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Spectroscopic Characterisation of Metallo-Cyclodextrins for Potential Chiral Separation of Amino Acids and L/D-DOPA

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Abstract

The derivatives 6-Deoxy-6-[1-(2-amino)ethylamino]-β-Cyclodextrin (CDEn), 6-Deoxy-6-[1-(3-amino)propylamino]-β-Cyclodextrin (CDPn) and 6-Deoxy-6-[1-(4-amino)butylamino]-β-Cyclodextrin (CDBn) were assessed with a view to demonstrating that increasing the chain length of the diaminoalkane moiety can affect the chiral selectivity of the metallo-complexes of these materials. It was shown that IR and Raman spectroscopies can be used to characterise these compounds. The results obtained from the electronic absorption spectra suggested the formation of CuCDAm binary complexes and that the derivatives CDEn and CDPn act as bidentate ligands while CDBn acts as a monodentate ligand due to its longer alkane chain. This study also showed that in the ternary complexes with DOPA there is further coordination of the metal ion to the amino nitrogen atom and the hydroxyl oxygen atom of the drug. On the basis of the results of the circular dichroic spectroscopic studies it was suggested that CuCDEn is the better enantioselective material for DOPA and it acts in a multi-site recognition manner, utilising the inclusion properties of the CD cavity in cooperation with the coordination properties of the metal ion.

Introduction

The processes of separation and detection of chiral compounds are extremely important in the pharmaceutical industry. In drug manufacture, drugs are rarely produced in single enantiomeric form. However, some enantiomers are undesirable because they demonstrate unwanted toxic effects. Due to the importance of drug safety, the undesirable enantiomer should be considered in the same manner as any other impurity and should be separated. In 1992 the Food and Drug Administration (FDA) issued a policy statement encouraging pharmaceutical companies to use recent advances in synthetic and separation techniques to develop single enantiomeric drugs. The strict regulatory controls, which have been imposed on the pharmaceutical industry, are placing the sector under severe pressure to ensure compliance. The sector therefore requires analytical techniques for the selective separation of enantiomeric pharmaceutical drugs, which will be cost effective, safe, simple and routine.

The drug under study in this work is the chiral drug DOPA, dihydroxyphenylalanine. L-DOPA is the most effective drug at present used to combat Parkinson's disease while D-DOPA is inactive. Parkinson's disease results from reduced levels of dopamine in the brain. Nerve cells convert L-DOPA to dopamine, thereby replenishing depleted supplies. This study aims ultimately to develop a safe and viable method for the separation of the two enantiomers of DOPA.

Cyclodextrins (CD) have in the past years been used to great effect as chiral selectors for the direct separation of many amino acids [1–3], amphetamines [4] and other drugs [5]. The most important aspect of cyclodextrin structure is the cavity (Figure 1), in which guests can be included to differing degrees, depending mainly on guest size and polarity and it is this property of inclusion which is used to great effect in chiral separations. Native cyclodextrins themselves are poor chiral discriminating agents [6] and it is therefore advantageous to modify the macrocycle. In order to improve the chiral selectivity, it is also desirable to have a metal ion centre present as well as the chiral cavity in the molecule [7]. However, native cyclodextrins are also inefficient coordinating ligands because of intramolecular hydrogen bonding. This function can be enhanced by forming cyclodextrin derivatives [8].

The amino derivatives of cyclodextrins have been noted to be particularly efficient first sphere ligands for a range of metal ions [9, 10]. For example a series of polyamine functionalised β-cyclodextrins were used as ligands for metal ions such as Cu(II), Zn(II) and

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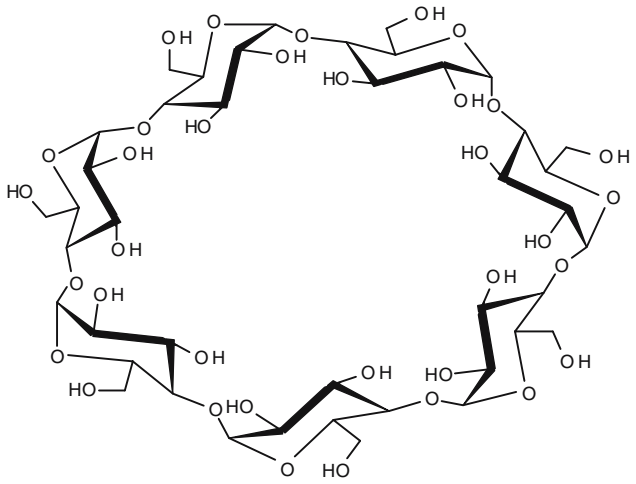

Figure 1. Structure of β-CD viewed from the secondary hydroxyl group face.

Mg(II) [11]. In the work reported here three diaminoalkane-type derivatives (CDAm) of β-CD were prepared from the tosylate (CDTs) intermediate. The derivatives synthesised were 6-Deoxy-6-[1-(2-amino)ethylamino]-β-Cyclodextrin (CDEn), 6-Deoxy-6-[1-(3-amino)propylamino]-β-Cyclodextrin (CDPn) and 6-Deoxy-6-[1-(4-amino)butylamino]-β-Cyclodextrin (CDBn).

Bonomo *et al*. [12] have shown using LEC and c.d. spectroscopy that the binary complex of CDEn with Cu(II) exhibits enantiomeric steroselectivity, although poor, towards the amino acids alanine, phenylalanine and tryptophan. Brown *et al*. [13, 14] studied the binary complexes of CDPn with Co(II), Ni(II), Cu(II) and Zn(II) and observed that enantioselection was largest for the Ni complex. Hydrophobicity, chirality and the restrictive nature of metal complexation all contribute to make metal complexes of CD amino derivatives good candidates for the separation of the DOPA racemate. The work presented here aims to show that increasing the chain length of the diaminoalkane moiety in CDAm species can affect the chiral selectivity. Ultimately this systematic study will permit a judicious choice of CuCDAm complex for the enantioselection of the chiral drug DOPA.

Experimental

Materials

β-Cyclodextrin was obtained from Wacker Chemie (Munich, Germany). All other materials were purchased from Sigma Aldrich (Dublin, Ireland) and were used without further purification. The amino acids studied in this work were phenylalanine (Phe), tyrosine (Tyr) and DOPA and all were stored below 4 °C. All solutions were prepared in distilled and Elga Millipore deionised water.

NMR measurements

^{1}H and ^{13}C NMR spectra were measured at 500 and 125 MHz, respectively, using a Varian Inova 500 NMR spectrometer. All spectra were recorded at 30 °C. ^{13}C NMR spectra were recorded in D_2O using 1,4 Dioxane as an internal standard, which gave a signal 67.19 ppm downfield from external TMS standard [15]. ^{1}H NMR spectra were recorded in D_2O and were referenced to the water peak, which gives a signal at 4.82 ppm [15]. Solutions were typically from 15 to 50 mmol dm^{-3}. Spectra of the derivative CDTs were measured in DMSO due to poor solubility in D_2O and both ^{13}C and ^{1}H NMR spectra were referenced to the DMSO solvent peaks at 39.52 and 2.5 ppm, respectively [15].

Vibrational spectroscopy

FTIR spectra were obtained from solid samples as KBr disks using a Perkin Elmer Spectrum GX, which is a single-beam, Michelson interferometer-based, Fourier transform infrared spectrometer. The spectra were measured over a range of 4000–400 cm^{-1} with a resolution of 4 cm^{-1}, an interval of 0.5 cm^{-1} and an accumulation of 16. Raman spectra of solid samples were obtained using an Instruments S.A. Labram 1B system. The Labram system is a confocal Raman imaging microscope system with both Helium–Neon (632.8 nm, 11 mW) and Argon ion (514.5 nm, 50 mW) lasers available as sources. The confocal, microscopic system allows measurement of powdered samples with no further sample preparation. Parameters used were: Time: 10 s, Accumulation: 10, Grating: 1800 lines/mm, Slit: 900 μm, Laser: 633 nm, Objective: ×100, Filter: 100%, Hole: 900 μm.

Elemental analysis

Analysis was carried out on neat samples using an Exeter Analytical CE440 CHN Analyser.

Electronic spectroscopy

Spectra were recorded using a Perkin Elmer Lambda 900 UV/Vis/NIR Spectrometer. The spectrometer is a double-beam, double monochromator ratio recording system with pre-aligned tungsten–halogen and deuterium lamps as sources. Parameters used were: Range: 200–1100 nm, Slit Width: 0.1 nm, Accumulation: 4. Spectra were taken of freshly prepared solutions of the binary and ternary complexes using quartz cells of either 10 or 1 mm path length.

Circular dichroic spectroscopy

Spectra were recorded using a Jasco J-810 Spectropolarimeter. Calibration of the c.d. instrument was performed with a 0.06% w/v ammonium d-Camphor-10-sulfonate solution in water using a 10 mm cell between 350 and 250 nm. A c.d. peak value of

+190.4 mdeg at 291.0 nm was found, which agrees well with reported values [16]. Experimental parameters used for the metallo-complexes were: Range: 825–200 nm, Sensitivity: Standard (100 mdeg), Data Pitch: 0.1–0.5 nm, Scanning Mode: Continuous, Scanning Speed: 50 nm/min, Response: 1, Bandwidth: 1 nm, Accumulation: 6–8. Spectra were recorded from 825 to 350 nm using a 10 mm cell and from 450 to 200 nm using a 1 mm cell, unless otherwise stated.

Solutions used for recording both electronic and circular dichroic spectra were prepared in a KH_2PO_4/NaOH buffer at a pH of 7.2. Binary and ternary complex solutions were prepared by mixing 2 cm^3 of each of $CuSO_4$ (0.01 mol dm^{-3} in water), CDAm (0.01 mol dm^{-3} in buffer) and the appropriate amino acid (0.01 mol dm^{-3} in buffer) in a 10 cm^3 volumetric flask and diluting to the mark with the buffer resulting in an overall complex concentration of 0.002 mol dm^{-3}.

Synthesis of derivatives. 6-O-Monotosyl-6-deoxy-β-Cyclodextrin

β-Cyclodextrin hydrate (10.01 g, 7.61 mmol) was dissolved in NaOH (100 cm^3 of 0.4 mol dm^{-3}). The solution was cooled in ice and *p*-toluenesulphonylchloride (3 g, 15.79 mmol) was added. The mixture was stirred for at least 3 h at 0–4 °C. The mixture was then filtered and the pH of the filtrate adjusted to 6.5 using HCl (~20 cm^3 of 1 mol dm^{-3}) and a precipitate formed. The filtrate was then maintained at 4 °C for at least 24 h. The product was removed by filtration, washed with acetone and recrystallised from water several times. The solid was recovered by filtration, washed with acetone and allowed to dry *in vacuo* at 60 °C for 4 h. It should be noted that rapid cooling to 60 °C of the mixture was employed during the recrystallisation to avoid significant hydrolysis [17]. Yield: 4.89 g, 44.8% (Based on hydrated materials). m.p.: 163 °C (dec.). Elemental Analysis: CDTs·$8H_2O$, Theory: 41.06% C, 6.47% H, 2.24% S (Based on hydrated materials), Found: 40.92% C, 5.48% H, 2.69% S.

^{1}H NMR (DMSO) δ (ppm): 7.74 (H-8), 7.44 (H-9), 5.68 (OH-2, OH-3), 4.84 (H-1), 4.46 (OH-6), 4.32 (H-6′$_\beta$)[1], 4.20 (H-6′$_\alpha$), 3.66 (H-6), 3.60 (H-3), 3.51 (H-5), 3.32 (H-4), 3.21 (H-2), 2.43 (H-11).

^{13}C NMR (DMSO) δ (ppm): 144.80 (C-10), 132.73 (C-7), 129.84 (C-9), 127.58 (C-8), 102.26 (C-1′), 101.96 (C-1), 101.31 (C-1″)[2], 81.70 (C-4′), 81.23 (C-4″), 81.54 (C-4), 73.08 (C-3), 72.76 (C2′ or C3′), 72.46 (C-2), 72.06 (C-5), 69.71 (C-6′), 68.94 (C-5′), 59.95 (C-6), 59.85 (C-6″), 21.08 (C-11).

FTIR (cm^{-1}): 1366, 1178, 838, 816 (These are new bands relative to β-CD).

Diaminoalkane-β-cyclodextrin derivatives

Matsui *et al*. published a synthetic route to 6-Deoxy-6-[1-(2-amino)ethylamino]-β-Cyclodextrin (CDEn) in 1976 [9]. A modified version of this synthesis was also reported by Singh *et al*. [18]. The diaminoalkane derivatives prepared here followed the route proposed by Singh, albeit modified slightly.

6-*O*-monotosyl-6-deoxy-β-cyclodextrin (CDTs) (19.28 g, 13.45 mmol) was dissolved in the appropriate diaminoalkane (1.05 mol) and refluxed under nitrogen for a minimum of 24 h at 70 °C. The mixture was then concentrated under vacuum and gave a clear, pale yellow viscous oil. The oil was then dissolved in a minimum volume of water:methanol (3:1) mixture. The solution was then slowly added to cold acetone (~500 cm^3) and a precipitate formed. The precipitate was recovered by filtration, washed with acetone and dried in air. The product was dissolved in a minimum volume of water (60 °C). A minimum volume of acetone was added to just initiate crystallisation and the mixture was cooled to 4 °C. The product was recovered by filtration, washed with acetone and dried at 60 °C *in vacuo* for 24 h.

6-Deoxy-6-[1-(2-amino)ethylamino]-β-cyclodextrin (CDEn)

Yield: 13.97 g, 76.52% (Based on hydrated materials). m.p.: 250 °C (dec.). Elemental Analysis: CDEn·$10H_2O$, Theory: 38.94% C, 7.13% H, 2.06% N, (Based on hydrated materials), Found: 38.74% C, 5.73% H, 1.55% N.

^{1}H NMR (D_2O) δ (ppm): 5.18 (H-1), 4.06 (H-3), 3.98 (H-6), 3.96 (H-5), 3.77 (H-2), 3.69 (H-4), 3.57 (H-4′), 3.16 (H-6′$_\alpha$), 2.95 (H-6′$_\beta$, H-b), 2.84 (H-a).

^{13}C NMR (D_2O) δ (ppm): 103.08 (C-1), 102.79 (C-1′), 84.72 (C-4′), 82.40 (C-4), 82.21 (C-4″), 74.35 (C-2), 74.26 (C-2′), 73.32 (C-3), 73.05 (C-5), 71.71 (C-5′), 61.56 (C-6), 50.65 (C-a), 50.41 (C-6′), 40.69 (C-b).

FTIR (cm^{-1}): 1586, 1492, 818 (These are new bands relative to β-CD).

6-Deoxy-6-[1-(3-amino)propylamino]-β-cyclodextrin (CDPn)

Yield: 2.54 g, 66.01%, (Based on hydrated materials). m.p.: 260 °C (dec.). Elemental Analysis: CDPn·$11H_2O$, Theory: 37.92% C, 7.25% H, 2.02% N, (Based on hydrated materials), Found: 38.77% C, 5.70% H, 1.62% N.

^{1}H NMR (D_2O) δ (ppm): 5.18 (H-1), 4.06 (H-3), 3.97 (H-6), 3.95 (H-5), 3.77 (H-2), 3.68 (H-4), 3.53 (H-4′), 3.15 (H-6′$_\alpha$), 2.90 (H-6′$_\beta$), 2.84 (H-c), 2.73 (H-a), 1.79 (H-b).

^{13}C NMR (D_2O) δ (ppm): 103.08 (C-1), 102.71 (C-1′), 84.87 (C-4′), 82.39 (C-4), 82.07 (C-4″), 74.32 (C-2), 74.23 (C-2′), 73.31 (C-3), 73.05 (C-5), 71.60 (C-5′), 61.50 (C-6), 50.68 (C-6′), 47.68 (C-a), 39.72 (C-c), 31.63 (C-b).

FTIR (cm^{-1}): 1572, 1485, 1472, 818 (These are new bands relative to β-CD).

6-Deoxy-6-[1-(4-amino)butylamino]-β-cyclodextrin (CDBn)

Yield: 2.814 g, 71.65%, (Based on hydrated materials). m.p.: 260 °C (dec.). Elemental Analysis: CDBn·$11H_2O$,

Theory: 38.39% C, 7.31% H, 1.99% N, (Based on hydrated materials), Found: 39.47% C, 5.84% H, 1.70% N.

^{1}H NMR (D_2O) δ (ppm): 5.18 (H-1), 4.06 (H-3), 3.97 (H-6), 3.95 (H-5), 3.77 (H-2), 3.69 (H-4), 3.53 (H-4′), 3.16 (H-6′$_\alpha$), 2.91 (H-6′$_\beta$), 2.87 (H-d), 2.71 (H-a), 1.64 (H-b, H-c).

^{13}C NMR (D_2O) δ (ppm): 103.08 (C-1) 102.57 (C1′), 84.68 (C-4′), 82.38 (C-4), 81.98 (C4″), 74.33 (C-2) 74.26 (C2′), 73.30 (C-3), 73.09 (C-5), 71.54 (C-5′), 61.55 (C-6), 50.57 (C-a), 49.67 (C-6′), 41.29 (C-d), 29.04 (C-c), 27.08 (C-b).

FTIR (cm^{-1}): 1634, 1489, 1472, 816 (These are new bands relative to β-CD) (Figure 2).

Results and discussion

NMR spectroscopy

^{1}H and ^{13}C NMR spectroscopy were both used in this study to characterise the CDTs and CDAm derivatives. The results obtained in both experiments compare favourably with previously published values for the CDTs derivative [12, 17–19] and the diaminoalkane derivatives [9, 19, 20].

Vibrational spectroscopy

Vibrational spectroscopy is generally of limited use in the investigation of CD inclusion complexes. [21]. The characteristic bands of β-CD are hardly influenced by inclusion and due to the relatively low concentration of the guest with respect to pyranose rings (1:7), its bands are usually obscured by the spectrum of the host. Nair and Dismukes [22] used IR spectroscopy to provide some evidence for the hydroxyl bridging system in the Mn_2CD complex. McNamara *et al.* [23] used both IR and Raman spectroscopy to provide structural information in a range of first sphere metallo-β-CD complexes. Therefore vibrational spectroscopy may be of use in studying derivatisation of CDs. For example, new bands due to the tosyl group or the amine group may be observed in both the IR and Raman spectra of the modified cyclodextrins.

The IR spectrum of CDTs has two new bands at 838 and 816 cm^{-1}, which may be assigned to vibrations of the para-disubstituted benzene ring. Also there is a marked change in intensity at 1366 cm^{-1} and a new band at 1177 cm^{-1}, which can be assigned to the asymmetric and symmetric stretching modes of the –SO_2–O– group, respectively. These results compare favourably with values previously published by this group for the CDTs derivative [3].

Primary amines are known to show two medium strength bands in the 3300–3500 cm^{-1} region caused by asymmetrical and symmetrical N–H stretching vibrations [15]. Secondary amines absorb weakly in this region. However, in the spectra of the CDAm species these bands are obscured by the strong OH absorption of the CD. Changes can be observed in the 1700–1500 cm^{-1} region in the spectra of all of the amino-CD derivatives. A new band occurs at 1586 cm^{-1} in CDEn, 1572 cm^{-1} in CDPn and 1634 cm^{-1} in CDBn. These bands can be assigned to the N–H bending vibration of primary amines. The band at 1642 cm^{-1} in the spectrum of β-CD can be assigned to HOH deformation modes of water molecules within the cavity [23a]. This activity may be perturbed by the presence of amino groups because of hydrogen bonding. In all cases this band is shifted to lower frequencies in the spectra of the amino-CD species. Changes also occur in the 1500–1200 cm^{-1} region in the spectra of the amino-CDs. In all cases there is a new absorption on the side of the 1415 cm^{-1} β-CD band, at 1492 cm^{-1} in CDEn, 1485 and 1472 cm^{-1} in CDPn and 1489 and 1472 cm^{-1} in CDBn. This may be assigned to the CH_2 scissoring mode of the aminoalkane group [15]. Other changes in this region may be assigned to the C–N stretching vibration. Bands due to this mode

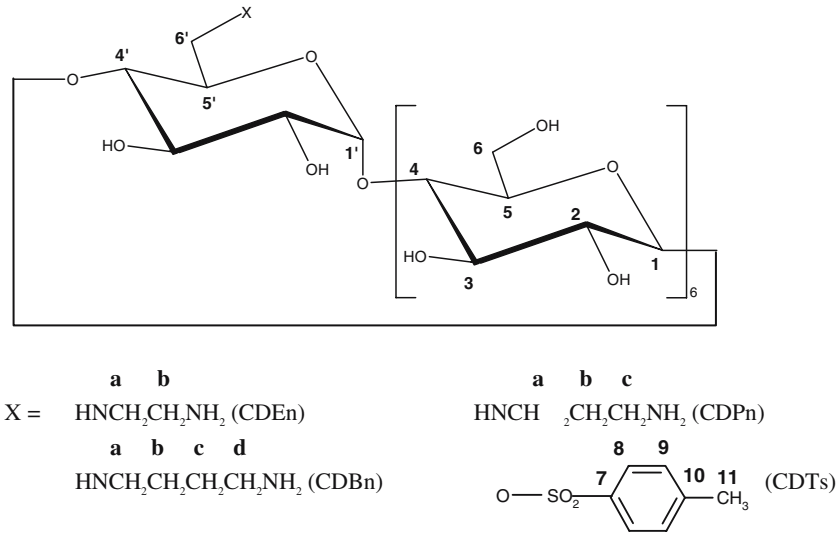

Figure 2. The numbering system used in assignment of NMR shift values for the cyclodextrin derivatives.

are generally of medium to weak intensity and may be masked by the absorption due to the CD group. There is also a new band at 818 cm^{-1} in CDEn and CDPn and at 816 cm^{-1} in CDBn, which can be assigned to the N–H wagging mode. Also the band at 1177 cm^{-1} in CDTs assigned to the $–SO_2–O–$ group is absent in the spectra of all the amino CD derivatives. These results suggest successful synthesis of CDTs and subsequent conversion to CDEn, CDPn or CDBn.

The Raman effect is a weak effect and CDs are weak scatterers. Even weak fluorescence can be much stronger than Raman scattering, easily overwhelming the weak Raman signal. Analysis showed that the CDAm compounds gave a strong fluorescence background at certain laser frequencies. Therefore fluorescence spectra were obtained of the amino species to determine at which excitation wavelengths they emit. It was observed that the CDAm species do not fluoresce at 633 nm. Subsequently all Raman spectra were obtained using the 633 nm line of the HeNe laser. Table 1 shows the main new bands observed in the spectra of the CD derivatives with assignments where possible [23, 24].

New bands can be seen in the Raman spectrum of CDTs at 3062, 1596, 1177 and 794 cm^{-1} all of which can be assigned to the tosyl moiety. The broad band at 3300 cm^{-1} observed in spectra of β-CD is assigned to O–H stretching. This band has been replaced by a sharp feature in the spectra of all the amino derivatives. This new feature at 3293, 3347 and 3300 cm^{-1} in CDEn, CDPn and CDBn, respectively can be assigned to NH_2 symmetric and asymmetric stretching modes. It is evident from the spectra of the amino cyclodextrins that the bands due to the tosyl moiety have disappeared. These results confirm those obtained from the NMR and IR studies and demonstrate the successful preparation of CDTs and conversion to the diaminoalkane species.

Electronic spectroscopy

Electronic spectroscopy has been successfully used to detect first-sphere coordination of transition metal ions by cyclodextrins and their derivatives [9, 23]. It has also been used to study the ternary complexes of metallo-CDs with amino acids [12–14]. Shifts in the position of the absorption bands with respect to that of the aquated metal ion have been taken as indicating coordination and a change in the strength of the ligand field. An increase in absorption intensity with respect to the aquated metal ion, measured in terms of the molar extinction coefficient ϵ, indicates a lowering in symmetry of the coordination sphere of the metal ion, with consequent relaxation of the Laporte rule. Electronic spectra obtained in this work are shown in Figure 3 and Table 2 summarises the data obtained for the binary and ternary complexes.

For the binary complexes the band in the visible region can be assigned to d–d transitions of the Cu(II) ion, while band(s) in the UV region are assigned to electronic transitions of the CDAm moiety. In all cases the electronic absorption spectra of the binary complexes show a shift downfield for the d–d transition to 671, 716 and 731 nm for CuCDEn, CuCDPn and CuCDBn, respectively from the value of 810 nm reported [25] for the hexaqua ion $[Cu(H_2O)_6]^{2+}$. The CDAm species therefore appear to be stronger ligands than H_2O, the ligand field strength increasing in the order CDBn < CDPn < CDEn. Cucinotta *et al*. [26] reported a shift to 656 nm at pH 7 for the Cu(II) complex of the 3^A,3^B-diamino-3^A, 3^B-dideoxy-β-cyclodextrin where A and B represent adjacent pyranose units. Similar complexes of 6^A,6^B-diamino-6^A, 6^B-dideoxy-β-cyclodextrin exhibit a shift in λ_{max} to 680 nm [27]. Matsui *et al*. [9] observed a shift to 570 nm for the $[Cu(CDEn)_2]^{2+}$ complex at pH 10.5. However, Bonomo *et al*. [12] have shown using potentiometric studies that the more favourable species in the pH range used in this work is $[Cu(CDEn)]^{2+}$. We therefore propose that the copper (II) ion is coordinated to one CDAm molecule with the coordination sphere completed by aqua ligands. The decrease in λ_{max} suggests that CDEn and CDPn are acting as bidentate ligands through coordination *via* the nitrogen atoms of the pendant group. The decrease in λ_{max} is less for CuCDBn. It is possible that in this case the metal ion may only be coordinated to one of the nitrogen atoms, the longer alkane chain preventing CDBn acting as a bidentate ligand. In all cases there is also an increase in the value of the molar extinction coefficient when compared to the value of 7 mol^{-1} dm^3 cm^{-1} obtained for the hexaaqua ion. This indicates a lowering in symmetry of the coordination sphere around the metal ion as expected for these complexes. There is very little change in the spectra in the UV region compared to spectra of the ligands alone, except for a slight broadening of bands.

In spectra of the ternary complexes, bands in the visible region are again assigned to d–d transitions of the metal ion. Assignments of bands in the UV region are complicated by the presence of different guest species. Spectra of the amino acids alone were also

Table 1. Raman vibrational bands assigned to the substituted moieties of the cyclodextrin derivatives

CDTs (cm^{-1})	CDEn (cm^{-1})	CDPn (cm^{-1})	CDBn (cm^{-1})	Assignment [23, 24]
	3292	3347	3300	Symmetric & asymmetric NH_2 stretch of amines
3062				Aromatic C–H stretch of alkyl benzene
1596				Ring stretch of benzene derivatives
1177				Symmetric SO_2 stretch
794				Ring vibration of para-disubstituted benzenes

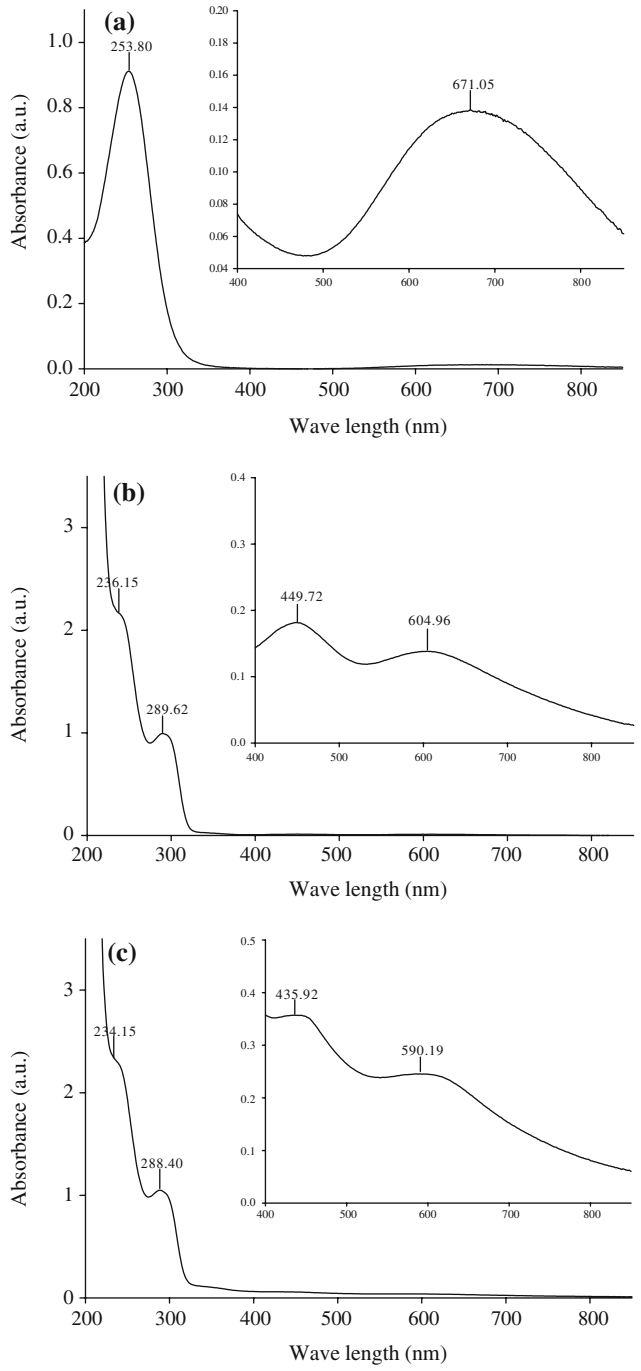

Figure 3. Electronic spectra of (a) the binary complex CuCDEn and the ternary complexes (b) CuCDEn L-DOPA and (c) CuCDEn D-DOPA recorded using 1 mm quartz cells. Insets show spectra recorded using 10 mm cells.

Table 2. Electronic absorption data for binary and ternary complexes

Binary complexes			
	λ (nm)	(ϵ) (mol^{-1} dm^3 cm^{-1})	
	Cu(CDEn)	Cu(CDPn)	Cu(CDBn)
	671 (69)	716 (79)	731 (70)
	254 (4557)	250 (3143)	242 (2179)
		230 (sh)	
Ternary complexes			
	λ (nm)	(ϵ) (mol^{-1} dm^3 cm^{-1})	
Guest	Cu(CDEn)	Cu(CDPn)	Cu(CDBn)
L-Phe	607 (67)	642 (63)	634 (62)
	248 (6250)	242 (4413)	239 (4413)
D-Phe	608 (69)	630 (65)	650 (40)
	248 (6184)	243 (4730)	239 (4045)
L-Tyr	643 (63)	652 (66)	632 (66)
	249 (6861)	260 (4133)	265 (3977)
	225 (11878)	224 (12194)	224 (12877)
D-Tyr	630 (65)	621 (81)	649 (58)
	249 (6733)	265 (4283)	267 (3366)
	225 (14560)	224 (12847)	224 (12367)
L-DOPA	605 (69)	624 (55)	615 (57)
	450 (91)	443 (83)	444 (105)
	290 (4968)	296 (5466)	298 (5553)
	236 (10895)	231 (13497)	232 (12195)
D-DOPA	590 (122.75)	627 (46)	631 (41)
	436 (178.51)	447 (80)	445 (83)
	288 (5250)	297 (5251)	298 (5641)
	234 (11680)	233 (11803)	234 (12229)

recorded and from these results the band between 248 and 238 nm in the spectra of the ternary complexes of phenylalanine can be assigned to the CDAm moiety, the phenylalanine itself only having weak absorption in this region. Similarly with ternary complexes of tyrosine, the band at ~222 nm can be assigned to a transition of the amino acid while the band at 250–265 nm is assigned to transitions of both the amino acid and the CDAm species. Again when DOPA is the guest species, bands at ~280 nm can be assigned to transitions of the drug while absorptions in the ~235 nm region are assigned to transitions of both the drug and CDAm species.

The electronic absorption spectra for the ternary complexes show a further downfield shift for the d–d transition. For example λ_{max} occurs at ~607 nm for [Cu CDEn Phe] and 640–630 nm for [Cu CDEn Tyr]. These results suggest further coordination of the copper(II) ion to the amino nitrogen atom and the hydroxyl oxygen atom of the amino acid. The values obtained for [Cu CDEn Phe] compare reasonably well with values reported by Bonomo *et al*. [12]. These workers also report [27] λ_{max} at 599–613 and 599–608 nm for the ternary complexes of Phe and Tyr, respectively with the copper complex of 6^A,6^B-diamino-6^A, 6^B-dideoxy-β-cyclodextrin. Cucinotta *et al*. [26] report similar shifts to 624 nm for the ternary complex of tyrosine with the Cu(II) complex of 3^A,3^B-diamino-3^A, 3^B-dideoxy-β-cyclodextrin.

The Cu(II) d^9 ground state in a psuedo octahedral field is the Jahn Teller unstable ^2E_g and therefore very few regular O_h Cu(II) complexes exist. For example $[Cu(en)_2Cl_2]H_2O$ is assigned to the D_{2h} point group and shows two bands in its electronic spectra at 667 and 552 nm [28]. Spectra of the ternary complexes of CuCDAm with DOPA as guest also show two bands in the visible region at ~590–600 and ~435–450 nm, again suggesting highly distorted or pseudo-O_h complexes.

The results obtained from the electronic absorption spectra therefore suggest the formation of CuCDAm binary complexes and that the derivatives CDEn and CDPn act as bidentate ligands and coordinate to the metal ion through the two nitrogen atoms of the diaminoalkane moiety. On the other hand it seems that CDBn acts as a monodentate ligand due to the longer alkane chain. This work also suggests that in the ternary complexes there is further coordination of the metal ion to the amino nitrogen atom and the hydroxyl oxygen atom of the amino acids.

Circular dichroism

Circular dichroism was used to examine inclusion of the enantiomers of the amino acids and DOPA within the cyclodextrin cavity of the CuCDAm species. The intensity of circular dichroism was measured in terms of the ellipticity θ in millidegrees (mdeg), which was subsequently converted into units of the circular dichroic molar extinction coefficient [16] $\Delta\epsilon$ in mol^{-1} dm^3 cm^{-1}. Increases in $\Delta\epsilon$, reflect the deeper inclusion of guests within the cavity [29]. Sample spectra are shown in Figure 4 and Table 3 summarises the data obtained.

In the binary complexes, the band present in the visible region can be assigned to d–d transitions of the metal ion. The bands in the UV region are due to the cyclodextrin moiety. The c.d. spectrum of the CuCDBn system does not show a band in the visible region, which may be due to poor complexation of the CDBn towards the copper(II) ion.

In the ternary complexes involving phenylalanine and tyrosine the bands in the visible region are once again assigned to d–d transitions of the metal ion. The bands present in the UV region are due to transitions of the CD moiety and the aromatic rings of the amino acids. The ternary complexes involving DOPA have similar electronic absorption spectra, i.e., two bands present in the visible region, which are assigned to d–d transitions of a highly Jahn Teller distorted Cu(II) ion. Bands in the UV region are again assigned to transitions of the CD moiety and the aromatic rings of the guest. The results shown in Table 3 are in agreement with previous results obtained for the ternary complex [Cu CDEn Phe] [12].

The circular dichroic spectra of the diastereomeric complexes of [Cu CDAm D/L-Phe], [Cu CDAm D/L-Tyr] and [Cu CDAm D/L-DOPA] were compared by calculating $\Delta(\Delta\epsilon)$ maximum values (where $\Delta(\Delta\epsilon) = |\Delta\epsilon(D)| - |\Delta\epsilon(L)|$). An increase in $\Delta\epsilon$ is taken as evidence of deeper inclusion of the aromatic ring of the guest within the cyclodextrin cavity [12]. Table 4 summarises the results obtained.

It can be seen from Table 4 that the binary complex CuCDEn gives the greatest value of $\Delta(\Delta\epsilon)$ with Tyr as guest, the D enantiomer giving the larger value of $\Delta\epsilon$. This suggests deeper inclusion of this enantiomer in the cyclodextrin cavity of CuCDEn when compared to the L isomer. This binary complex is also the best

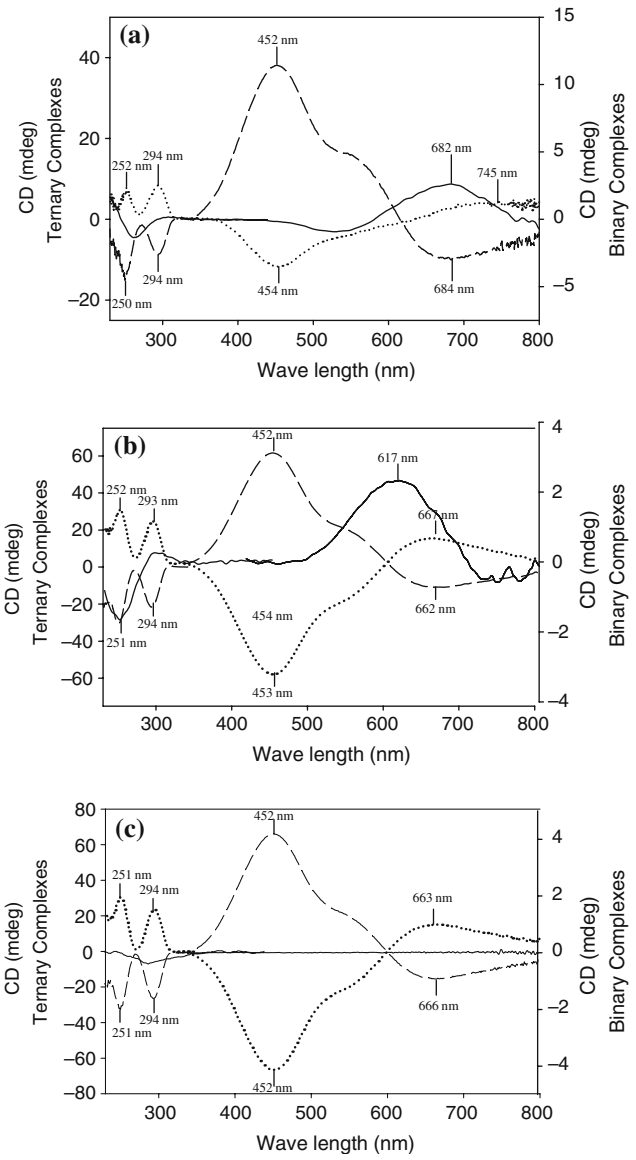

Figure 4. Circular dichroic spectra of the binary and ternary complexes of (a) CDEn, (b) CDPn and (c) CDBn with DOPA as guest. Spectra of binary complexes are shown as solid lines, ternary complexes with the D enantiomer as dashed and with the L enantiomer as dotted lines.

enantioselective material of those studied for the drug DOPA, again with the D enantiomer giving the larger value of $\Delta\epsilon$. Only a small difference in $\Delta(\Delta\epsilon)$ was observed for the complexes of CuCDPn and CuCDBn with this drug.

It can also be ascertained from these results that the CuCDEn binary complex is the better enantioselective material for phenylalanine. The order of enantioselectivity towards the different guests can be shown in series as: CuCDEn > CuCDPn > CuCDBn for phenylalanine and DOPA while for tyrosine the order is: CuCDEn > CuCDBn > CuCDPn. In all cases there is preferential inclusion of the D isomer.

On the basis of these results it is suggested that CuCDEn is the better enantioselective material and is acting in a multi-site recognition manner, utilising the

Table 3. Circular dichroism data for binary and ternary complexes

Binary complexes			
	λ (nm)	($\Delta\epsilon$) (mol^{-1} dm^3 cm^{-1})	
	Cu(CDEn)	Cu(CDPn)	Cu(CDBn)
	682 (0.04)	617 (0.04)	288 (−0.06)
	529 (−0.01)	296 (0.26)	
	262 (−0.21)	250 (−0.25)	
Ternary complexes			
	λ (nm)	($\Delta\epsilon$) (mol^{-1} dm^3 cm^{-1})	
Guest	Cu(CDEn)	Cu(CDPn)	Cu(CDBn)
L-Phe	561 (−0.16)	577 (−0.11)	595 (−0.20)
	283 (−0.21)	279 (−0.54)	277 (−0.29)
	239 (0.87)	241 (0.84)	240 (0.88)
D-Phe	587.0 (0.19)	591 (0.25)	588 (0.22)
	277 (0.45)	–	284 (0.10)
	228.0 (−0.12)	244 (−1.29)	240 (−1.32)
L-Tyr	551 (−0.13)	566 (−0.06)	595 (−0.10)
	301 (−0.20)	295 (−0.33)	294 (−0.17)
	269 (0.59)	248 (0.71)	264 (0.39)
D-Tyr	583 (0.17)	595 (0.21)	592 (0.16)
	293 (0.42)	306 (0.24)	293 (0.16)
	276 (−0.43)	266 (−0.91)	267 (−0.59)
L-DOPA	745 (0.06)	667 (0.23)	663 (0.24)
	537(sh) (−0.06)	536(sh) (−0.32)	533(sh) (−0.35)
	452 (−0.18)	453 (−0.89)	452 (−1.01)
	294 (1.27)	293 (3.45)	294 (3.60)
	252 (1.18)	251 (4.34)	251 (4.64)
D-DOPA	684 (−0.15)	662 (−0.19)	666 (−0.23)
	537(sh) (0.25)	536(sh) (0.31)	533(sh) (0.36)
	452 (0.58)	452 (0.90)	452 (1.02)
	294 (−1.34)	294 (−3.30)	294 (−3.93)
	250 (−2.27)	251 (−4.65)	251 (−4.85)

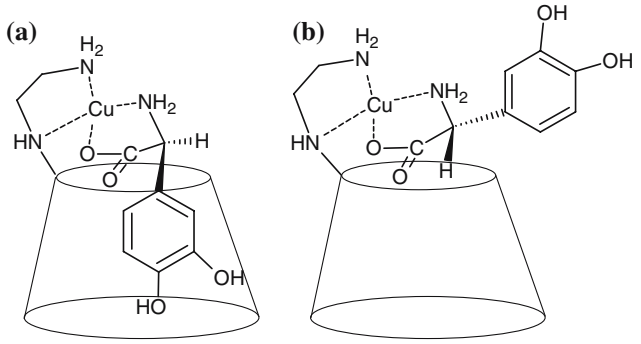

Figure 5. Proposed structures of the diastereoisomers of (a) [CuCDEn(D-DOPA)]$^+$ and (b) [CuCDEn(L-DOPA)]$^+$ showing the different orientations of the enantiomer guests.

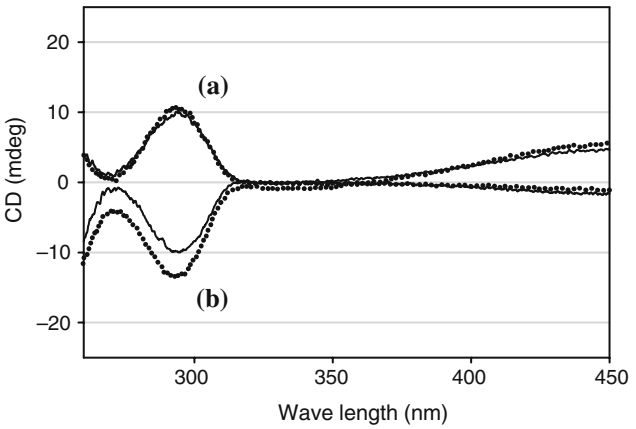

Figure 6. c.d. Spectra of the ternary complexes of CuCDEn with (a) L-DOPA and (b) D-DOPA. The solid lines represent spectra of the complexes [0.002 mol dm^{-3}] with no 1-adamantanol present. The dotted lines represent spectra of the complexes [0.002 mol dm^{-3}] with 0.008 mol dm^{-3} 1-adamantanol added as a competitive guest.

inclusion properties of the CD cavity in cooperation with the restrictions of the coordination sphere of the metal ion as discriminating factors. In line with previous proposals by Maccarone *et al.* for tryptophan [30] we now suggest the formation of two diasteriomeric complexes along the lines shown in Figure 5 for the [Cu CDEn DOPA] species. The ability of the D enantiomer to achieve significant inclusion relative to the L enantiomer is paramount.

Adamantanol has been reported to be a very good competitive guest for inclusion in cyclodextrins [31] and it does not coordinate with the copper ion. Increasing concentrations of 1-adamantanol were therefore added to solutions of the ternary complexes [Cu CDEn L-DOPA] and [Cu CDEn D-DOPA] with a view to excluding the included guest from the cavity. Figure 6 shows the results obtained for this study. It can be clearly seen that the addition of 1-adamantanol to solutions of the ternary complexes alters the circular dichroic spectra in both cases. However, the greatest change was observed for the [Cu CDEn D-DOPA] ternary complex; which suggests that inclusion of the guest in the cavity is inhibited by adamantanol. This study provides further evidence for the structures proposed in Figure 5.

Table 4. $\Delta(\Delta\epsilon)$ Values in the UV region for the ternary complexes of CuCDAm with the guests phenylalanine, tyrosine and DOPA

	CuCDEn	CuCDPn	CuCDBn
Phe	0.75	0.45	0.44
Tyr	2.54	0.80	1.31
DOPA	1.09	0.31	0.21

Conclusions

On the basis of the results obtained in this study, it is suggested that of the three metallo-complexes, CuCDEn is the most enantioselective material for Phe, Tyr and DOPA. The enantioselection of CuCDEn is believed to be strongly influenced by the inclusion of the D-enantiomer within the CD cavity. Cu(II) will have a tetragonally distorted octahedral structure due to its d^9 configuration and therefore structural restrictions imposed by coordination of the drug to the copper(II) ion inhibit inclusion in the case of the L-enantiomer. In the case of CuCDPn this enantioselection may not be

possible due to the structure of the 1,3-diaminopropane moiety, which may be sufficiently flexible to allow interaction of both enantiomers with the cavity. For CuCDBn it is suggested that the length of the alkane chain prevents 1,4-diaminobutane acting as a bidentate ligand, which again facilitates inclusion of both enantiomers in the cavity. Although the results presented here do not provide direct evidence for selection, the data strongly suggests marked enantioselection of DOPA by CuCDEn, which will allow the development of chiral separation and detection systems for the pharmaceutical sector.

Notes

1. α and β represent diastereotopic hydrogens.
2. It should be noted that the single primes correspond to atoms on the substituted pyranose ring, whilst the double primes refer to atoms present on a pyranose unit adjacent to the substituted ring. Other minor shifts were found but are omitted for clarity.

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