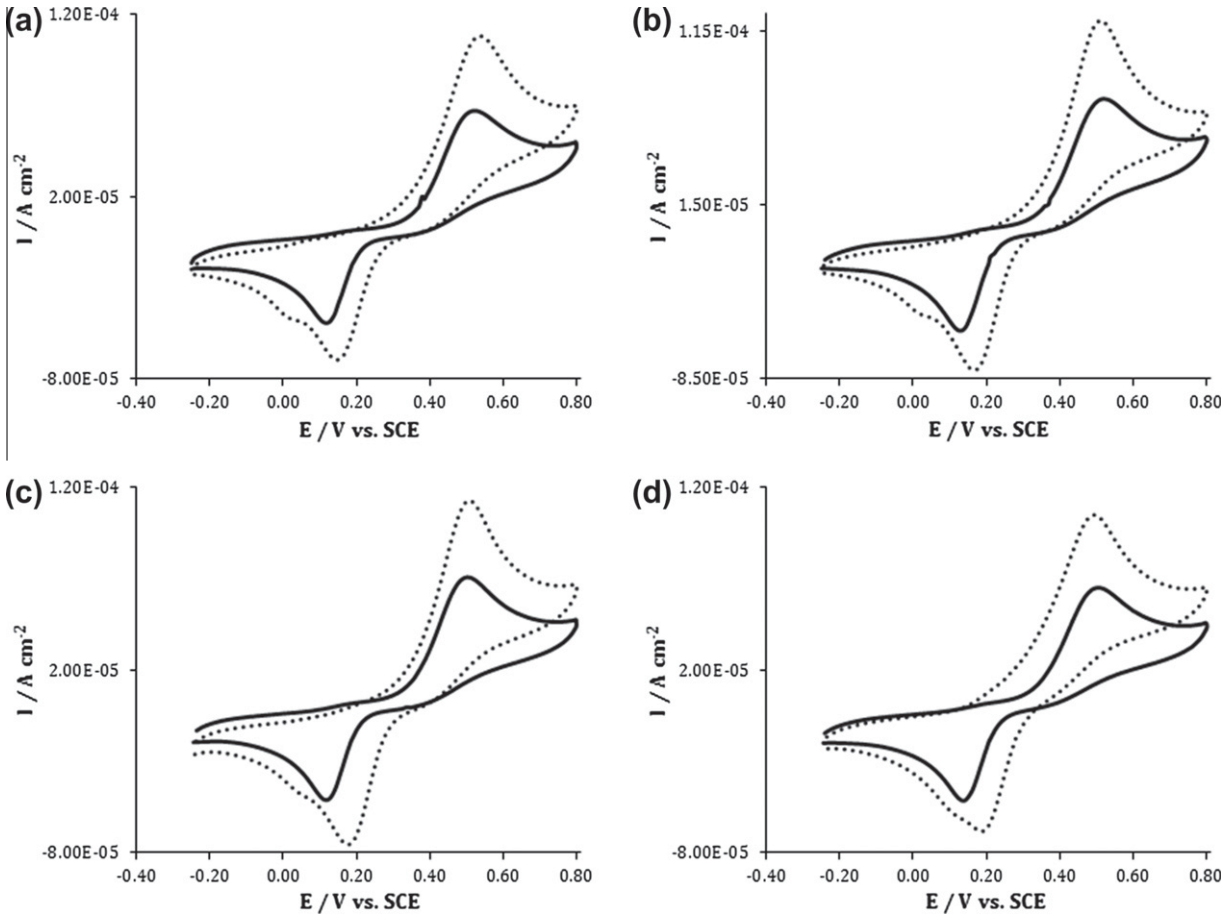


Fig. 8. Cyclic voltammograms recorded at a GC electrode at 50 mV s^{-1} in 5.0×10^{-4} mol dm^{-3} DA in the absence (- - - - -) and presence of 0.02 mol dm^{-3} sulfonated β-CD (——) in (a) 0.2 mol dm^{-3} NaCl, (b) 0.2 mol dm^{-3} KCl, (c) 0.2 mol dm^{-3} $CaCl_2$ and (d) 0.2 mol dm^{-3} NH_4Cl.

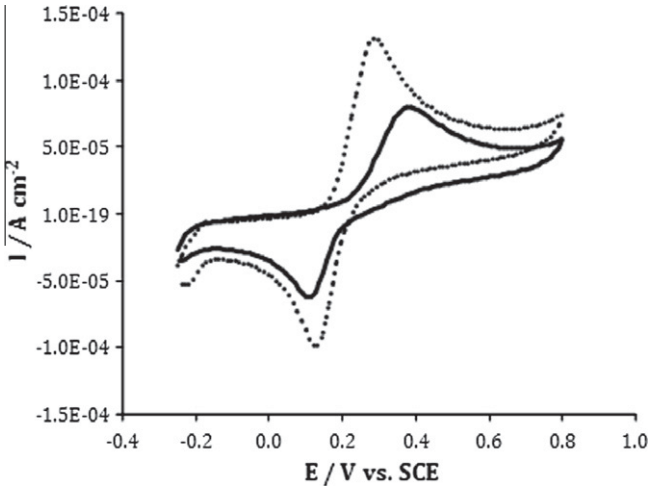


Fig. 9. Cyclic voltammograms recorded at a GC electrode at 50 mV s^{-1} in 5.0×10^{-4} mol dm^{-3} DA in the absence (- - - - -) and presence of 0.02 mol dm^{-3} sulfonated β-CD (——) in citrate–phosphate buffer, pH = 6.0.

electrochemistry of the dopamine-quinone oxidation product, with a greater cathodic shift in the reduction potential of the dopamine-quinone in the presence of Ca^{2+}. On the other hand, the anion of the supporting electrolyte plays a significant role on the oxidation of DA, as shown from a comparison of Figs. 8 and 9. In Fig. 8, the voltammograms recorded in a 5.0×10^{-4} DA solution in a citrate–phosphate buffer solution in the absence and presence of 0.02 mol dm^{-3} sulfonated β-CD are compared. In this citrate–phosphate electrolyte, the $i^{A}_{p\,(DA)}/i^{A}_{p\,(DA+S\beta-CD)}$ is 1.92, while a significant shift in the half-wave potential is observed, with ΔE = 120 mV. Similar experiments carried out in a Na_2SO_4 supporting electrolyte give an $i^{A}_{p\,(DA)}/i^{A}_{p\,(DA+S\beta-CD)}$ value of 1.77 and a ΔE of 85 mV. These data do indeed show that the citrate–phosphate medium facilitates formation of the inclusion complex, with the extent of complexation varying in the order K_f (citrate–phosphate) > K_f(sulfate) > K_f(chloride).

In an attempt to gain more information on the nature of this anion effect, voltammograms were recorded and compared for the oxidation of DA in chloride, sulfate, citrate and phosphate salts (0.2 mol dm^{-3} and constant pH of 5.0). It was found that the electrochemical oxidation of DA was more strongly suppressed when chloride was used as a supporting electrolyte than in the case of phosphate, sulfate or citrate. The DA peak oxidation potentials, E^{A}_{p} were found as 368 mV, 411 mV, 475 mV and 524 mV vs. SCE for the Na_2HPO_4, $Na_2H(C_3H_5O(COO)_3)$, Na_2SO_4 and NaCl supporting electrolytes, respectively. The I^{A}_{p} values were determined as 13.2 mA cm^{-2}, 12.8 mA cm^{-2}, 10.7 mA cm^{-2} and 10.8 mA cm^{-2} for the Na_2HPO_4, $Na_2H(C_3H_5O(COO)_3)$, Na_2SO_4 and NaCl electrolytes, respectively. One possible explanation for these observations is the size and polarisability of the anions. The small chloride and sulfate anions have a stronger electrostatic binding with the protonated DA, making it more difficult to oxidise the DA molecule. The larger more diffuse anions, where the negative charge is delocalised, have a lower attraction for the protonated DA, enabling the oxidation of DA at a slightly lower potential. If this is indeed the case, then it will be more energetically favourable for the DA to remain free and uncomplexed in the sulfate and chloride media than in the citrate–phosphate medium. In contrast, the larger citrate/phosphate species have a lower stabilisation effect on the protonated DA and it is now the formation of the complex that is more energetically favoured.

4. Conclusions

The question of inclusion phenomena between DA and Sβ-CD was introduced and examined in this paper. It has been shown that the DA forms an inclusion complex with the CD in solution. Although, there is a difference in analysing the complexation properties of CDs in solution and on the surface, many papers have examined both these processes and have attained similar observations. This implies that we can safely say that the DA forms an inclusion complex with the Sβ-CD. UV spectra showed a distinct shift in the wavelength and an increase in the absorbance of the DA, in the presence of excess Sβ-CD, confirming a change in the environment of the DA and verifying complexation. The association constant, K_f, was computed as 336.92 ± 24.83 using UV data.

Some indication of the structure of the inclusion complex was obtained from NMR studies and concluded that the aromatic DA ring was included inside the cavity, while the protonated amine was bound through electrostatic interactions to the sulfonated groups on the rim of the CD. It was found also that from the NMR data, using a non-linear curve fitting method, the K_f value was determined as 384.5 ± 164.8 and 394.39 ± 163.8 for the a−H and b−H protons, respectively. This is in good agreement with the value obtained with the spectrophotometric approach. In all techniques the formation constants evaluated were in very close agreement, which not only validated the results, but confirmed that the methods examined for the complexation could be recommended as a reliable option in determining the formation constants of the inclusion complex of Sβ-CD, with other guest molecules. In comparing the data obtained in these experiments to data found in the literature for the neutral β-CD, K_f = 95.06 [4] it can be concluded that the negatively charged sulfonate groups on the CD play an important role in the complexation and increase the binding affinities in the case of protonated DA.

In the electrochemical studies, the DA oxidation potentials shifted to higher potentials, while, the peak current decreased upon the addition of Sβ-CD. These traits were once more attributed to the formation of an inclusion complex due to the DA being harder to oxidise inside the cavity and the decrease in the diffusion coefficient of DA due to the less mobile bulky complex. Examinations on the influence of supporting electrolyte established that a change in the anion had an influence on the DA-Sβ-CD complexation while no change was observed for the cation.

Acknowledgments

This project was supported by the Irish Research Council for Science Engineering and Technology (IRCSET) and the John and Pat Hume Scholarship at the National University of Ireland Maynooth.

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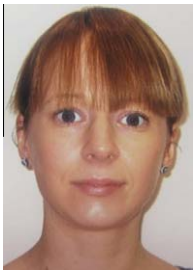

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