Journal of Thermal Analysis and Calorimetry, Vol. 80 (2005) 419–424

THERMAL CHARACTERIZATION OF NATURAL AND MODIFIED CYCLODEXTRINS USING TG-MS COMBINED TECHNIQUE*

Zsuzsanna Éhen[1], *F. Giordano*[2], *J. Sztatisz*[1], *L. Jicsinszky*[3], *Cs. Novák*[4]**

[1]Institute of General and Analytical Chemistry, Budapest University of Technology and Economics, Szt. Gellért tér 4, 1111 Budapest, Hungary
[2]Department of Pharmacy, University of Parma, Parco Area delle Scienze 27/A, 43100 Parma, Italy
[3]CYCLOLAB R&D Lab. Ltd., 1525 Budapest, P.O.B. 435, Hungary
[4]Hungarian Academy of Sciences, Budapest University of Technology and Economics, Research Group of Technical Analytical Chemistry, Szt. Gellért tér 4, 1111 Budapest, Hungary

Thermoanalytical techniques (TG, DSC) are frequently used in the investigation of the thermal properties of cyclodextrins and their inclusion complexes. However, the above techniques do not provide information on the chemical composition of the evolved fragments upon the thermal decomposition. In this study α-, β- and γ-cyclodextrins and 4 methylated and 3 ethylated β-CD derivatives were investigated with a TG-MS combined thermoanalytical technique in order to get information about their fragmentation behaviour.

By comparison of the TG/DTA curves, a different thermal behaviour was found for each of the native and the chemically modified cyclodextrins. Except for the water loss profiles and the solid-solid phase transformations, the thermal behaviour of the (investigated) native CDs do not show remarkable differences. However, the chemical modification of the native β-CD resulting in a new compound may change the strength of interactions between host and guest causing differences in the thermal stabilities of the derivatives. The mass spectrometry results supported the observed thermal differences and showed significant alterations in the fragmentation of ethylated and methylated compounds.

The investigated natural CDs possess a very similar fragmentation profile, due to the common α-D-glucopyranose building units. In the case of modified CDs characteristic signals of the substituents are present.

Keywords: *combined technique, cyclodextrin, mass spectrometry, TG-MS, thermal analysis*

Introduction

Cyclodextrins are the products of the enzymatic transformation of starch [1]. These natural or synthetic cyclic oligosaccharides possess an inner space able to host foreign molecules. Among several natural cyclodextrins, α-, β- and γ-cyclodextrins (CDs) – containing 6, 7, 8 glucopyranose units respectively – have the highest industrial relevance.

Cyclodextrins are typical host molecules. Their peculiar cyclic structure and physico-chemical properties allow the entrapment of other guest molecules. This process is sometimes referred to as encapsulation.

They can entrap molecules with the size of one or two benzene rings (even larger ones if the guest molecule has a proper side chain or functional group), which are included inside the cyclodextrin cavity and interact with the internal surface of the CD ring. The resulted compound is called cyclodextrin inclusion complex.

The applications of cyclodextrins and their complexes play a significant role in many fields as food, cosmetics, agriculture, chemistry, analytical chemistry and pharmaceutical technology [2].

The production of inclusion complexes is a useful tool to improve the stability and water solubility of the hosted compound, to modify the release of drugs [3–5] and/or turn liquid substances, as essential oils, into stable and free-flowing powders.

The increasing number of marketed cyclodextrin products also underline the importance of these agents in the listed cases.

An example of the advantages offered by the preparation of inclusion complexes is observed for the essential oils, which are volatile liquids obtained from a large variety of plants. These multicomponent systems, when exposed to oxygen, light, moisture and high temperatures, are oxidized, decompose and become resinous or evaporate. The complexation with cyclodextrins can improve the thermal and chemical stability and at the same time also facilitates their handling and the preparation of new dosage forms.

β-CD is the most frequently used term for the preparation of complexes, despite its relatively low water solubility. Using the proper substituents its water solubility can be substantially increased, the hy-

* *Dedicated to the memory of Prof. J. Szejtli*

** Author for correspondence: cs-novak@mail.bme.hu

1388–6150/$20.00

Akadémiai Kiadó, Budapest, Hungary
Springer, Dordrecht, The Netherlands

drophilic character can be changed or the toxicity can be decreased. The most frequently used cyclodextrin derivatives are the acetylated, methylated, ethylated, hydroxypropylated and maltosyl compounds [6].

In the present study our aim was to investigate the three native CDs and some of their substituted forms using combined thermoanalytical/mass spectrometry technique to characterize the investigated samples and to follow their way of fragmentation [7].

Experimental

Materials

Together with the α-, β-, γ-CDs, 7 substituted β-cyclodextrins were investigated.

α-, β-, γ-CDs were provided by CYCLOLAB Res. & Dev. Ltd. Budapest, Hungary.

RAMEB (DS~12.5) was purchased from Wacker Chemie GmbH, Munich, Germany, and used without additional purification.

Preparation of (2-hydroxy)propyl β-cyclodextrin (HPβCD)

β-Cyclodextrin (1 mol) was dissolved in water (2.5 L) containing sodium hydroxide (3 mol) and 1,2-propylene oxide (5 mol) was added below 20°C. When the reaction completed, sodium was removed with strong cation exchanger (3 kg) and product was obtained by freeze-drying. Yield: 80%, calcd. for DS 3.4, detd. ^{1}H-NMR.

Preparation of (2-hydroxy)ethyl β-cyclodextrin (HEβCD)

β-Cyclodextrin (1 mol) was dissolved in water (2 L) containing sodium hydroxide (6 mol) and 2-chloroethanol (5 mol) was added dropwise at 60–65°C. When the reaction completed, the reaction mixture was neutralized with 2N hydrochloric acid, water was evaporated and the obtained residue was treated with MeOH (3 L). Solids were filtered off, washed with MeOH (3×25 cm^3), and the filtrate was dialyzed twice against deionized water (2×50 L). The solution of the product was clarified with charcoal and freeze dried, with a yield of 68%.

Preparation of heptakis(2,6-di-O-ethyl) β-cyclodextrin (DIETβCD)

Freshly dried β-cyclodextrin (0.1 mol) was dissolved in dried DMSO (700 cm^3). Powdered sodium hydroxide (30 mol) was added to the solution and heated at 80–85°C for 30 min, when the sodium hydroxide was practically dissolved. The reaction mixture was cooled below 40°C and iodoethane (29 mol) was added slowly but not dropwise using water bath to maintain the temperature below 40°C. The reaction mixture was stirred for 72 hrs at room temperature and then poured onto deionized water (7 L). The formed precipitate was collected by filtration. The filtered solid was dissolved in *n*-hexane (approx. 1 L), and allowed to stand for crystallization for two days. Solids were filtered off, and filtered through a silicagel bed (1 kg) in *n*-hexane and *n*-hexane/acetone mixtures. Product was isolated by evaporation of the combined pure fractions (>90% of DIETβCD). Yield: 37%.

Preparation of ethylated β-cyclodextrin (ETβCD)

The mother liquor of *n*-hexane crystallization of DIETβCD was filtered through a silicagel bed (500 g), washed with *n*-hexane and *n*-hexane-acetone mixture. The product containing fractions were combined and solvents were removed by evaporation. The obtained sticky foam was dissolved in acetone, clarified with charcoal, and poured onto water (1 L) dropwise. The obtained precipitate was collected by filtration and dried over P_2O_5 at room temperature, in vacuo. Yield: 40%, calcd. for DS 13.5, detd. ^{1}H-NMR.

Preparation of heptakis(2,6-di-O-methyl) β-cyclodextrin (DIMEB)

DIMEB was prepared by the known method using barium hydroxide octahydrate, dimethyl sulfate, and sodium hydroxide in DMSO and THF. The obtained product had about 50% of pure isomer accompanied by over- (~15% DS=15, ~10% DS=16) and undermethylated (~15% DS=13) β-cyclodextrins and ~10% of regioisomers. DIMEB has >90% purity could be obtained after 5–6 consecutive recrystallizations from acetone and methanol. Yield: 10%.

Preparation of heptakis(2,3,6-tri-O-methyl) β-cyclodextrin (TRIMEB)

RAMEB (0.1 mol) was methylated with sodium hydroxide (1.4 mol) in DMSO (700 cm^3) and dimethyl sulfate (1.4 mol). When the reaction completed the excess of dimethyl sulfate was decomposed with cc. ammonia (150 cm^3). The formed precipitate was filtered off, washed with DMSO (200 cm^3) and *n*-hexane (2×100 cm^3). The obtained crude product was recrystallized several times from water. Yield: 77%.

Preparation of partially methylated β-cyclodextrin, DS=4-6 (PMβCD46)

β-Cyclodextrin (1 mol) was dissolved in water (2.5 L) containing calcium hydroxide (6 mol) and dimethyl sulfate (6 mol) was added dropwise below 20°C. When

the reaction completed, the unreacted calcium hydroxide was removed by filtration, the filtrate was solidified with evaporation, and dissolved in MeOH (3 L) and treated with strong cation exchanger (3 kg) and anion exchanger (2 kg). Removal of MeOH and crystallization from water resulted in the targeted product. Yield: 74%, calcd. for DS 4.8, detd. ^{1}H-NMR.

Table 1 Systematic names of the substituted samples and their abbreviations

Systematic name	Abbreviation
(2-Hydroxy)ethylated-β-cyclodextrin	HEBCD
Ethylated-β-cyclodextrin	ETBCD
Heptakis (2,6-*di*-O-Ethyl)-β-cyclodextrin	DIETBCD
Heptakis (2,6-*di*-O-Methyl)-β-cyclodextrin	DIMEB
Heptakis (2,3,6-*tri*-O-Methyl)-β-cyclodextrin	TRIMEB
Partially methylated β-cyclodextrin	PMBCD
Randomly methylated β-cyclodextrin	RAMEB

The systematic names of the β-CD derivatives and their abbreviations are given in Table 1.

Instruments and methods

The thermoanalytical measurements have been carried out using TA Instruments (Newcastle, Delaware, USA) STD 2960 simultaneous TG-DTA unit under helium purging. The STD 2960 unit was connected through a heated silica capillary inlet to a Balzers Thermostar GSD 300T quadrupole mass spectrometer for the evolved gas analysis measurements.

The mass spectrometer can operate in two modes.

When the 'Scan Analog' mode is used, the ion current belonging to a previously adjusted specific mass/charge range is continuously measured. The recorded values describe a three-dimensional data block, where the *x*, *y*, *z* axes belong to *m*/*e* units, ion currents and time – which is related to the temperature – respectively.

In the 'Multiple Ion Detection (MID)' mode the ion currents belonging to the previously selected individual *m/e* units are detected and plotted as a function of time thus resulting in a two-dimensional graph.

Table 2 Experimental parameters

	Parameters	CD samples
TA	Flow rate/ L h^{-1}	10
	Heating rate/K min^{-1}	10
	Temperature interval/°C	35–400
	Sample mass/mg	8–8.5
SCAN mode	Observ. interval/amu	Selected channels between 10–140
SCAN/ MID mode	Observ. time/s	0.2 per channel

The experimental conditions are collected in Table 2.

X-ray

The equipment was a Jena-Zeiss HZG4 Freiberger Präzisions Mechanik Diffractometer with CuK_α (λ 1.5405 Å and 2θ 2 to 44°), operating at room temperature under settings of 30 kV and 25 mA.

Results and discussion

Depending on the way of preparation and origin, some differences can be found in the water content of CDs. In this respect, in the ambient temperature – 150°C range, TG and DTA/DSC methods are suitable to perform a careful characterization of water release kinetics from the samples.

On the other hand the TG/DTA-MS combined technique provides not only the mass loss values but also information on the chemical composition of the produced fragments can be obtained.

Native cyclodextrins

As expected from their chemical structure and physical properties, the general thermal behaviour of natural CDs is similar. Differences were obviously observed in the water contents and therefore in the mass loss values.

Thermoanalytical results

The investigation of the three most common natural CDs was in the focus of many previous papers [8–10]. The TG and DTA curves of the native CDs (Fig. 1) can be divided into three main parts.

Water loss between ambient and 120°C is the first representative feature, and, depending on the examined compound, 9–14% mass loss can be ob-

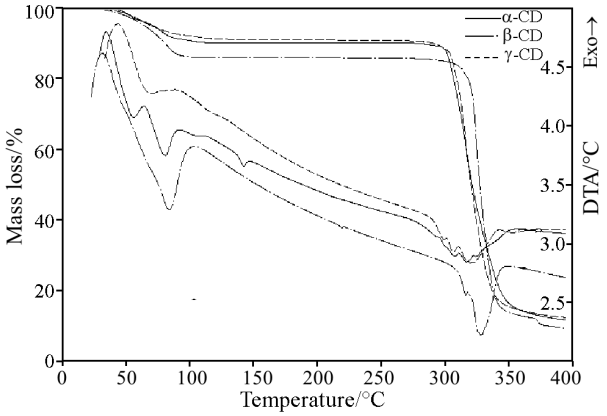

Fig. 1 TG/DTA curves of native cyclodextrins

served. In the second stage (120–250°C) a plateau region appears and no further mass loss can be seen in the TG curves. However, the corresponding DTA curves exhibit a small endotherm peak at 142°C for α-CD and another small one at about 210°C for β-CD, which can be attributed to structural transformations [5–7]. The thermal degradation of the cyclodextrins becomes remarkable over 250°C causing a mass loss of the initial sample up to 78%. The degradation starts in the solid phase and a simultaneous melting/decomposition effect takes place.

Mass spectrometry results

Since α-, β- and γ-CDs are built up from the same glucopyranose unit, one can suppose that, disregard of water loss and the solid-solid phase transformation, their thermal fragmentation would take place in a similar way and substantially consistent with the fragmentation of the α-D-glucopyranose itself.

Figure 2 indicates the most remarkable MID curves of β-CD. It is worth to underline that above 250°C the thermal fragmentation of α- and γ-CD is practically the same as for β-CD, and, therefore, the MID profiles of the former ones are not shown. The key fragments are identified as the 18, 22, 31, 44 *m*/*e* units. The 18 *m*/*e* fragment is obviously related to the formation of water while the others correspond to the fragments of the glucopyranose units (see the relevant assignments in Table 3). In agreement with the thermal traces of β-CD, the MID curves confirm the three-stage decomposition pattern. Owing to the loss of absorbed water the ion current intensity of the *m*/*e*=18 unit represents a smaller peak up to 120°C. The evaporation of the strongly bound and structural water takes place only in the third stage, over 310°C, resulting a higher ion current. The water formation in the melting/decomposition region can also be reasonably attributed to concomitant reactions among by-products of the thermal decomposition. Supporting the TG results, the thermal fragmentation of the sugar part starts above 250°C.

Assignments of the formed fragments related to the decomposition of α-D-glucopyranose are listed in Table 3.

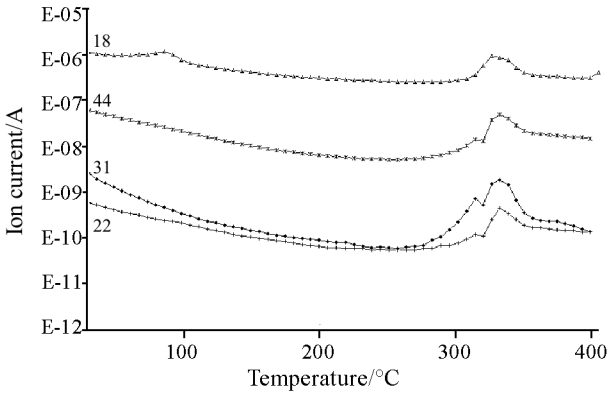

Fig. 2 MID curves of β-cyclodextrin

Table 3 Fragments related to the decomposition of α-D-glucopyranose

Fragment ions	*m*/*e* unit
OH_2^+	18
CH_2OH^+	31
$OHCH_2CH^+$	44
$OHCH_2CH^{2+}$	22

β-*cyclodextrin derivatives*

Thermoanalytical results

The chemical modification of the native CD can lead, as already mentioned, to the change of the strength of the interaction between host and guest. In addition, the introduction of functional groups could result in new interactions among decomposition products of the cyclodextrin molecule, due to differences in the thermal stabilities, as it can be seen in Figs 3 and 4.

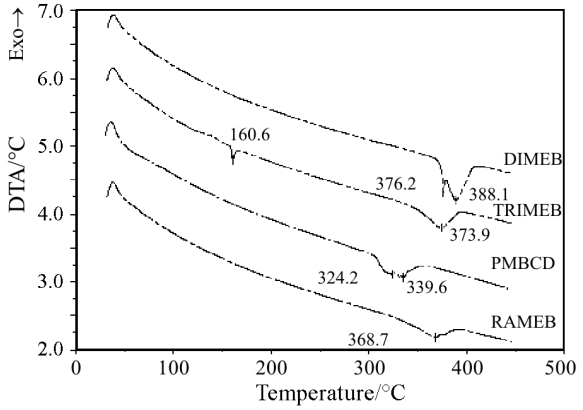

Fig. 3 DTA curves of methylated cyclodextrin derivatives

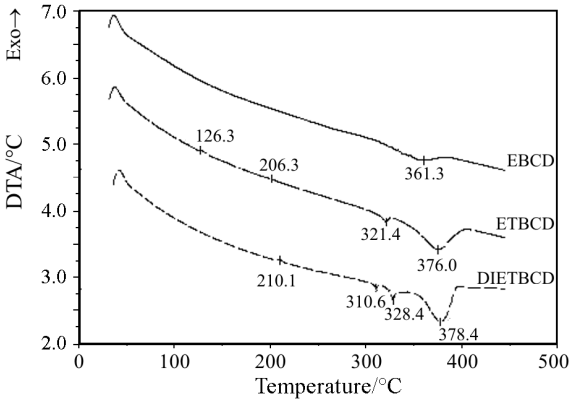

Fig. 4 DTA curves of ethylated cyclodextrin derivatives

In particular, the investigated methylated and ethylated cyclodextrin derivatives show a lower mass loss (1–5%) up to 120°C, compared to the corresponding native CD, related to the evaporation of water. Therefore, the corresponding endotherm peaks on the DTA curves are hardly visible.

The lower water content is the obvious consequence of the chemical modification, and might be attributed to the decrease of free hydroxyl groups, which are partially responsible for the formation of the hydrogen bonds with water.

In the case of TRIMEB the sharp endothermal peak at 160°C (Fig. 3) indicates the melting of the compound, as reported by Szejtli [1].

Comparing the ethyl and methyl substituents, it can be seen that the decomposition of the four methyl derivatives takes place in one or two overlapping steps, resulting in a 88–95% mass loss up to 300°C, except from PMBCD, where the degradation process starts only above 300°C. The corresponding DTA peaks can be found in the 368–390°C range.

By comparing the TG and DTA curves of the methylated derivatives (Fig. 3), the highest thermal stability was observed for the DIMEB while PMBCD showed the lowest thermal stability decomposing above 324°C.

Accordingly, the X-ray powder diffraction pattern of PMBCD confirmed the amorphicity of this compound, thus supporting the DTA results, which did not evidence any melting endotherm.

In a previous investigation on the ethylated and methylated disubstituted derivatives it was reported [10] that, compared to the native CDs, the cavity of the modified cyclodextrin is partially obstructed by a hydrophobic lock formed by the alkyl substituents and it is somehow responsible for the absence of crystal water (Table 4) in DIETBCD, DIMEB, RAMEB, TRIMEB, and the highly reduced water contents for HEBCD, ETBCD and PMBCD.

Comparing the thermal profiles of DIMEB and DIETBCD, the observed differences can also be explained with the intra- and intermolecular interactions caused by the two different alkyl groups. Due to the longer alkyl chain, the ethyl substituent is able to establish stronger dispersional interactions than the methyl group. Starting from the larger size and ability to form stronger dispersional interaction at the ethyl substituent one can expect that the ethyl group would hinder to a higher extent the formation of the hydrophobic lock [11, 12].

The characteristic temperature intervals, the corresponding mass losses and peak temperatures for all derivatives are reported in Table 4.

By the comparison of the starting temperatures of the main decomposition steps of methyl derivatives, their stabilities can be arranged in the following order: PMBCD < RAMEB < TRIMEB < DIMEB. The low thermal stability of PMBCD can possibly be explained also with its amorphous structure. The stability order for the ethylated compounds is HEBCD < ETBCD < DIETBCD.

Regarding the effect of the alkyl substituents on the thermal stability of the CD derivatives, the decomposition of the methylated compounds started at least 15 K above the decomposition temperature of the ethyl derivatives.

Mass spectrometry results

In Figs 5 and 6, the MID curves of TRIMEB and ETBCD, as methyl and ethyl derivatives, are given respectively. The specific mass/charge units at *m/e*= 18, 22 and 44 are reported indicating the water loss and the fragmentation of the glucopyranose unit, similarly to the case of the native β-CD ring.

The MID curves of the methylated derivatives show a single-step decomposition pattern. Besides the main characteristic fragments of CD, a signal at *m*/*e*=15 also appeared, due to the decay of the methyl groups (Fig. 5). All fragmentation processes took place between 245 and 400°C (see assignments in Table 5).

Table 4 Thermal characteristics of the observed CD derivatives

	Mass loss/%				*T_{dec}/°C	Peak temperature/°C			
Sample	Step1 35–120°C	Step2 120–200°C	Step3 200–270°C	Step4 270–400°C		DTA1	DTA2	DTA3	DTA4
HEBCD	2.5	2.2	0.6	84.2	270	–	–	–	361.3
ETBCD	1.0	2.1	0.2	91.7	273	–	–	321.4	376.0
DIETBCD	–	0.8	0.3	95.1	275	–	310.6	328.4	378.4
DIMEB	–	–	–	93.1	349	–	–	376.2	389.1
TRIMEB	–	–	–	94.5	301	160.6	–	–	373.9
PMBCD	3.3	–	77.3	–	253	–	–	324.2	339.6
RAMEB	–	–	–	88.4	291	–	–	–	368.7

*T_{dec} represents the initial temperature of the main decomposition step

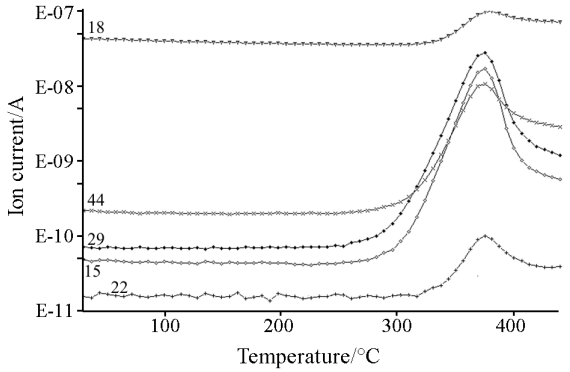

Fig. 5 MID curves of TRIMEB

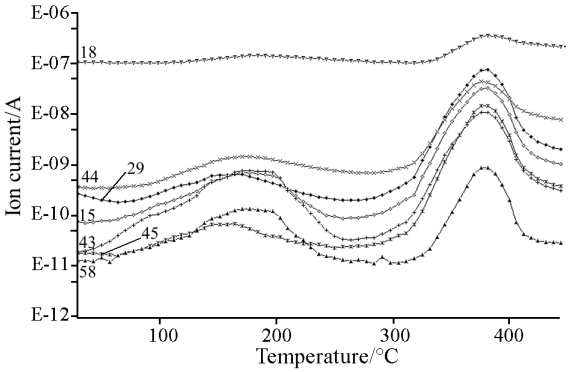

Fig. 6 MID curves of ETBCD

By the evaluation of the MID curves of the ethylated compounds (Fig. 6), it can be stated that the most characteristic signals appear at *m*/*e*=15, 29, 43, 45 and 58 representing the fragmentation of the alkyl substituent. *M*/*e*=15 belongs to the methyl, 29 to the ethyl and 45 to the O-ethyl fragments (from which signal 43 derives). Considering the ion current intensities, *m*/*e*=58 should be attributed to a recombination product originated by *m*/*e*=15 and 43 fragments.

The temperature intervals for the fragmentation of methyl and ethyl derivatives are summarized in Table 5.

Table 5 Temperature intervals for the fragmentation of modified cyclodextrins

Sample	Methyl group	Ethyl group
	Temp. range/°C	Temp. range/°C
HEBCD	–	80–280 and 280–400
ETBCD	–	70–260 and 260–400
DIETBCD	–	290–400
DIMEB	335–400	–
TRIMEB	265–400	–
PMBCD	245–400	–
RAMEB	260–400	–

The MID curves show that the two-step ethyl fragmentation starts below 100°C as indicated also by the TG curves.

Conclusions

The combined DTA/TG-MS experiments allow a general description of thermal behaviours of native and modified cyclodextrins.

Native cyclodextrins show minor differences in their water contents while their decomposition pattern is rather similar. The differences in the number of glucopyranose units do not influence remarkably the mass spectrometric behaviour.

Lower water content was observed for all substituted products. It is interesting to note that a thermal stability scale could be traced for all tested derivatives.

Methylated samples are more stable while the ethylated products were less stable than the native β-CD.

By the application of the TG-MS combined method the fragmentation of the native and different CD derivatives can be followed and distinguished from each other.

Acknowledgements

The financial support of OTKA Grant No. 26459 is gratefully acknowledged.

References

1 J. Szejtli, Cyclodextrins and their inclusion complexes, Akadémiai Kiadó, Budapest 1982.
2 J. Szejtli, Cyclodextrins in food, cosmetics and toiletries, 34 (1982) 379.
3 P. Mura, F. Maestrelli, M. Cirri, S. Furlanetto and S. Pinzauti, J. Therm. Anal. Cal., 73 (2003) 635.
4 F. Hirayama and K. Uekama, Adv. Drug Del. Rev., 36 (1999) 125.
5 B. McCormack and G. Gregoriadis, Int. J. Pharm., 112 (1994) 249.
6 M. D. Veiga, M. Merino, D. Fernandez and R. Lozano, J. Therm. Anal. Cal., 68 (2002) 511.
7 L. P. Fernandes, Zs. Éhen, T. F. Moura, Cs. Novák and J. Sztatisz, J. Therm. Anal. Cal., 78 (2004) 557.
8 G. Bettinetti, A. Gazzaniga, F. Giordano and M. E. Sangalli, Eur. J. Pharm. Biopharm., 40 (1994) 209.
9 J. Sztatisz, S. Gal, J. Komives, A. Stadler-Szoke and J. Szejtli, J. Thermal Anal., 2 (1979) 487.
10 G. Bettinetti, Cs. Novák and M. Sorrenti, J. Therm. Anal. Cal., 68 (2002) 517.
11 Y. Sayed, L. A. Wallace and H. W. Dirr, FEBS Letters, 465 (2000) 169.
12 S. Makedonopoulou, K. Yannakopoulou, D. Mentzafos, V. Lamzin, A. Popov and I. M. Mavridis, Acta Cryst., B57 (2001) 399.