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Effect of methylation of β-cyclodextrin on the formation of inclusion complexes with aromatic compounds. An ionspray mass spectrometry investigation

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Abstract

The investigation of the inclusion complexes obtained with heptakis-(2,6-di-*O*-methyl)-β-cyclodextrin (DM-β-Cdx) or heptakis-(2,3,6-tri-*O*-methyl)-β-cyclodextrin (PM-β-Cdx) and either 1-anilinonaphthalene-8-sulfonate (ANS) or 2-*p*-toluidinylnaphthalene-6-sulfonate (TNS) was carried out by means of ionspray mass spectrometry, both in the positive and in the negative ion mode. All the data collected showed that the heptakis-(2,3,6-tri-*O*-methyl)-β-cyclodextrin interacted to a very little extent with TNS and not at all with ANS. On the contrary, heptakis-(2,6-di-*O*-methyl)-β-cyclodextrin formed complexes with both aromatic molecules showing a more effective interaction with TNS. Small variations in the number of methoxy substituents in the DM-β-Cdx molecule did not affect the complexation behaviour significantly. The spectra recorded at different orifice potentials indicated that the complex of heptakis-(2,6-di-*O*-methyl)-β-cyclodextrin with TNS is more stable than the one formed with ANS. These results agreed on one hand with the conformations of both the heptakis-(2,6-di-*O*-methyl)-β-cyclodextrin and heptakis-(2,3,6-tri-*O*-methyl)-β-cyclodextrin, established by X-ray diffraction studies, and, on the other hand, with the different complexation behaviour of the guest aromatic molecules due to their own geometry. © 1997 Elsevier Science Ltd.

Keywords: Methylated β-cyclodextrins; Ionspray mass spectrometry; Inclusion complexes

Abbreviations: ANS, 1-anilinonaphthalene-8-sulfonate ; DM-β-Cdx, heptakis-(2,6-di-*O*-methyl)-β-cyclodextrin; ISMS, ionspray ionisation mass spectrometry; ISV, ionspray voltage; PM-β-Cdx, heptakis-(2,3,6-tri-*O*-methyl)-β-cyclodextrin; PPG, polypropylene glycol; OR, orifice voltage; TNS, 2-*p*-toluidinylnaphthalene-6-sulfonate

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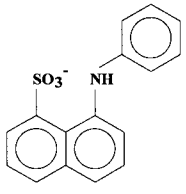
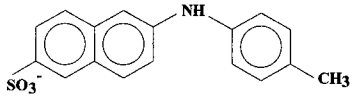
1. Introduction

The development of soft ionisation procedures has prompted a number of investigations on the detection of mass spectrometric peaks produced by molecular associations and possibly by specific interactions [1]. In this context cyclodextrins, having a relatively low molecular weight, are good model systems since they can host specific chemical groups into their hydrophobic cavity, notably aromatic molecules and long aliphatic chains [2,3]. These host–guest interactions have been extensively investigated by means of a variety of experimental techniques, such as NMR spectroscopy [4,5], circular dichroism [6], fluorescence spectrometry [7,8], calorimetry [9,10], and mass spectrometry [11–13]; therefore the inclusion complexation has a sound experimental basis.

ANS



TNS



The possibility to detect inclusion complexes of cyclodextrins with aromatic molecules (ANS, 1-anilinonaphthalene-8-sulfonate; TNS, 2-*p*-toluidinylnaphthalene-6-sulfonate) was recently investigated by means of ionspray mass spectrometry (ISMS) [14]. In this study, the interference due to simple electrostatic adducts was eliminated by applying a high voltage at the orifice in order to produce disruption of week interactions. The results obtained showed that indeed the electrostatic adducts disappeared for small increase of the orifice voltage, whilst ions belonging to inclusion complexes were detected up to orifice voltage (OR) of 130 V and OR = −180 V, in the positive and in the negative ion mode, respectively. Moreover, in the same paper it has been shown that, for the above mentioned systems, the spectra recorded in the negative ion mode contained more information than the ones obtained in the positive mode.

In this paper, ionspray mass spectrometry data on heptakis-(2,6-di-*O*-methyl)-β-cyclodextrin (DM-β-Cdx), on heptakis-(2,3,6-tri-*O*-methyl)-β-cyclodextrin (PM-β-Cdx) as well as on their inclusion complexes with ANS and TNS are presented.

2. Results and discussion

The search for DM-β-Cdx and PM-β-Cdx inclusion complexes was conducted both in the positive and in the negative ion mode. The mass spectra of DM-β-Cdx and PM-β-Cdx, recorded in the positive mode, are shown in Fig. 1a and b, respectively. In both spectra the peaks relative to singly and doubly charged species, of the ammonium and sodium adducts, are clearly detected. Moreover, the spectrum of DM-β-Cdx (Fig. 1a) was rather complicated by the presence of pseudomolecular ions differing from each other by 14 amu. They were assigned to DM-β-Cdx molecules bearing one or two extra methoxy groups on the C-3's of the glucosyl residues. The same pattern was also evident for the doubly charged species, where peaks differing by 7 amu were detected. The PM-β-Cdx sample did not exhibit any peak relative to a lower methylation degree.

When DM-β-Cdx was mixed with TNS, the recorded mass spectrum clearly showed the presence

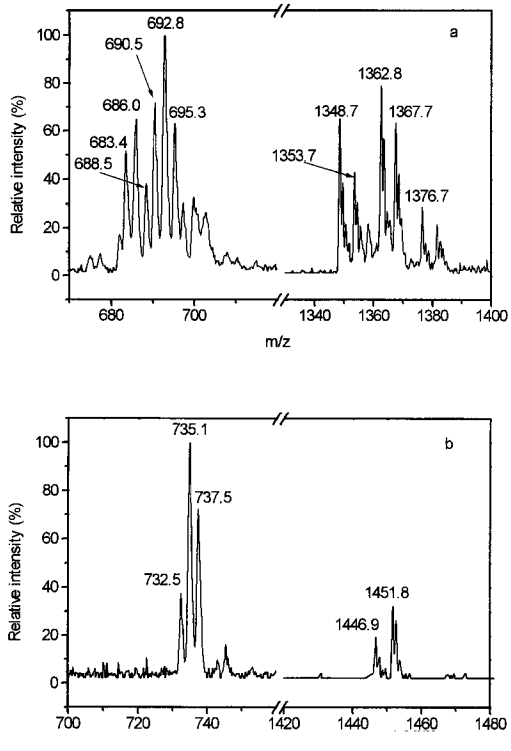


Fig. 1. Positive detection mode ISMS of DM-β-Cdx (a) and PM-β-Cdx (b).

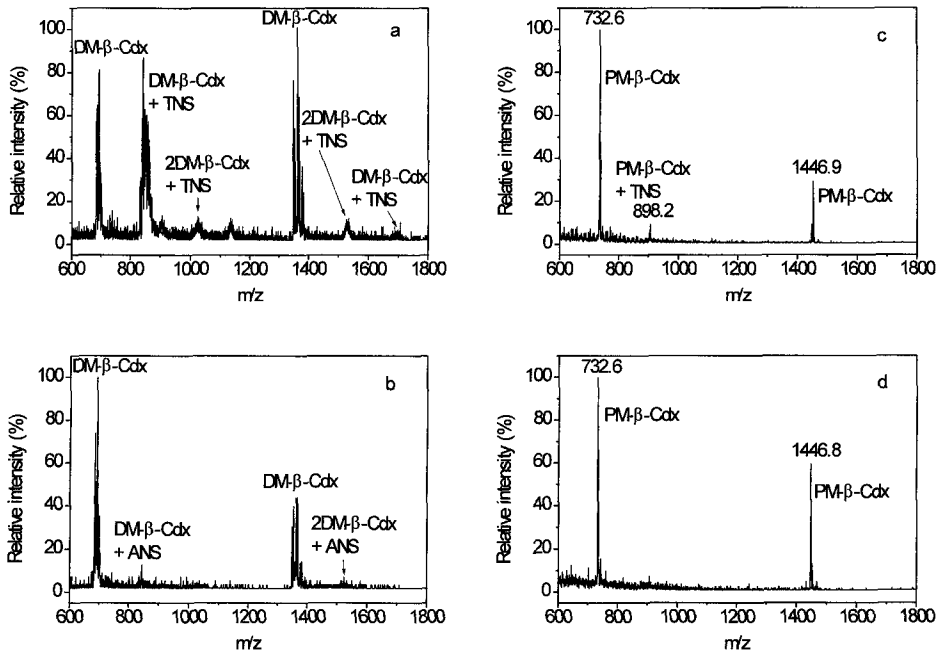


Fig. 2. Positive detection mode ISMS of the mixtures: DM-β-Cdx and TNS (a), DM-β-Cdx and ANS (b), PM-β-Cdx and TNS (c), and PM-β-Cdx and ANS (d). For Fig. 2a and b, a more detailed assignment is given in Table 1. In Fig. 2c and d, the peaks at 732.6 m/z and 1446.8 m/z correspond to the singly and doubly charged PM-β-Cdx species, respectively.

of new clusters (Fig. 2a); they were relative to the complex of DM-β-Cdx with the aromatic molecule and were present as singly and doubly ionised species (Table 1). The presence of multiple peaks for each ionic species was due on one hand to the occurrence of extra methoxy groups onto the DM-β-Cdx ring and on the other hand, to the presence of different ionising species and counterions (H^+, NH_4^+, Na^+, and K^+). Also the presence of a complex consisting of two DM-β-Cdx and one TNS molecules was detected. The spectrum obtained in the presence of DM-β-Cdx and ANS showed similar results (Fig. 2b). However, the ions belonging to the latter complexed species exhibited a much lower intensity with respect to that relative to the TNS's complexes, thus suggesting a lower degree of complexation. The greater capacity of TNS to fit into the cavity of the substituted cyclodextrin is in good agreement with previous experimental findings obtained by means of different experimental techniques on mixtures of na-

Table 1
Assignment of the observed ions for the experiments carried out on the mixtures of DM-Cdx with TNS and with ANS

Mixture	Figure	m/z	Charges [a]	Assignment
DM-β-Cdx-TNS	2a	670–710	2	DM-β-Cdx
DM-β-Cdx-TNS	2a	820–870	2	DM-β-Cdx + TNS
DM-β-Cdx-TNS	2a	1000–1050	3	2DM-β-Cdx + TNS
DM-β-Cdx-TNS	2a	1340–1390	1	DM-β-Cdx
DM-β-Cdx-TNS	2a	1500–1550	2	2DM-β-Cdx + TNS
DM-β-Cdx-TNS	2a	1680–1750	1	DM-β-Cdx + TNS
DM-β-Cdx-ANS	2b	670–710	2	DM-β-Cdx
DM-β-Cdx-ANS	2b	830–870	2	DM-β-Cdx + ANS
DM-β-Cdx-ANS	2b	1340–1390	1	DM-β-Cdx
DM-β-Cdx-ANS	2b	1500–1550	2	2DM-β-Cdx + ANS

[a] Number of charges of the observed ions.
The ions present as counter ions of TNS and ANS, and as charging species can be H^+, NH_4^+, Na^+, K^+.

tive cyclodextrins and either TNS or ANS [9]. Such experimental data were also supported by molecular modelling calculations [9]. The rather large difference in the intensity of the peaks relative to DM-β-Cdx/TNS and to DM-β-Cdx/ANS complexes might lead to the conclusion that the presence of the methoxy groups on the cyclodextrin ring makes the complexation behaviour of the two aromatic molecules even more different, when compared to that exhibited by native cyclodextrins. When PM-β-Cdx was used, the complex with TNS was still detected at 898.2 amu (Fig. 2c), although to a very low extent. On the contrary, no ions due to complex formation could be detected when the mixture PM-β-Cdx and ANS was investigated (Fig. 2d). These latter findings could be explained taking into account not only the difference in size and shape between TNS and ANS, but also looking at the structures of the methylated cyclodextrins as obtained by means of X-ray diffraction on single crystal. Both the X-ray structures of the heptakis-(2,6-di-*O*-methyl)-β-cyclodextrin [15] and heptakis-(2,3,6-tri-*O*-methyl)-β-cyclodextrin [16] exhibited the methoxy groups occurring on C-6 of the glucose residues, bowing from the same rim of the cyclodextrin ring and interacting within each other forming a cap on the cyclodextrin cavity. In this way, the structure of permethylated cyclodextrin resembles more a 'bowl' than a 'crown'. In addition to this, the investigation of the crystal structure of PM-β-Cdx showed that one glucose residue exhibited the unusual $^{1}C_{4}$ conformation, thus causing a slight reduction of the size of the hydrophobic cavity [16]. Probably, the 'bowl' structure prevent the oligosaccharidic ring from fitting properly the ANS and TNS molecular shape. For the sake of comparison, the crystal structure of the 1:1 complex of PM-β-Cdx with ethyl laurate [17] showed that the inclusion of such a guest molecule (in this case a long aliphatic chain) modified the conformation of the ring. In fact, after complexation all the glucose residues exhibited the $^{4}C_{1}$ chair conformation, and all the primary methoxy groups were turned so to remove the 'cap' from the cyclodextrin cavity.

The net negative charge on the guest molecules suggested to carry out the same set of experiments in the negative ion mode, thus avoiding the presence of different counter ions. The results were similar to those attained in the positive ion mode.

The spectra obtained with equimolar mixture of PM-β-Cdx and either ANS or TNS confirmed that the permethylated cyclodextrin does not interact at all with ANS, and only slightly with TNS (data not shown). On the contrary, the spectra recorded with DM-β-Cdx (Fig. 3a and b) indicated the presence of ions exhibiting the m/z values corresponding to the inclusion complexes. However, only in the presence of ANS the peaks of the free heptakis-(2,6-di-*O*-methyl)-β-cyclodextrin species could be detected, whilst in the presence of TNS only the peak of the complex appeared in the spectrum, thus suggesting a larger degree of complexation. In both spectra, the presence of one, two and three extra methoxy groups on the DM-β-Cdx molecule was clearly revealed by ions differing from each other by 14 amu, even if the most substituted DM-β-Cdx was present in very small amount and therefore neglected in the following considerations. The relative intensity ratio of DM-β-Cdx, DM-β-Cdx with one extra methoxy group and DM-β-Cdx with two extra methoxy groups was about 80:100:40. Interestingly enough, this ratio was maintained also in the complexed species, thus indicating that these few extra methoxy groups on the C-3's of the glucosyl residues did not interfere in the formation of the inclusion complex with either TNS or ANS, while the full substitution of hydroxyl groups with methoxy groups (PM-β-Cdx) prevented the formation of inclusion complexes.

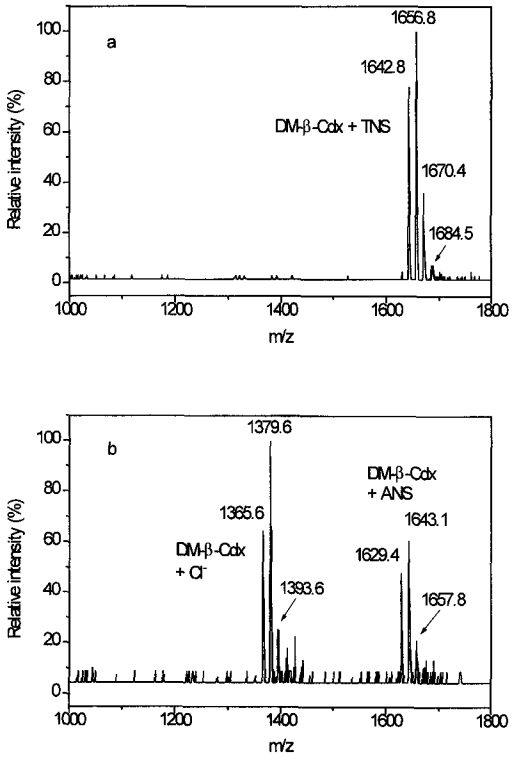


Fig. 3. Negative detection mode of the mixtures DM-β-Cdx and TNS (a), and DM-β-Cdx and ANS (b). In Fig. 3b, the free DM-β-Cdx is present as Cl^- adduct.

In order to exclude the presence of 'false positives', simply due to electrostatic adducts, two experimental approaches were used. In the first experiment, DM-β-Cdx was mixed with equimolar amounts of acetate ions and no ions were detected, thus suggesting the absence of non-specific interactions (due to the presence of negative charges). When DM-β-Cdx was mixed with equimolar concentration of acetate and either TNS or ANS, the spectra recorded were identical to the ones which contained no acetate ions, further confirming the presence of specific association between DM-β-Cdx and both TNS and ANS.

The second experimental approach consisted in the investigation of the relative stability of the complexes DM-β-Cdx/TNS and DM-β-Cdx/ANS in the negative mode by increasing the voltage at the orifice. In the case of the mixture DM-β-Cdx and TNS the ions corresponding to the complex were present for orifice potential up to −180 V. As expected, the total intensity decreased, but the peak relative to the free DM-β-Cdx molecules never appeared even at the highest OR potential applied, indicating that such potentials were not able to dissociate the complex. When the mixture DM-β-Cdx and ANS was studied, for increasing negative values of the orifice potential (from −50 V up to −180 V) the peak of free DM-β-Cdx was always present. In addition to this, the intensity ratio of the peak DM-β-Cdx/ANS to the free DM-β-Cdx peak exhibited a diminishing trend. In conclusion, the complex DM-β-Cdx/TNS was relatively more stable than the DM-β-Cdx/ANS one. This finding could be easily explained by considering the different molecular shape and the consequent mode of interaction of the two organic molecules. In fact, the more elongated TNS molecule seemed to better fit into the methylated cyclodextrin cavity.

3. Experimental

Ionspray mass spectrometry.—The mass spectra were recorded on a API-I PE SCIEX quadrupole mass spectrometer equipped with an articulated ion spray and connected to a syringe pump for the injection of the samples. The instrument was calibrated using a polypropylene glycol mixture (3.3×10^{-5} M PPG 425, 1×10^{-4} M PPG 1000 and 2×10^{-4} M PPG 2000), 0.1% acetonitrile and 2 mM ammonium formate in 50% aq MeOH. The samples were injected at a flow rate of 0.2 mL/hr. When the analyses were conducted in the positive mode, the ionspray voltage (ISV) was 5000 V and the OR was 50 V. In the negative mode, the ISV was set at −5000 V and the OR at −50 V. The spectra were recorded using a step size of 0.1 amu. Some experiments were performed varying the OR from −50 V to −180 V in the negative ion mode.

Preparation of the samples.—The samples of DM-β-Cdx (MW: 1331.8) and PM-β-Cdx (MW: 1430.0) (a kind gift of Dr. Szejtli, Cyclolab, Cyclodextrin Research and Development Laboratory, 1026 Budapest, Enrõdi S. 38/40 Hungary), as well as ANS and TNS (see Formula 1) were dissolved in 50% aq acetonitrile. The final concns used in the positive ion mode experiments were 2×10^{-5} M in DM-β-Cdx and 1×10^{-6} M in PM-β-Cdx. For the negative ion mode experiments, the DM-β-Cdx was used at a final concn of 1×10^{-3} M and PM-β-Cdx at 5×10^{-5} M. The concns of ANS and TNS were equimolar to the methylated cyclodextrins with which they were mixed. Ammonium acetate (0.6×10^{-4} M) was used as ionising agents in the positive ion mode.

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