ELSEVIER

International Journal of Pharmaceutics 131 (1996) 219–228

international journal of pharmaceutics

Inclusion complexation of amide-typed local anaesthetics with ß-cyclodextrin and its derivatives. I. Physicochemical characterization

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Received 14 August 1995; accepted 26 September 1995

Abstract

Qualitative aspects of the inclusion complexation of two local anaesthetics of the amide-type (LAs), bupivacaine (BVC) and lidocaine (LDC) with three cyclodextrins (CDs), ß-cyclodextrin (ßCD) and its alkylated derivatives 2-hydroxypropyl-ß-cyclodextrin (HPßCD) and heptakis (2,6-di-*o*-methyl)-ß-cyclodextrin (DMßCD), were studied in the solid state by infrared spectroscopy (IR) and differential scanning calorimetry (DSC). The LDC-ßCD couple was also investigated in aqueous solution by nuclear magnetic resonance (^{1}H-NMR and ^{13}C-NMR). This first part of a study dealing with improvement in LA administration provided clear indications about LA-CD complexation. Qualitative modifications in a number of peaks or bands obtained from spectral methods as well as thermal analysis signed the inclusion, showing that the freeze-drying method was suitable for obtaining inclusion complexes of LAs with CDs.

Keywords: Local anaesthetics; Cyclodextrins; Inclusion compound; IR; DSC; ^{1}H-NMR; ^{13}C-NMR

1. Introduction

The use of local anaesthetics (LAs), either via central administration (spinal or epidural routes) or via peripheral administration is currently increasing (Howell, 1992, Levinson, 1992) for regional anaesthesia upon surgery as well as for regional control of acute and chronic pain (Benumof, 1992), allowing a decrease in morbidity. However, there is a need to reduce toxicity of these local anaesthetics because of their systemic entry. Thus, regional administration of LAs could be improved by developing drug-delivery systems of such drugs that would allow a controlled release leading to a longer duration of action, a lower uptake in the systemic circulation and, so, an improvement of the therapeutic index of these anaesthetics (Stanley, 1988). Among the various ways of obtaining such delivery systems, complex-

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SSDI 0378-5173(95)04334-7

ation with cyclodextrins (CDs) may provide an easy one. Moreover, pharmacodynamic approaches have shown that CDs can modify the effectiveness (increase in duration of action) of some spinally administered endogen molecules like endorphinic and opioids peptides (Yaksh et al., 1991; Jang et al., 1992), as well as spinally or epiduraly administered analgesics, as shown by a much longer presence time at the spinal level (Meert et al., 1992), suggesting interaction between these substrates and CDs.

CDs are torus-shaped oligosaccharides produced by enzymatic hydrolysis and cyclization of starch, containing six (αCD), seven (ßCD) or eight (γCD) α-1,4-linked D-glucose units (Duchêne, 1987), with a hydrophilic hydroxyl group on their outer surface and a relatively hydrophobic central cavity. All the primary hydroxyl groups are located on the small-diameter side while all secondary hydroxyl groups are located on the large-diameter side of these cone-shaped molecules. During the last decades, CDs and their derivatives have been increasingly investigated in the pharmaceutical field because of their ability to entrap entirely, or at least partially, drug molecules of appropriate size and polarity in their cavities (Brewster et al., 1989; Duchêne, 1987) to form non covalent inclusion compounds (Szejtli, 1982; Saenger, 1980; Bekers et al., 1991). This may lead to useful modifications of the physical and chemical properties of the inside guest molecule allowing the improvement of stability (Bekers et al., 1988; Uekama et al., 1983; Szejtli et al., 1979; Fujioka et al., 1983), solubility for a poorly water soluble drug (Van Doorne et al., 1988; Szejtli, 1984), dissolution rate (Erden and Celebi, 1988; Hassan et al., 1990), membrane permeability (Lin and Yang, 1986) and bioavailability (Erden and Celebi, 1988; Jones et al., 1984; Duchêne and Wouessidjewe, 1990a). The complexation may also reduce volatility, unpleasant taste or smell, local irritancy or untoward side effects associated to the inside drug (Saenger, 1980; Jones et al., 1984; Irie and Uekama, 1985; Szejtli, 1992). Thus, CDs application as excipients in drug formulation and drug delivery systems is currently very active (Hirayama et al., 1988; Hassan et al., 1990). ßCD has been studied extensively despite a very low aqueous solubility but its alkylated derivatives, heptakis (2,6-di-*o*-methyl)-ß-cyclodextrin (DMßCD) and 2-hydroxypropyl-ß-cyclodextrin (HPßCD), have attracted growing interest due to their improved complexing abilities and their greater solubility abilities (Duchêne and Wouessidjewe, 1990b; Uekama and Irie, 1987b; Müller and Brauns, 1985).

The aim of the current was to investigate the nature of the interaction process between two amide-typed LA drugs among the most used in therapeutics, i.e. bupivacaine (BVC) and lidocaine (LDC), and three CDs, i.e. ßCD, DMßCD and HPßCD. Spectral methods (i.e. NMR for LDC-ßCD couple and IR for all LDC and BVC couples with these precited CDs) and thermal analysis (DSC for all LDC and BVC couples with ßCD and HPßCD) were used.

2. Materials and methods

2.1. Materials

ßCD (KLEPTOSE®, ref. no. 472408, M.W. = 1135 g/mole) was kindly supplied by Roquette Frères (Lestrem, France), DMßCD (ref. no. 03483/01, M.W. = 1331 g/mole) was purchased from AVEBE (Veendam, The Netherlands) and HPßCD (ENCAPSIN®, ref. no. 30.221.54, MS = 0.47, M.W. = 1300 g/mole) was purchased from Janssen Biotech (Olen, Belgium). All CDs were used as received after considerating their water content, determined by the Karl Fisher method with a Methrom E408 A apparatus and found to be 11.34%, 4.58% and 0.85% for ßCD, HPßCD and DMßCD, respectively (each value is the mean of three determinations).

Both local anaesthetics (BVC and LDC, Fig. 1) were supplied by Laboratoire Astra (Nanterre, France) in their therapeutic used hydrochloride form because of the very low aqueous solubility of the active base form. The bases were obtained by precipitation from an alkaline (3% aqueous NH_4OH solution) saturated solution of the corresponding hydrochlorides. The precipitates were rinsed by distilled water until a neutral pH filtrate

was obtained. The bases were then dried (+ 40°C) before their purity was compared to hydrochloride standards by HPLC. All other reagents and solvents (E. Merck, Darmstadt, Germany) were of analytical grade. Freshly prepared distilled water was used as medium throughout the study.

2.2. Preparation of the solid complexes of LAs with CDs

Solid complexes of LDC and BVC with all CDs were prepared after dissolving the exact amounts giving a molar ratio of 1:1, by lyophilizing aqueous solutions of the complexes, according to Erden and Celebi, 1988. LDC or BVC (30 mg) and the CD, in 1:1 molar ratio, were added to 7 ml of water in 10 ml glass tubes (Sovirel, France) stoppered with PTFE septum. The mixtures were shaken at 25°C for 24 h, then filtered to remove the drug excess and lyophilized to give white crystalline powders (Alpha model, Chriss, Osterode/Harz, Germany). Physical mixtures of each complex components were also prepared. LAs and CDs were pulverized in a ceramic mortar, sieved separately (100 μm) and the calculated and exactly weighted (1:1 molar ratio) amounts of both compounds were then vortexed for 30 s and mixed with a spatula. The freeze-dried samples obtained were used for thermal analysis and infra-red spectroscopy.

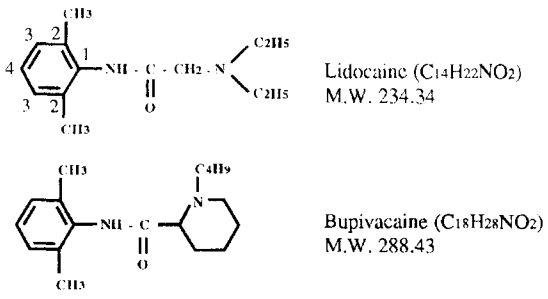

Fig. 1. Chemical structures of lidocaine and bupivacaine. The numbering is identical for LDC phenyl protons for which NMR behavior is the same.

2.3. Infrared spectroscopy

The Fourier Transformation-Infrared (FT-IR) spectra of LDC, BVC, CDs, complexes and physical mixtures were measured with a Perkin Elmer 16PC FT-IR spectrophotometer (Perkin Elmer, Norwalk, CT, USA). The samples were ground and mixed thoroughly with KBr, an infrared transparent matrix, using a 1% dilution.

2.4. Differential Scanning Calorimetry (DSC)

DSC scans were recorded on a Perkin Elmer DSC-4 differential scanning calorimeter, equipped with a Perkin Elmer model 10 atomic spectroscopy data system and a system 4 thermal analysis microprocessor (Perkin Elmer, Norwalk, CT, USA). Indium (99.99%, m.p. 157.6°C, Perkin Elmer, Norwalk, CT, USA) was used to calibrate the apparatus. All samples (2-4 mg) were placed in crimped aluminium pans and hermetically sealed, before being heated under dry nitrogen flow, at a scanning rate of 50°C/min from 0°C to 200°C.

2.5. Nuclear Magnetic Resonance (NMR) studies

The experiments were performed at 300.134 MHz and 75.469 MHz for proton NMR (^{1}H-NMR) and carbon NMR (^{13}C-NMR), respectively. Spectra of LDC, ßCD and LDC-ßCD complex were recorded on solutions in deuterium oxide (D_2O) at ambient probe temperature of 25° ± 0.5°C with a Bruker AM300 WB spectrometer (Bruker, Karlsruhe, Germany). An average of 200 accumulations with 32768 data points were made over a spectral width of 4000 Hz and 17857 Hz, for ^{1}H-NMR and ^{13}C-NMR, respectively. All chemical shifts were assigned values based on an external standard of sodium dioctyl sulfosuccinate (Docusate sodium, DSS), and were automatically calibrated with an accuracy of ± 0.001 p.p.m. and 0.01 p.p.m. for ^{1}H and ^{13}C-NMR spectra, respectively.

3. Results and discussion

3.1. DSC study

Some evidence of inclusion complexation was obtained from thermal analysis study. When guest molecules are embedded in the CDs cavities, their melting or sublimation point generally shift to a different temperature or disappear within the temperature range where the CD is decomposed. Fig. 2 shows the DSC thermograms of the pure components, the physical mixtures and the inclusion complexes of BVC and LDC systems with ßCD and DMßCD, for example. The thermograms of intact LDC and BVC showed a characteristic endothermic peak at 72.2°C and 114.6°C, respectively, corresponding to their melting point. The thermogram of ßCD exhibited a very broad endothermic peak between 90 and 170°C (maximum at 127.1°C), corresponding to the release of the water molecules (Hassan et al., 1990), while the thermogram of DMßCD exhibited no thermal event in the same range. Concerning the physical mixtures of LDC with ßCD and DMßCD, the endothermic peak characteristic of pure LDC was found at 70.2°C and 72.1°C, respectively. In the case of LDC-ßCD physical mixture, the broad thermal rise corresponding to pure ßCD was also found (maximum around 125°C), as if those thermograms were the superposition of those of the components analysed separately. Thus, we can considerate the absence of interaction between LDC and CDs in such systems. However, in the DSC curves of the freeze-dried complexes, complete disappearance of the endothermic peak of LDC were observed. Concerning BVC, the thermal characteristic peak appeared around 110°C in both physical mixtures with ßCD (109.2°C) and DMßCD (110.4°C). These little changes relative to the peak of the intact BVC may suggest a weak interaction during heating for DSC scanning (Erden and Celebi, 1988). However, in the solid freeze-dried complexes implicating BVC, the

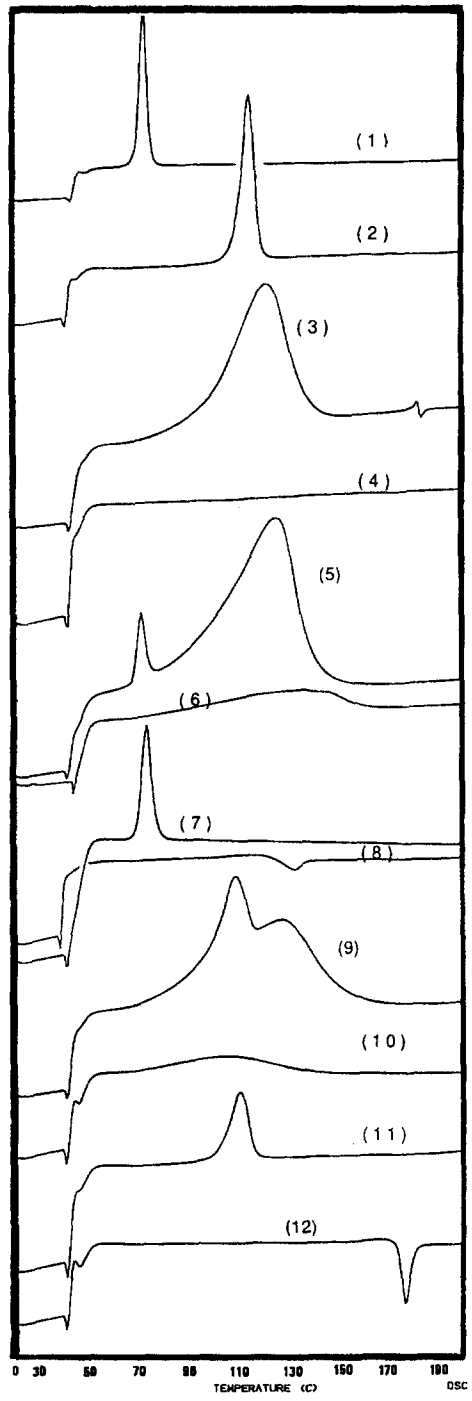

Fig. 2. Differential scanning calorimetry thermograms of LDC and BVC systems with ßCD and HPßCD. (1) LDC alone; (2) BVC alone; (3) ßCD alone; (4) DMßCD alone; (5) physical mixture of LDC and ßCD; (6) LDC-ßCD solid complex; (7) physical mixture of LDC and DMßCD; (8) LDC-DMßCD solid complex; (9) physical mixture of BVC and ßCD; (10) BVC-ßCD solid complex; (11) physical mixture of BVC and DMßCD; (12) BVC-DMßCD solid complex.

Table 1
FT-IR characteristic bands (cm^{-1}) for physical mixtures (P.M.) and solid complexes (S.C.) of LDC with βCD, DMβCD and HPβCD.

CYCLODEXTRIN						
	βCD		HPβCD		DMβCD	
	P.M.	S.C.	P.M.	S.C.	P.M.	S.C.
NH elongation	3274	3324	3290	3334	3408	3410
C = O elongation	1664	1664	1662	1664	1664	1686
C-N elongation	1498	1508	1496	1502	1496	1496

Table 2
FT-IR characteristic bands (cm^{-1}) for physical mixtures (P.M) and solid complexes (S.C.) of BVC with βCD, DMβCD and HPβCD

CYCLODEXTRIN						
	βCD		HPβCD		DMβCD	
	P.M.	S.C.	P.M.	S.C.	P.M.	S.C.
NH elongation	3334	3306	3326	3342	3410	3414
C = O elongation	1648	1654	1648	1654	1650	1686
C-N elongation	1526	1526	1526	1526	1526	1526

curves lacked the endothermic peak. These results clearly indicate the existence of interactions between LAs and CDs in the solid state to form inclusion complexes (Fujioka et al., 1983).

3.2. *Infrared spectroscopy*

More evidence of complex formation was obtained from FT-IR study. Table 1 and Table 2 list the wavelengths (cm^{-1}) of some characteristic bands for LDC and BVC systems with CDs, respectively. These characteristic stretching bands are the N-H elongation (ν N-H from 3000 to 3500 cm^{-1}); the carbonyle elongation (ν C = O around 1650 cm^{-1}) and the C-N elongation probably masking the ν (C = C) elongation band of the benzene ring, around 1650 cm^{-1}. The N-H bands which were observed in the spectrum of LDC and BVC at 3252 and 3166 cm^{-1}, respectively, shifted to a longer wavelength and hid themselves by broadening in both physical mixtures and freeze-dried complexes, with a decrease in intensity. The carbonyle stretching vibrations (1664 and 1648 cm^{-1} for LDC and BVC, respectively) did not change in the physical mixtures with all CDs. Complexation with ßCD as well as HPßCD did not produce any shift for LDC, while slight shifts were obtained with BVC. However, with DMßCD, the carbonyle stretching bands shifted to a longer frequency, i.e. 1686 cm^{-1} for both LDC and BVC, suggesting the breakdown of the intramolecular hydrogen bonding associated with the LAs molecules and the formation of strong interactions (hydrogen bonds) between LAs and DMßCD in the freeze-dried complexes (Bellamy, 1958; Otagiri et al., 1983). The role of the methoxyl groups of DMßCD in these shifts may be predominant. In addition, other shifts may have resulted from the inclusion of the LAs molecules within the CD cavities. Fig. 3 shows the FT-IR spectra of the LDC-DMßCD system, for example. The characteristic carbonyl stretching band at 1664 cm^{-1} appeared in both the drug and the physical mixture samples; conversely, the band shifted to a higher wavelength, i.e. 1686 cm^{-1} in the inclusion complex sample, suggesting the dis-

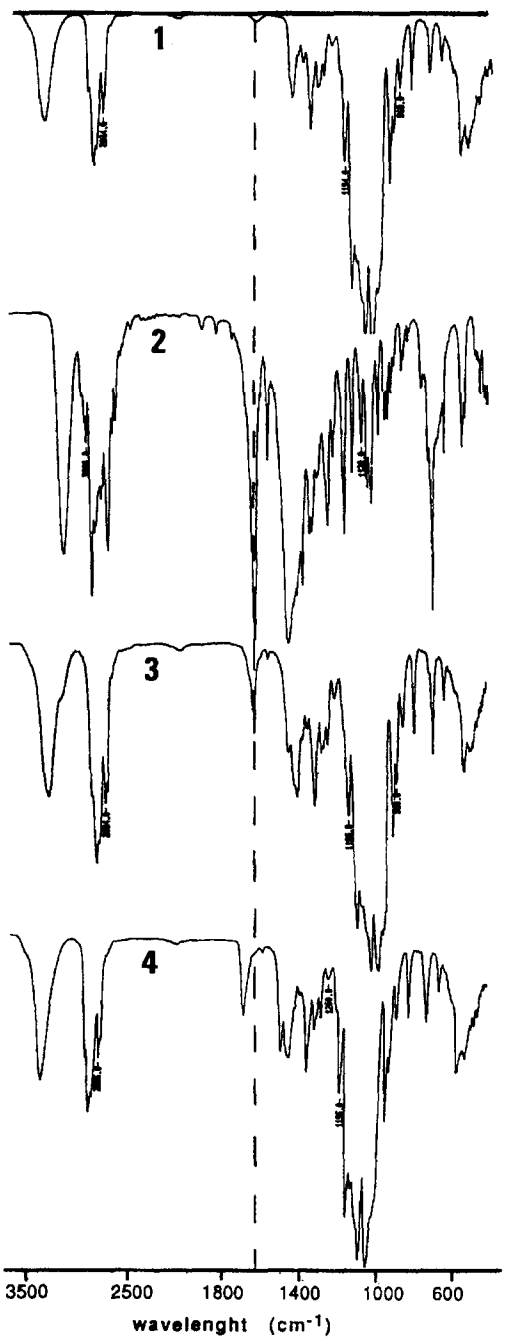

Fig. 3. FT-IR spectra of the LDC-DMßCD system. (1) DMßCD alone; (2) LDC alone; (3) physical mixture of LDC and DMßCD; (4) LDC-DMßCD solid complex (freeze-drying method).

sociation of the intramolecular hydrogen bonds of LDC through the complexation.

All these above results obtained by DSC and IR, showing differences between freeze-dried complexes and physical mixtures of LAs-CDs systems, clearly indicate that inclusion complexes exist in the solid state, even if it was difficult to visualize which parts of the LAs were actually included within the cavity of the CDs.

3.3. NMR study of the LDC-ßCD inclusion complex

NMR analyses provide information about the orientation of the guest molecule and its conformation within the host. $^{1}H-NMR$ evidence for the formation of an inclusion complex between a CD and a guest molecule is provided by changes in the chemical shifts of the protons of both partners, based on the shielding of the CD and drug protons (Thakkar and Demarco, 1971), while the locations of such changes pinpoint the regions of the molecules implicated in the association. ^{13}C-NMR spectroscopy affords considerable information on the environment of individual carbons and intermolecular interactions and therefore is very useful for analyzing inclusion phenomena.

^{1}H and ^{13}C-NMR spectra were examined in order to clarify the mode of inclusion of LDC within the ßCD cavity in aqueous solution. ^{1}H as well as ^{13}C-NMR signals of both LDC and ßCD were straightforwardly assigned. Fig. 4 shows the ^{1}H-NMR spectra of the LDC-ßCD system. ^{1}H chemical shifts for ßCD were in good agreement with those obtained by Casy and Mercer (1988). Table 3 shows the effect of ßCD on the ^{1}H chemical shifts of LDC in D_2O. On inclusion, all protons of LDC were shifted downfield, while not significantly. The signals of the benzyl protons determined as a whole because of the multiplet structure were also shifted downfield by inclusion. These shifts were not greater than those of the aliphatic protons, as if no part of the LDC molecule was interacting preferentially with the ßCD cavity, indicating only a decreased freedom of rotation for the whole LDC molecule upon inclusion. The magnitudes of the displacements

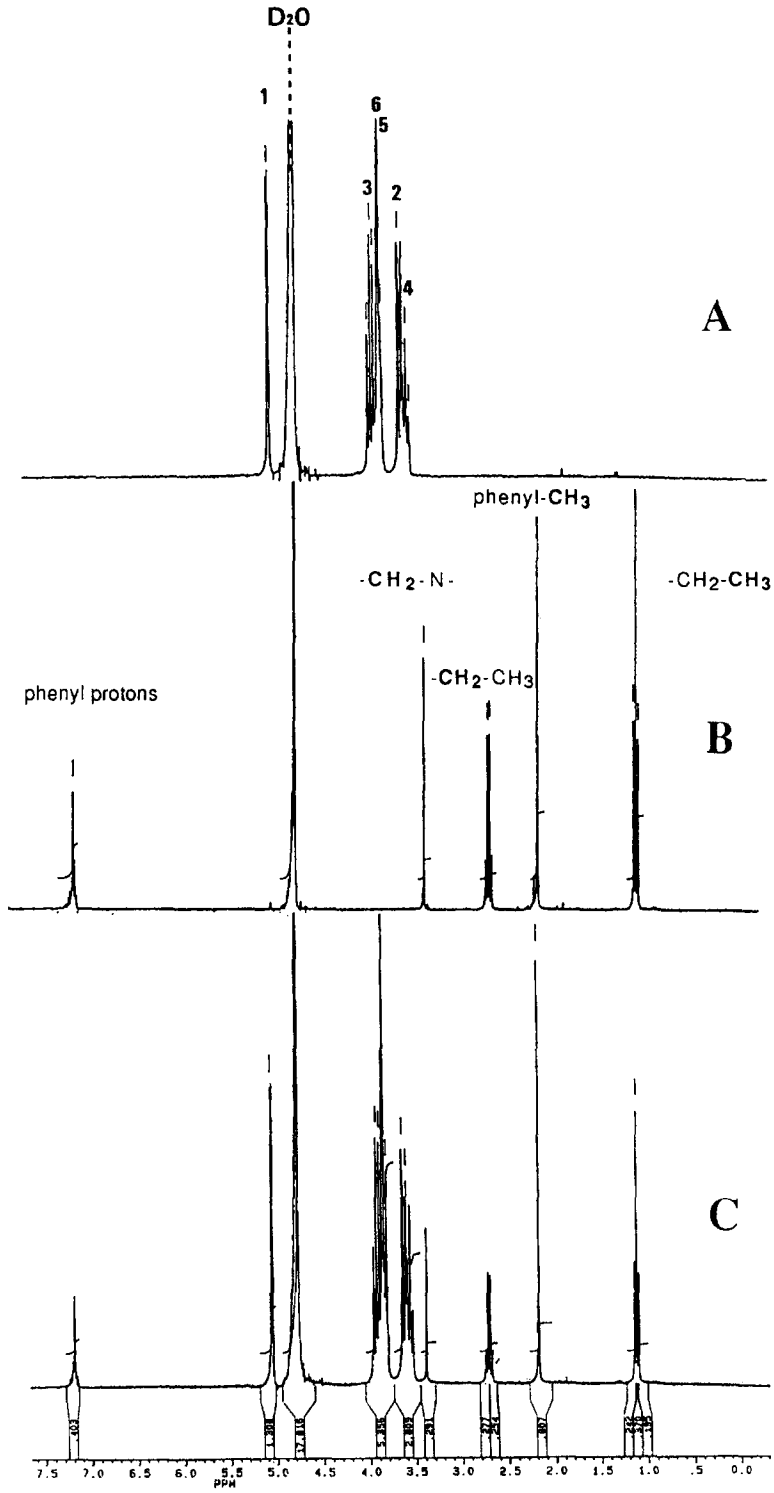

Fig. 4. 1H-NMR spectra of the LDC-ßCD system. (A) ßCD alone; (B) LDC alone; (3) LDC-ßCD complex in D_2O.

Table 3
βCDinduced ^{1}H and ^{13}C chemical shifts of LDC

Proton or Carbon	$\Delta\delta$ ^{1}H (ppm)	$\Delta\delta$ ^{13}C (ppm)
-CH_2-$\underline{CH3}$	0.0285	0.149
-$\underline{CH_2}$-$\overline{CH3}$	0.0285	0.083
-$\overline{CH_2}$-N-	0.01	0.485
$\underline{\text{Phenyl-CH}_3}$	0.0265	0.216
Phenyl protons as a whole	0.0234	
C_1^*-N		0.007
C_3		−0.007
C_2		−0.117
N-C = 0		−0.289
C_4		−0.063

$\Delta\delta$ is the difference in chemical shifts of LDC in the presence and absence of ßCD. Negative signs indicate upfield displacement, positive signs indicate downfield displacement. * numbering of LDC carbons is shown in Fig. 1

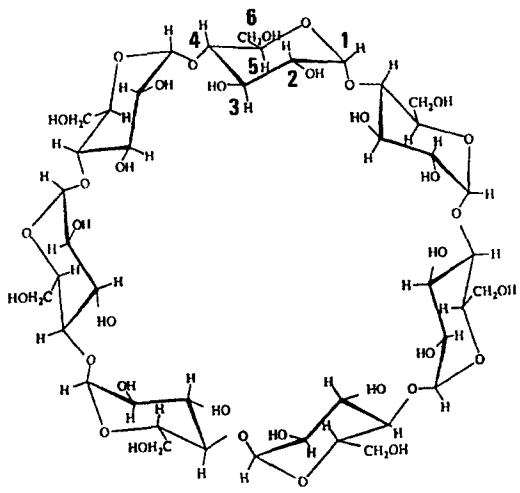

Fig. 5. Structure of ß-cyclodextrin.

were rather small, perhaps due to a loose inclusion of LDC in ßCD. Table 4 shows the effect of LDC on the ^{1}H chemical shifts of ßCD in D_2O. It is clearly evident that LDC caused an upfield displacement of all signals in ßCD. The chemical structure of ßCD is shown in Fig. 5. Significant changes of chemical shifts which are assigned to inner protons of ßCD (H3 and H5), as well as proton near the cavity (H6 on the rim of the torus), were observed. By contrast, no appreciable shifts were detected for protons located outside the cavity, such as H1, H2 and H4, indicating a lower probability of interaction with LDC. This clearly shows that LDC molecules are well inside the ßCD cavity, forming inclusion complexes. The upfield shift of H3 and H5, directed towards the interior, were significantly larger in comparison with that of the interior H6 proton, located at the rim of the small-diameter side, and were considered to be induced by the diamagnetic anisotropy of the LDC aromatic moiety (Thakkar and Demarco, 1971). The greater shift of the H3 proton in comparison with H5 may indicate that more LDC molecules entered the ßCD torus from the H3 side, which is in fact the large-diameter side. The proton-NMR shifts obtained are in agreement with Thakkar and Demarco, 1971 who mentioned that upfield shifts were observed for CD protons and downfield shifts for drug protons upon hydrophobic interactions between both partners.

Table 4
LDC induced ^{1}H and ^{13}C chemical shifts of βCD

Proton or Carbon	$\Delta\delta$ ^{1}H (ppm)	$\Delta\delta$ ^{13}C (ppm)
C_1-H	−0.0026	0.058
C_2-H	−0.0024	0.0014
C_3-H	−0.0183	0.064 or 0.0674*
C_4-H	−0.0020	0.044
C_5-H	−0.016	0.0674 or 0.064*
C_6-H	−0.0082	0.013

$\Delta\delta$ is the difference in chemical shifts of ßCD in the presence and absence of LDC. Negative signs indicate upfield displacement, positive signs indicate downfield displacement. *^{13}C chemical shifts for C_3 and C_5 couldn't be precisely attributed due to overlapping.

The inclusion was also confirmed with carbon-NMR. Table 4 shows the effect of LDC on the ^{13}C chemical shifts of ßCD. LDC caused a downfield displacement of all signals in ßCD, with significant changes of chemical shifts for C1, C3, C4 and C5. The smallest shift was obtained for C2, because of its situation at the outermost position of the ßCD cavity. The magnitudes of the $\Delta\delta$'s for C3, C4, C5 and C6 situated in the direction from the large to the small-diameter side, respectively, showed that the carbon nearer to the large diameter-side, i.e. C3 was shielded more strongly, and this may support the conclusion based on proton-NMR results, that LDC

enters the ßCD cavity from the large-diameter side. Lastly, Table 3 shows the effect of ßCD on the ^{13}C chemical shifts of LDC. Significant downfield chemical shifts were obtained for aliphatic carbons, but significant downfield displacements were also obtained for aromatic C2 as well as for the C = O near the phenyl group. This may, again, support that the whole LDC molecule is interacting with the cavity of ßCD.

These NMR chemical shifts behaviors suggested that the benzene ring of LDC was not predominantly included within the cavity of ßCD and that all part of LDC molecule may interact with ßCD. NMR studies also provided evidence that the interaction was an inclusion phenomenon since the modifications obtained for ßCD signals involved hydrogens and carbons that were oriented towards the cavity.

4. Conclusion

The freeze-drying method is suitable for giving inclusion complexes between LAs and CDs, as shown by modifications in the complexes spectra obtained from spectral methods and thermal analysis, when compared to pure components. These results allow us to envisage a possible use of LA-CD complexes as a drug-delivery system resulting in controlled release of the LA drug. In a subsequent paper, the interaction between five LAs and these CDs will be quantified with the determination of the affinity constants. Then, bupivacaine transfer rate from an aqueous to an organic phase will be evaluated, with or without CDs, using an in vitro two phase liquid-liquid system.

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