Journal of Inclusion Phenomena and Macrocyclic Chemistry (2005) 52:213–221
DOI 10.1007/s10847-004-7239-7

On Chloralose-Cyclodextrin Complexes by *ESI*-Mass Spectrometry

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(Received: 6 April 2004; in final form: 6 December 2004)

Key words: cyclodextrin inclusion complexes, encapsulation of chloraloses by cyclodextrins, ESI-MS *versus* molecular modelling

Abstract

α-Chloralose is a useful mild anaesthetic, providing stable but not deep anaesthesia. Host–guest complexes of α-chloralose with α-, β- and γ-cyclodextrin (CD) were studied using electrospray ionisation (ESI-MS) mass spectrometry and molecular modelling (MD). As it is currently administered, chloralose is transported in a convenient water-soluble complex, and released to react on a physiological level with the reactor sites. It is believed that the chloraloses (α and its isomer β) are encapsulated within the CD cavity. However, the ESI spectra did not reveal such the presence of inclusion compounds. Searching of alternative mechanisms of transportation of these anaesthetic agents, we found that outer-cavity complexes of inserted chloraloses, as found from MD calculations, do have a reasonable stability.

Drug design has reached new heights with the massive use of combinatorial chemistry. This new trend in drug discovery, based on a 'shotgun' approach, does not necessarily produce larger quantities of the desired substance, and additionally poses the problem of separation of complex mixtures. However, it does allow huge libraries of new products to be tested, and their active components isolated, quickly and easily.

A second trend in new drug development is the search for new methods of transportation of drugs of known, proven physiological characteristics. One such method is the use of molecular encapsulating agents *via* inclusion complexes, such as those formed from cyclodextrins of various cavity sizes. An increasing number of papers deal with the structure, chemical reactivity, and biological interactions of cyclodextrin complexes [1]. The encapsulation of drugs *via* cyclodextrin inclusion complexes is one of the simplest applications of supramolecular technology to find medical use. A cyclodextrin's apolar cavity can easily accept many different guest molecules, both organic and inorganic, because of its variable size (cavity depth, circumference of both ends). Due to its hydrophilic hydroxyl groups, complexes of cyclodextrin are, in general, water-soluble.

Cyclodextrins (CD) are macromolecular oligomers composed of six (α-CD), seven (β-CD), or eight (γ-CD) glucose units (α-D,(+)), connected *via* [1–4]-glycosidic bonds to form toroidal wide-mouthed baskets, with a slightly larger circumference for the top opening than for the bottom. The secondary hydroxyls on C_2 and C_3 of each glucose form the upper rim of the basket, while the primary C_6 hydroxyls form the base of the basket, and are oriented out of its bottom. The glucose ring oxygens are oriented toward the inside of the molecule (truncated cone). The apolar cavity size of such a CD ranges from ca. 5 to 10 Å, the basket wall thickness is ca. 4.5 Å, and its total height is 7.9 Å. Many of the CD's hydroxyls can be selectively derivatised: for instance, in this study the CD investigated included a hepta 6-(2-hydroxypropyl) β-CD (2HPβ-CD). Derivatisation usually leads to the modification of the physicochemical properties and, therefore, of the inclusion selectivity. Both the size of the cavity and the flexibility of the CD increased, from α to β and from β to γ. These molecules were studied *via* molecular modelling to evaluate the stability of their major and most probable conformations, and to calculate the dimensions of their apolar cavities.

Both the conformer stability and the cavity dimension are necessary to study the insertion of α-chloralose [(1,2-0-2,2,2-trichloroethylidene)-α-D-glucofuranose] or β-chloralose [(1,2-0-2,2,2-trichloroethylidene)-β-D-glucofuranose] into a cyclodextrin. The cyclodextrin's apolar cavity acts as a cache for the inserted molecule, which can be charged or neutral. This 'guest' molecule is transported as a water-soluble complex, and is released upon its dilution by the liquids of the physiological environment (plasma, etc). In this way, α-chloralose, which is minimally soluble in water at room temperature, could be delivered to its site of action without having had its solubility increased by heating. This is significant because heating can lead to α-chloralose's partial hydrolysis, and (perhaps more

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importantly) to the formation of the toxic convulsant isomer, β-chloralose. Although α-chloralose is soluble in urethane, in glycerol, and in polyethylene glycol, these are either inconvenient or risky [2–4].

From this perspective, the room-temperature stability of α-chloralose-CD complexes within a wide range of concentrations is an interesting feature of its wider anaesthetic application [5, 6] *via* intravenous injection.

When the chloralose enters the CD-cavity, there is enough space for at least one guest molecule per cavity, as well as for several solvent molecules. In CD-water solution, water molecules in the cavity can be replaced by an α-chloralose. Under further dilution, however, the chloralose itself can be replaced by further solvent molecules (water, or – depending on the experimental conditions – spectrometric solvents such as methanol, ethanol, or acetonitrile).

The β-CD derivative hepta 6-(2-hydroxypropyl)-βCD was offered by Sigma as a host for α-chloralose, and is commercially available. The structure of this host–guest entity was presented by Sigma as an insertion complex (Sigma Product # C-8849) [7, 8]. Since the effects of chloralose formulations on animal subjects have already been evaluated, and since cyclodextrins have many potential applications as vectorisation agents for other pharmaceuticals, we decided to study the structure of chloralose-CD complexes.

In this study, the structures of twelve complexes of six different CD (α, β, γ-CD and their hexa, hepta and octa 6-(2-hydroxy propyl)-derivatives, 2HP-α, β, γ -CD) with two chloraloses (α and β) were investigated using ESI-MS and molecular modelling. In both techniques, several hypotheses of complex structures were considered according to the ratio of host to guest reagents (1:1, 1:2, and 2:1), and to the type of insertion of the guest within the host, which greatly increased the number of possible structures.

The structures of inclusion compounds such as chloralose-CD complexes can be studied using different spectroscopic techniques, such as mass spectroscopy based on electrospray ionisation (ESI) or on atmospheric pressure ionisation (API). Both techniques allow us to study the complexes in aqueous media using soft ionisation. We chose to use positive-ion (PI) Electrospray Ionisation Mass Spectrometry (ESI-MS) to study these complexes.

ESI-MS allowed us to detect cyclodextrin complexes of various organic or inorganic compounds, and, to a certain extent, to study the speciation of these products. Although controversy still exists concerning speciation under ESI-MS [9–17], the insertion products from sugar-related physiologically-neutral hosts (CD) and physiologically-active guests (α-chloralose) should, in principle, be observed under protonation as mono- or polycharged cations. The ESI- mass spectra of all products (all CD and both chloraloses as well as complexes made in different solvents and commercial complexes) were recorded.

Electrospray ionisation mass spectroscopy study

First, the spectra of commercial cyclodextrins were recorded (Figure 1). The MH^+ ions were not observed; we did, however, observe a less intense series of polyprotonated MHn^{+n} ions, as well as a mixed series of metal cation ions and protonated hydrate ions for α-, β- and γ-CD. When the cone voltage was optimized, the higher voltage (e.g. 90 V) led to fragmentation of the CD skeleton, with the successive loss of glucose units (Figure 2). For the 2HP-CD, the MH^+ molecular ion was not observed. Careful examination of these spectra revealed the presence of peaks separated by 58 Da, which are a series of Na^+ (mono or double) charged ions corresponding to different degree of substitution on the primary hydroxyl of CD. For example (Figure 1), for 2HP-α-CD, peaks at 1111, 1169, 1227, 1285, 1343, and 1401 corresponded to MNa^+ ions of (2HP)n-α-CD with $n = 2$–6, and in a Mna_2^{+2} series, at 567, 596, 625, 654, 683, as well as even more substituted peaks of 712 or 741, with $n = 2$–6. This confirmed the presence of complex mixtures resulting from the random reaction of the CD with epoxides or halohydrins.

The ESI-MS spectra of α- and β-chloralose displayed pseudomolecular protonated and cationized ions at the expected masses, within a wide range of solvents. The double protonation of these compounds does not take place.

After recording the ESI-MS spectra of the substrates, we then recorded the spectra of CD-chloralose complexes prepared according to Sigma's specifications – i.e., in ethanol. CD-chloralose complexes of different formulations of six CD and two chloraloses were measured at several cone voltages, from 10 to 90 V. However, even at lower voltages we were not able to observe any complex ions for any substrate ratios (1:1, 2:1 and 1:2).

We failed to observe any complex ions corresponding to CD-chloralose products in various other solvents, despite the use of both sonication and heating. Variations in substitute ratio yielded no signs of complexation in either cationic or anionic spectra. Table 1 contains the diagnostic ions expected for different protonation level for these four complexes. Following this investigation we also evaluated the commercial complex hepta (2-hydroxypropyl) β-CD-α-chloralose (Sigma)[1] (2HP-β-CD-α-chloralose).

The loss of the water molecules from the cavity was observed in the presence of several solvent systems studied such as water, acetonitrile, methanol, ethanol, trifluoroethanol, and mixtures thereof. This follows logically from the easy expulsion of the water molecules from the cavity, and is also affected by the presence of other solvents such as methanol or ethanol. It has also been observed that the addition of methanol to the CD complexes lead to further destruction of complexes [18].

However, the α- and γ-CD, hosting both chloralose substrates, yielded the expected molecular ions (Table 1, Figures 3 and 4). For the α-CD, the protonated

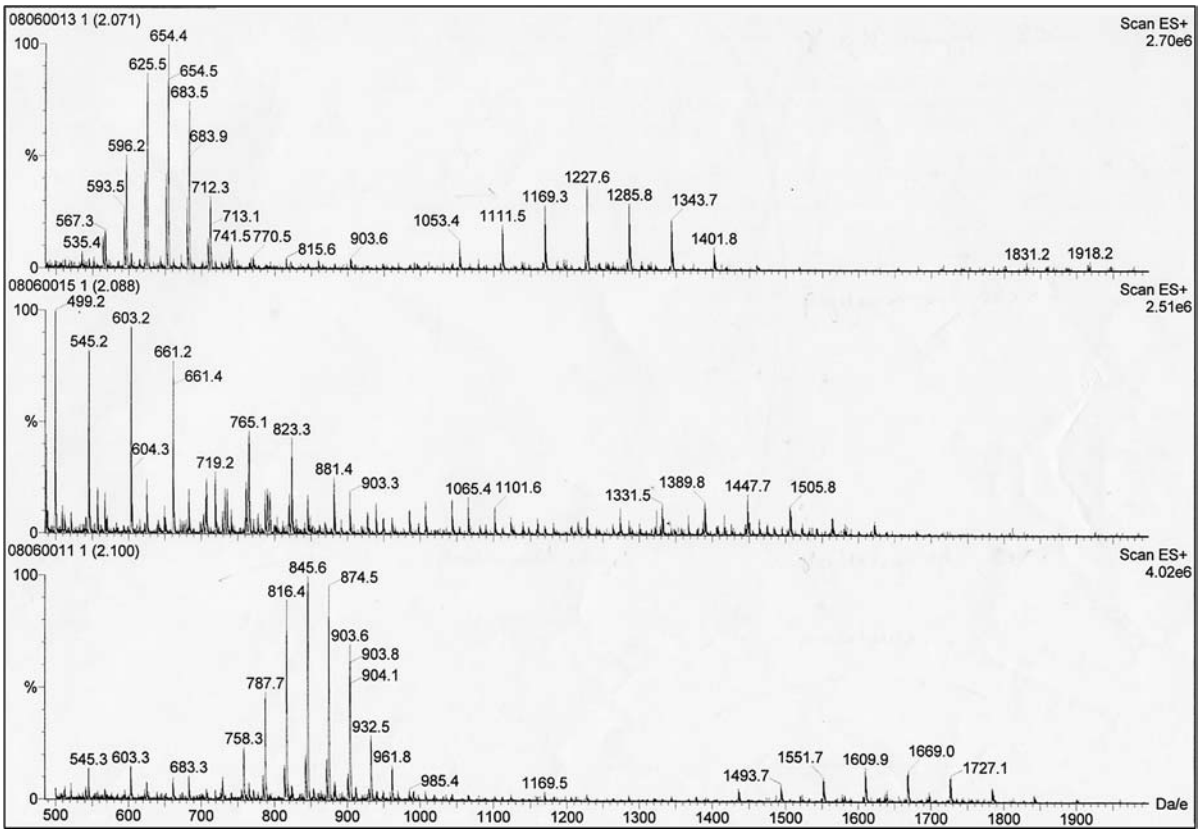


Figure 1. ESI mass spectra of commercial 2HP-CD (Aldrich Chemicals) upper run 2-HP α-CD, median 2-HP β-CD, bottom 2-HP γ–CD at 80 V.

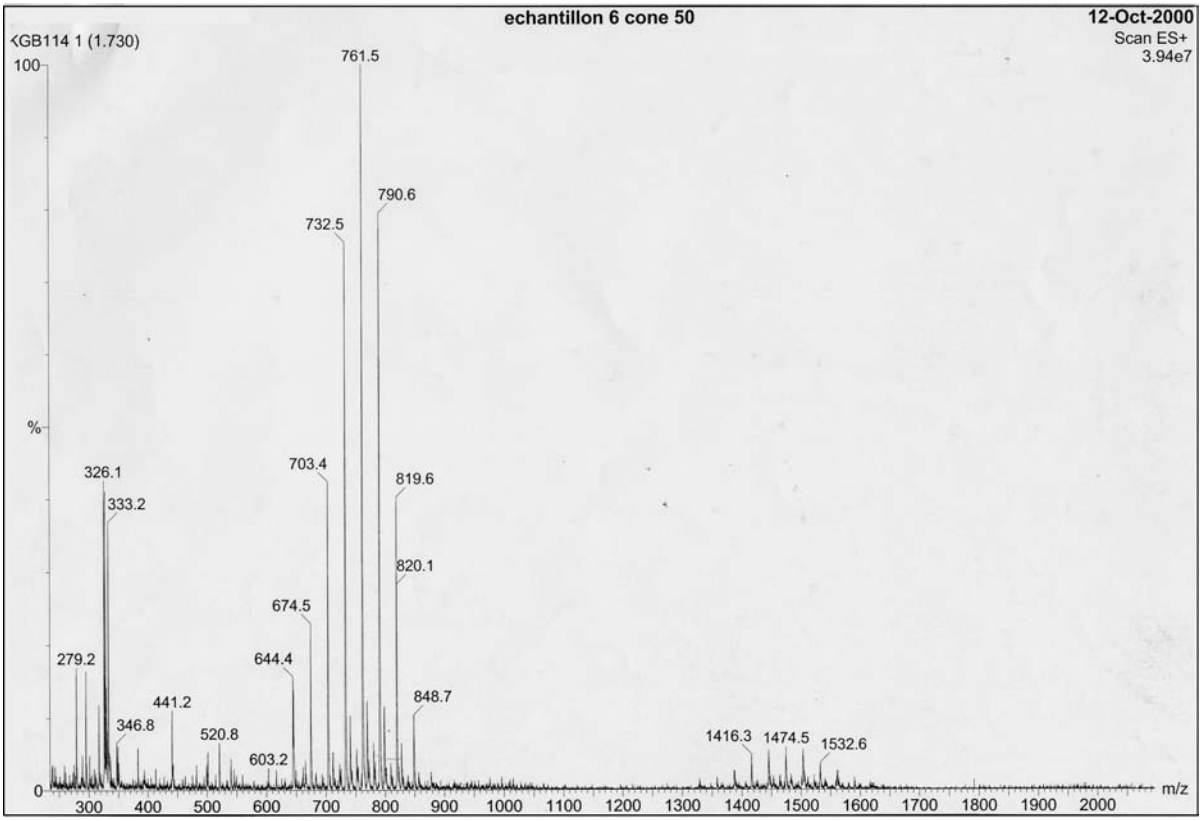


Figure 2. ESI mass spectra of commercial (Sigma, RBI) 2HP β-CD-α-chloralose complex at 50 V.

Table 1. Diagnostic ions for 1:1, 1:2 and 2:1 complexes of CD and chloralose (MHn^{+n}, m/z, Th)

α or β-chloralose		h-CD			2HP n-CD		
		α (n = 6)	β (n = 7)	γ (n = 8)	α (n = 6)	β (n = 7)	γ (n = 8)
Alone	309	973	1135	1297	1320	1540	1760
Complex type (chloralose:CD) ratio	Ions						
1:1	MH^+	1283	1445	1607	1630	1850	2070
	MH_2^{+2}	642	723	804	815.5	925.5	1035.5
	MH_3^{+3}	428.3	482.3	536.3	544	617.3	690.7
	MH_4^{+4}	321.5	362	402.5	408.3	463.3	518.3
1:2	MH^+	1592	1754	1916	1939	2159	2379
	MH_2^{+2}	796.5	877.5	958.5	970	1080	1190
	MH_3^{+3}	531.3	585.3	639.3	647	720.3	793.7
	MH_4^{+4}	398.8	439.3	479.8	485.5	540.5	595.5
2:1	MH^+	2256	2580	2904	2950	3390	3830
	MH_2^{+2}	1128.5	1290.5	1452.5	1475.5	1695.5	1915.5
	MH_3^{+3}	752.7	860.7	968.7	984	1130.7	1277.3
	MH_4^{+4}	564.8	645.8	726.8	738.3	848.3	958.3

* Nominal mass only shown.

molecular ion at m/z 973 is observed at 20 and 35 V and completely disappears at higher cone voltage. The γ-CD MH^+ ion is observed at m/z 1297 Th at similar cone voltage, together with Na^+ adduct at 1319 Th in several solvents such as water, methanol–water 1:4, etc.

It is interesting to notice the formation of these host–guest complex ions with neutral guest molecules, as opposed to the more popular charged guests (protonated amines, etc.). Detection of a complex of a nominally neutral guest, such as chloralose, means that some of these complexes were actually present in solution and survived the electrospray process. The absence of these peaks (corresponding to complex), however, is not absolute proof of the complexes' absence.

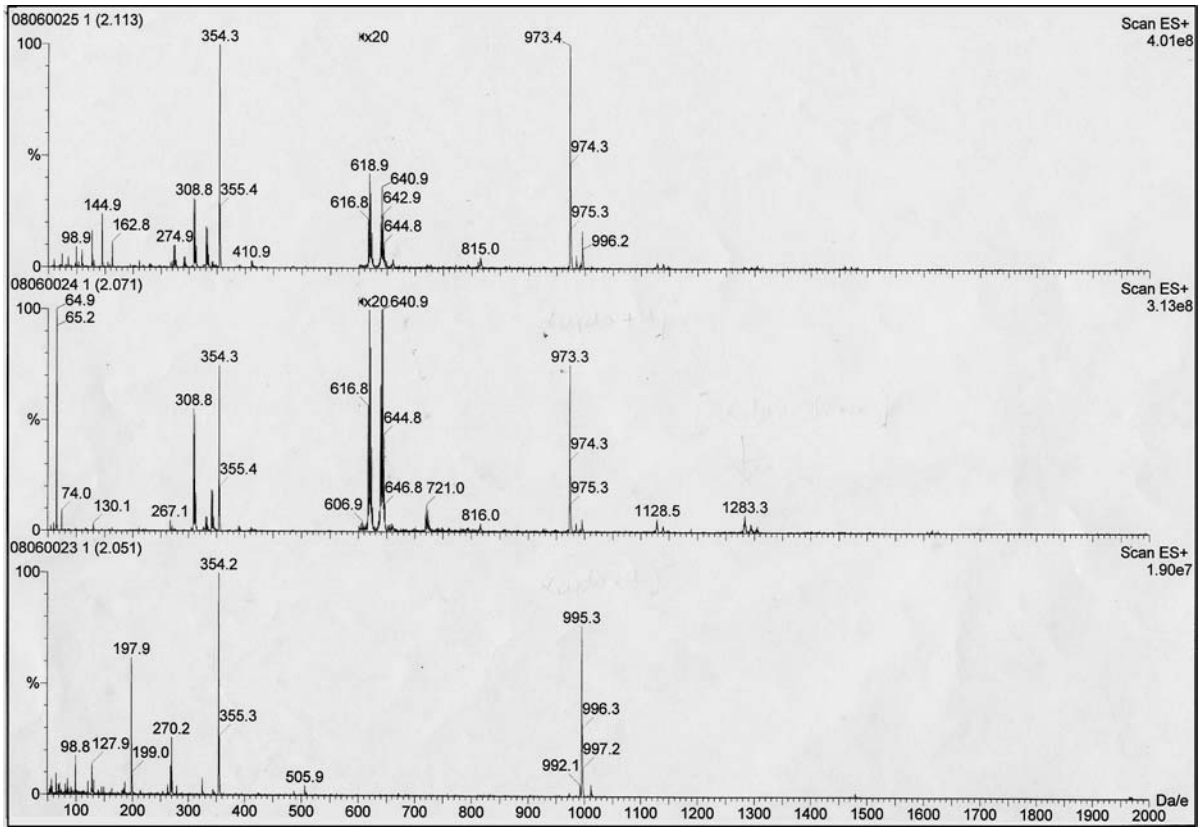


Figure 3. ESI mass spectrum of α-CD-α-chloralose complex, upper run at 35 V(m/z 973 MH^+), median run at 20 V (m/z 1283 of complex, 973 α-CD MH^+, 309, α-chloralose MH^+), bottom run α-CD alone at 20 V (m/z 973 MH^+, 995 MNa^+).

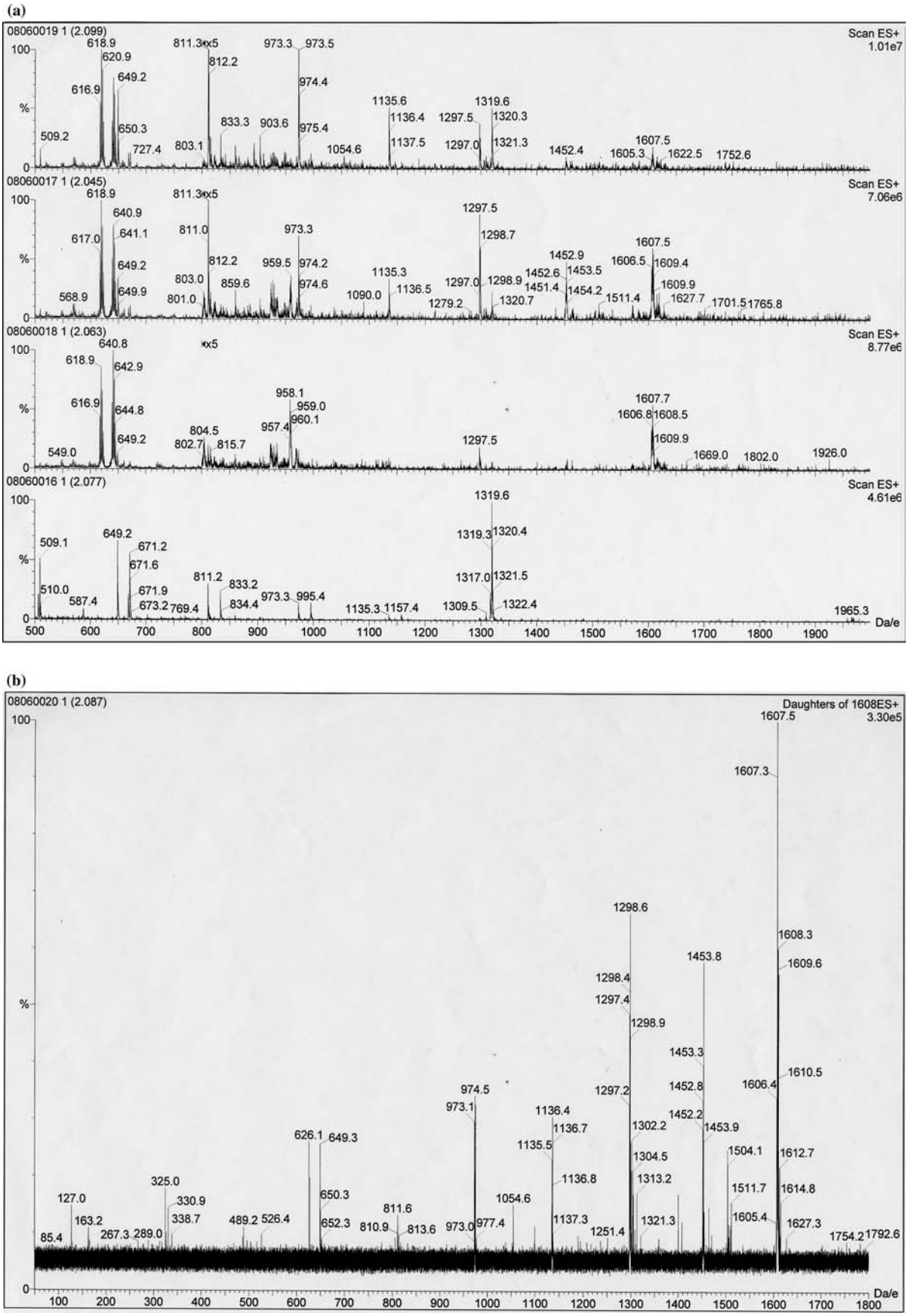


Figure 4. ESI mass spectrum of γ-CD α-chloralose complex. (a) upper runs m/z 1607.5 MH^+ of complex at 80, 50 and 35 V, together with 1297, (MH^+) and 1319 (MNa^+) of γ-CD, bottom run γ-CD alone (m/z 1319.6, MNa^+). (b) MS/MS daughter ions of complex γ-CD α-chloralose at m/z 1607 (m/z 1298 γ-CD MH^+, 649.3 γ-CD $MH_2{}^{+2}$). Series of ions m/z 1298-n (162) loss of glucose units: 1298, 1136, 974, 812 is also observed.

The two other complexes observed in this series were weak adduct ions for α-CD and α-chloralose at m/z 1283 Th, and γ-CD and α-chloralose at 1607 Th, both corresponding to 1:1 complexes of these substrates, and observed from 35–50 V. When a search for the daughter ions of this last complex was performed, we observed only the intense MH^+ ion for γ-CD (Figures 3 and 4).

Increased cone voltage applied to the CD usually leads to the losses of sugar units, one by one, and, for the (2-hydroxypropyl) CD series, to the dealkyation of primary alcohol groups only. The absence of any other positive indications for complex formation from mass spectra in ESI-MS could also be explained by the fact that these compounds are, in fact, mixtures of several products.

This conclusion validates the failure of ESI to identify the presence of single-unit CD in the mixtures of these products. The absence of the complex-originating ions could also be a result of their low stability, or of unexpected host–guest structures. This last conclusion leads us to further molecular modelling calculations, assuming different inclusion models [5].

Under this hypothesis, we elucidated the structure of superficially-inserted chloralose in the core of the CD, which allowed us to preserve Sigma's hypothesis and explain the drug delivery *via* CD. This structure, resulting from the capping of either rim of the CD by the chloralose, is an alternative explanation for the lack of results in ESI-MS, and a compromise solution to fully-inserted complexes. From the mass spectrometric standpoint, such a capped structure – although it cannot be recorded even at low cone voltage – should display a reasonable stability, relative to the energy of such a complex (as calculated using molecular modelling).

Molecular modelling study

The two types of half-inserted (exo-cavity) structures (with the voluminous trichloromethyl head protruding either from the upper, larger opening, or from the lower, narrower one) were calculated separately. Two other types (with the pentose tail protruding), were likewise calculated separately [18].

The calculations, presented in Table 2A (α-chloralose) and B (β-chloralose), were performed for three CD's (α, β, γ) as well as for three 2-hydroxypropyl CD (α, β, γ). The calculated structures were created according to the following procedure [19–23].

First, for the 1:1 complexes, eight starting structures were calculated (A–H); these were formed from the insertion of chloralose *via* the trichloromethyl head (A) or tail (B) into the larger rim of any given CD. Two analogous structures were calculated with chloralose being inserted *via* the narrower rim of the CD/(C and D). Then, capping structures were calculated with the C—Cl_3 head of chloralose being oriented toward (F) or outside (E) larger rim of the CD, according to the bent shape of the chloralose. The next two structures were calculated for narrow rim of the CD in analogous manner (structures G and H).

Then, four 2:1 CD-chloralose complexes were calculated. Structures J and L represent the complex with chloralose immobilised between two CD oriented with similar rim sides, and the other two 2:1 complexes represent two other (I and K) possibilities of orientation.

Finally, four 1:2 CD-chloralose complexes were calculated, in which the chloraloses are oriented into the CD either in a symmetrical manner (i.e. heads

Table 2A. Molecular modelling

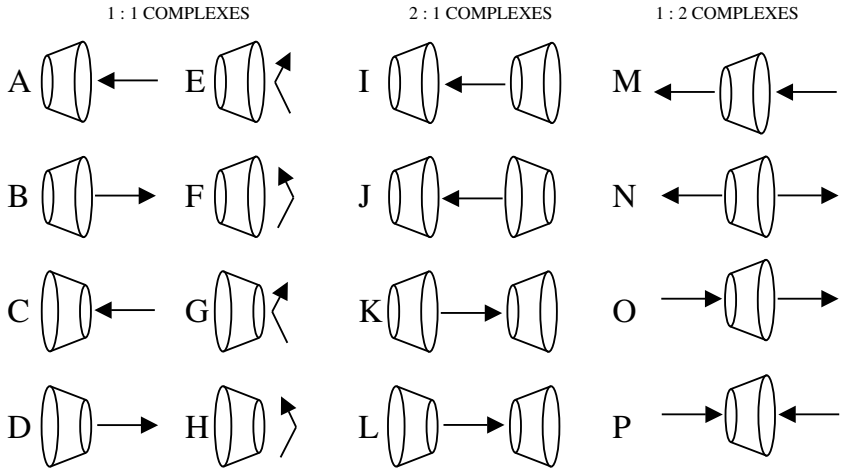
	Calculated total energies (Kcal/mol) and final structure codes* for the final conformations					
Structure code	α-CD	β-CD	γ-CD	2HP α-CD	2HP β-CD	2HP γ-CD
1:1 (CD: chloralose)						
A	64.5/**c**	73.4/**p**	79.0/**i**	85.0/**c**	96.2/**c**	110.6/**i**
B	66.6/**c**	68.6/**i**	80.4/**i**	86.4/**p**	96.8/**p**	111.6/**i**
C	70.3/**c**	74.0/**c**	81.8/**i**	88.6/**c**	107.3/**c**	120.9/**c**
D	69.9/**p**	73.9/**i**	78.9/**i**	89.6/**p**	109.1/**c**	112.6/**i**
E	66.8/**c**	70.9/**c**	80.1/**i**	87.7/**c**	97.9/**c**	111.1/**p**
F	65.3/**c**	76.3/**c**	80.4/**i**	86.2/**c**	97.8/**c**	118.6/**c**
G	71.7/**c**	79.6/**c**	79.8/**i**	89.1/**c**	105.3/**c**	120.2/**c**
H	71.1/**c**	80.0/**c**	85.3/**p**	92.8/**c**	117.8/**c**	122.3/**c**
2:1 (2 CD: 1 chloralose)						
I	118.8/**cc**	118.4/**pp**	136.8/**pp**	159.3/**cc**	179.3/**cc**	202.7/**pp**
J	113.1/**cc**	114.8/**pp**	129.9/**pp**	155.5/**cc**	167.9/**cc**	204.9/**pp**
K	110.9/**cp**	117.7/**pp**	134.3/**ic**	156.5/**cc**	188.3/**cp**	206.6/**pp**
L	125.9/**cc**	123.7/**pp**	134.9/**ic**	167.2/**cc**	193.3/**cc**	209.4/**ic**
1:2 (1 CD: 2 chloraloses)						
M	67.8/**cc**	74.4/**cp**	82.3/**ic**	87.5/**cc**	99.3/**cc**	108.7/**pp**
N	68.4/**cp**	73.0/**pp**	80.1/**ic**	88.3/**pp**	97.5/**cp**	107.8/**ic**
O	64.1/**cp**	72.1/**ic**	78.5/**ic**	85.7/**pp**	95.5/**cp**	111.8/**ic**
P	71.1/**cc**	70.6/**cp**	77.1/**ic**	86.8/**cc**	97.9/**cp**	110.0/**pp**

α-Chloralose complexes with CD and 2-hydroxypropyl CD structures according to insertion mode.

Table 2B. Molecular modelling

Structure code	Calculated total energies (Kcal/mol) and final structure codes* for the final conformations					
	α-CD	β-CD	γ-CD	2HP α-CD	2HP β-CD	2HP γ-CD
1:1 (CD: chloralose)						
A	67.1/**c**	74.2/**p**	81.3/**i**	87.6/**c**	99.9/**c**	113.4/**i**
B	69.1/**p**	72.3/**i**	82.2/**i**	88.4/**c**	98.7/**i**	112.2/**i**
C	76.0/**c**	82.7/**c**	82.3/**i**	99.8/**c**	112.3/**c**	110.1/**i**
D	75.2/**c**	77.2/**c**	84.1/**i**	92.4/**c**	110.3/**c**	109.8/**i**
E	69.7/**c**	73.9/**c**	84.4/**c**	91.2/**c**	100.7/**c**	112.0/**i**
F	65.3/c	74.3/**p**	81.5/**i**	85.5/**c**	101.3/**c**	112.1/**i**
G	73.1/**c**	80.1/**c**	82.6/**i**	98.1/**c**	109.7/**c**	116.3/**c**
H	71.4/**c**	80.4/**c**	89.7/**c**	94.2/**c**	105.7/**c**	118.1/**c**
2:1 (2 CD: 1 chloralose)						
I	121.5/**cc**	121.2/**ic**	135.4/**pp**	159.3/**cc**	178.6/**cc**	197.3/**pp**
J	113.1/**cc**	120.0/**ic**	134.8/**pp**	156.7/**cc**	187.4/**cc**	193.7/**pp**
K	130.5/**cp**	121.5/**pp**	136.8/**ic**	163.5/**cp**	176.6/**cp**	203.7/**pp**
L	135.5/**cc**	124.2/**pp**	136.5/**ic**	175.9/**cc**	199.0/**cc**	206.8/**cp**
1:2 (1 CD: 2 chloraloses)						
M	2.2/**cc**	75.8/**cc**	82.7/**cp**	102.5/**cc**	105.0/**cc**	110.5/**cp**
N	72.7/**cc**	77.5/**cp**	84.7/**pp**	93.9/**cp**	100.4/**cp**	115.3/**pp**
O	69.9/**cp**	77.4/**cp**	86.1/**ic**	90.0/**cp**	100.4/**cp**	116.5/**cp**
P	74.8/**cc**	76.7/**cc**	81.2/**ic**	95.9/**cc**	112.1/**cc**	110.9/**cp**

β-Chloralose complexes with CD and 2-hydroxypropyl CD structures according to insertion mode.
**Final structure codes*
For the 1:1 complexes
c – Capping.
p – Partial insertion.
i – Insertion.
For the 2:1 complexes
cc – Capping of both CDs by the same chloralose.
cp – Capping of one CD and partial insertion on the other CD by the same chloralose.
ic – Insertion in one CD and capping of the other CD by the same chloralose.
pp – Partial insertion in both CDs by the same chloralose.
For the 1:2 complexes
cc – Capping on both sides of the CD by the two chloraloses.
cp – Capping on one side of the CD by one chloralose and partial insertion on the other side by the other chloralose.
ic – Insertion on one side of the CD by one chloralose and capping on the other side by the other chloralose.
pp – Partial insertion on both sides of the CD by the two chloraloses.



Scheme 1. Starting *insertion patterns.*

toward Px or tails towards CD structure N), or in opposite directions with head, tail (structure M with CD oriented as shown in Scheme 1), and tail, head (structure O).

All these starting structures were optimised, and their final structure energies are presented in Table 2A (for α-chloralose) and 2B (for β-chloralose). Eleven types of final structures were detected. When we considered only

one chloralose and one CD, we obtained: capping (**c**) on one side of the CD, partial insertion (**p**), and full insertion (**i**). When we considered one chloralose with two CD's, we obtained: capping by the chloralose of both CD's (**cc**), capping of one CD by the chloralose and partial insertion in the other CD (**cp**), partial insertion in both CDs (**pp**), and full insertion of the chloralose in one CD and capping of the second CD (**ic**). When we considered two chloraloses with one CD, we obtained: capping on both sides of the CD (**cc**), capping on one side of the CD and partial insertion on the other side (**cp**), partial insertion on both sides of the CD (**pp**), and full insertion by one of the chloraloses and capping by the second one (**ic**).

After computation, the chloralose was either in an inserted position (most of the chloralose was inside the cage), a partially-inserted position (less than half of the chloralose was inside the cage) or in a capped position (the chloralose was above the cage opening, covering the entrance, in a parallel orientation to it).

In general, in the 1:1 complexes, capping was more prevalent with the smaller CD, while insertion was more frequently observed for the larger CD.

In the 1:2 complexes, the most common cases were those with two chloraloses capping both entrances of the CD, and those in which one chloralose is partially inserted in one side and the other chloralose is capping the second entrance.

In the case of 2:1 complexes, both CD's were sandwiching the chloralose. The most common cases were those with the chloralose capping both CD's at once, and the chloralose being partially inserted in both CD's. In all cases, the chloralose could be seen as being *inside* the complex.

The comparison of calculated total energies for several complexes leads to the following conclusions. The most stable complex of all calculated structures for the α-chloralose is that for the 1:1 complex with α-CD, which most likely indicates a good fit of the cavity size and the guest molecule (the mass spectrum for these two molecules confirmed the formation of 1:1 complex).

All final optimised complexes figures are available on request from CKJ (in colour or B/W).

Conclusions

ESI spectra of the α-chloralose-2-hydroxypropyl-β-CD complex (commercially available from Sigma Chemicals) in several solvents generally failed to show the presence of inclusion ions. The fact that ESI loses its sensitivity when a complex mixture of products are treated does not alone explain the lack of expected ions for α-chloralose-HBC inclusion compounds [18, 24].

The commercial sample was prepared by inserting α-chloralose at room temperature into HBC in boiling [9] ethanol. We have tried this preparation as well as several different solvents (e.g. water, alcohols, ethylene glycol, acetonitrile, and trifluoroethanol, both separately and in mixtures), and varying inclusion temperature from room temperature to 80 °C, without being able to obtain the ion corresponding to these complexes from the ESI spectra in different solvents. Even if both components (CD and chloraloses) were well heated for a longer period of time, the inclusion ions were not observed. β-chloralose in particular (together with β-cyclodextrins) should be heated for a long time in order to achieve complete dissolution.

Curiously enough, Sigma Chemicals' procedure for the commercial preparation of α-chloralose-2-hydroxypropyl β-CD uses ethanol as a solvent; consequently, the release of the guest molecule from the apolar cavity should be expected. This release shows some variation, when compared to the CD-cavity size. This means that the stability constants of complexes in water and in ethanol are slightly different. It is possible that some other complexation mechanisms are involved. However, from our modelling it is clear that the chloralose transportation and delivery could be easily achieved as (for instance) an inserted complex with two cyclodextrins sandwiching the guest molecule, to carry it to the active site.

Experimental

All six cyclodextrins as well as the C8849 complex and α- and β-chloraloses were purchased from Sigma–Aldrich Chemical C. (St Louis, MO, U.S.A.) and were used without prior purification, as received. The solvents used for preparation of complexes were bought from Merck Chemicals.

Molecular modelling

Molecular modelling calculations were performed on HyperChem 6 Mm + (UdeM) in gas phase and in solvent box (ethanol) modes. The minimisation of energy was performed using the technique described in the HyperChem instruction manual, and in ref. [20]. In a first phase, 1,600 Polak-Ribler iterations were applied. A small incremental energy was applied in a second phase (0.001 ps for a total of 1 ps, 370 K) for selected insertion models only. All calculated complex structures (1:1, 2:1 and 1:2 stoichiometry) and energy data are available upon request from CKJ (UdeM) and according to ref. [18].

Mass spectroscopy

Electrospray ionsisation mass spectroscopy (ESI-MS) experiments were performed on a Quattro II Micromass (U.K.) spectrometer (CEN de Saclay). Samples were injected at 10 ml/min, with a source temperature of 80 °C and capillary tension maintained at + 3.35 KV. The cone voltage ranged from 10 to 70 V; at 20 V the skimmer voltage was 1.9 V. In a typical experimental

setting, 100 μl of an ethanolic solution of Nile Red and g-CD (1:1 or 2:1 mole ratio) was prepared and introduced through a Harward Apparatus syringe pump. The ions were detected by scanning the first quadrupole and the mass range was monitored from $m/z = 80$–2000 in 7 s. At least 50 scans were averaged to obtain representative spectra [18].

Acknowledgements

This work was realized with the help of small research grant from the FESR (U de Moncton). The help in recording of some of the mass spectra by Drs. C. Lamouroux and B. Amekraz is gratefully acknowledged. We would like to thank Dr. J. Quick, Sigma, for helpful discussion.

Note

[1] According to Sigma's brochure, the CD-chloralose complexes (C8849) are formed from a 'mixture of products of unspecified purity'. This alone explains the difficulty in recording the mass spectrum of such a mixture.

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