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Sulfoalkyl Ether β-Cyclodextrin Derivatives: Synthesis and Characterizations

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Abstract

A series of sulfoalkyl ether β-cyclodextrin derivatives, including sulfoethyl, sulfopropyl and sulfobutyl ethyl β-cyclodextrins, have been synthesized and characterized. Each sulfoalkyl ether β-cyclodextrin is a mixture of various degrees of substitution and different positional isotherms. Elemental analysis, ^{1}H NMR, MS, and Differential Scanning Calorimetry analysis were used to determine the average degree of substitution for each β-cyclodextrin derivative. The average degrees of substitution are 3.4, 1.6 and 2.5 for sulfoethyl, sulfopropyl and sulfobutyl ether β-cyclodextrin, respectively. The water solubility of these derivatives is substantially higher than that of β-cyclodextrin. ^{1}H NMR indicates that sulfoethyl ether β-cyclodextrin may have major substitution on the secondary hydroxyl group while the major substitution in sulfopropyl and sulfobutyl ether β-cyclodextrin could be on the primary hydroxyl group. MS spectra show that no more than one substitution occurred on a single glucose unit.

Introduction

More than a century ago, Villier [1] isolated cyclodextrins (CDs) from the enzymatic reaction of starch with a particular kind of enzymes called cyclodextrin-glycosyl- transferases (CGTases). Today, more than 95% of cyclodextrin research is about β-CD [2]. Numerous chemical modifications of β-CD have been made to improve its water solubility and to modify its physicochemical properties and inclusion capability [3–5]. Among the three OH groups in a glucopyronase unit, the primary OH on C6 has the greatest reactivity, especially when bulky substitution regents are used, and the secondary OH on C3 has the least reactivity. This has been attributed to the hydrogen bonds formed between the protons of the hydroxyl group on C3 and the oxygen atoms of hydroxyl groups on C2 [6]. The same hydrogen bonds also cause the hydroxyl groups on C2 to be the most acidic of the three hydroxyl groups with a pK_a of 12.1. The OH groups on C3 can only react after all the primary and secondary hydroxyl groups are protected. The functional groups used in the modifications of cyclodextrins included alkyl-, hydroxyalkyl-, carboxyalkyl-, amino-, glucosyl-, etc. [7].

Since most syntheses of cyclodextrin derivatives give mixtures of products with different substitution degrees and positional isomers, the determination of the composition of the mixture has not been an easy task. Some derivatives, obtained from some sophisticated separation process and considered to be pure compounds, have been shown to be mixtures by later studies [7]. Common methods to determine the average substitution degree in cyclodextrin technology include FAB-MS spectroscopy [8], elemental analysis [9–11], ^{1}H NMR spectroscopy [10, 12], ^{13}C NMR spectroscopy [10, 11], and differential scanning calorimetry [13]. DSC analysis has been claimed to be a cheap, simple and rapid method for the determination of substitution degree [13].

One approach to prepare cyclodextrin derivatives with desired properties is to introduce sulfonated alkyl groups directly to the ring of β-CD instead of to methylated or hydroxyalkylated β-CD. The alkyl group in the sulfoalkyl substituent is directly attached to the CD structure and the anionic sulfonate group is separated from the cavity by the alkyl group. The sulfoalkyl ether β-CDs should have strong solubilizing capacity, very high water solubility and low toxicity due to the anionic nature of the substituents. Sulfoalkyl ether β-cyclodextrins may also have significant advantages over other β-cyclodextrin derivatives, such as hydrophobic and anionic cyclodextrin derivatives [14]. The first sulfoalkyl ether β-CD, sulfobutyl ether β-cyclodextrin, was reported in 1990 [15]. The derivatives synthesized in the present work are mixtures of randomly substituted heterogeneous sulfoalkyl ether β-CD with substitution degree ranging from 1 to 5 (Figure 1). In this work, sulfoethyl ether β-cyclodextrin, sulfopropyl ether β-cyclodextrin and sulfobutyl ether β-cyclodextrin have been prepared by reacting β-CD with 2-bromoethylene sulfonate, 1,3-propane sultone and 1,4-butane sultone, respectively, in 1,4-dioxane/water mixture solvent. ^{1}H NMR, MS, elemental analysis and DSC were used in the characterizations of these β-CD derivatives. Previous work has shown that at low alkali concentration the secondary OH groups on C2 have higher reactivity than the primary OH group on C6 while, at high alkali concentration the primary OH groups on C6 have higher reactivity than the secondary OH groups on C2 [11, 16–19]. Our results indicated a similar phenomena.

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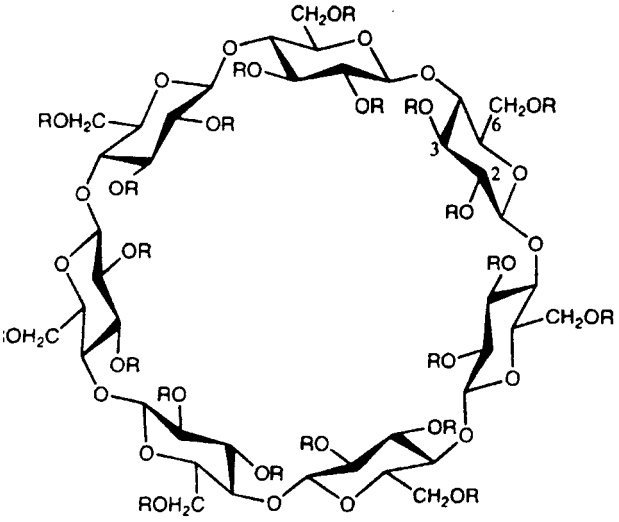


1	Sulfoethyl Ether β-CD:	R= **-$CH_2CH_2SO_3Na$**	or **H**
2	Sulfopropyl Ether β-CD:	R= **-$CH_2CH_2CH_2SO_3Na$**	or **H**
3	Sulfobutyl Ether β-CD:	R= **-$CH_2CH_2CH_2CH_2SO_3Na$**	or **H**

Figure 1. Structure of sulfoalkyl ether β-cyclodextrins.

Materials and methods

β-Cyclodextrin (98%) was kindly provided by American Maize-Products Company, Hammond, Indiana, as a gift. 2-Bromoethane sulfonic acid, sodium salt (98%), 1,3-propane sultone (98%), 1,4-butane sultone (98%) and 1,4-dioxane (>99%) were used as received from Aldrich Chemical Company. Cellulose Ester membrane with 500 MWCO was purchased from Spectrum Medical Industries, Inc., Houston, Texas. The membrane was rinsed by deionized water before use.

Preparation of sulfoalkyl ether β-cyclodextrins

The procedure is illustrated with the preparation of sulfoethyl ether β-cyclodextrin. To prepare this β-cyclodextrin derivative, 5 g β-cyclodextrin was added to 25 mL dioxane while stirring, and then 10 g 50% NaOH was added dropwise. After stirring about 15 minutes, 5 g $BrCH_2CH_2SO_3Na$ was slowly added to the solution and the mixture was allowed to reflux overnight. When the reaction was complete, two phases formed. The liquid phase was discarded and the amorphous phase was washed by CH_3OH and dried. The dry solid was dissolved in 100 mL deionized water and the solution was neutralized with 1 M H_2SO_4. The solution was then filtered with an ultrafiltration cell through a membrane with a 500 MW cut-off to wash out the small molecular inorganic salts and to concentrate the solution. The retentate solution (about 50 mL) was filtered by filter paper to remove the precipitate formed in the concentration process. The above process was then repeated several times until there was no precipitation when the permeate solution was tested by 0.01 M $BaCl_2$ solution. Then the retentate solution was freeze-dried to give 4.75 g of sulfoethyl ether β-cyclodextrin. The other sulfoalkyl ether β-CDs were prepared with same procedure. 1,3-propane sultone and 1,4-butane sultone were used as sulfonation reagents, respectively.

Mass spectrometry

Spectra were obtained by using low-resolution fast atom bombardment mass spectrometry (FAB-MS) with a VG Instruments ZAB-E spectrophotometer. The FAB ion source was operated in the negative ion mode. Triethanolamine was used as liquid matrix for Neg. FAB.

NMR spectroscopy

The ^{1}H NMR spectra of β-cyclodextrin and its sulfonate derivatives were recorded on a Varian XL-300 spectrometer. D_2O was used as solvent and the solvent peak was used as reference. All the NMR measurements were performed at room temperature.

Differential scanning calorimetry (DSC) analysis

DSC was carried out using the Metler-Toledo 820 workstation. The size of samples used in the DSC analysis varied between 5 and 15 mg. A nitrogen atmosphere was applied to the DSC cell and samples were always heated from 50 °C to 400 °C with the heating rate of 10 °C min^{-1}.

Elemental analysis

Samples of CD derivatives were freeze-dried for over 24 hours in vacuum before analysis. The analysis was performed by Atlantic Microlab, Inc., Norcross, Georgia.

Results and discussion

The products, denoted **1**, **2**, and **3** (Figure 1), respectively, were white, water soluble powders. All three compounds are readily soluble in water, more than 33% (w/w). Phenolphthalein forms a 1:1 complex with β-cyclodextrin and the adsorption at $\lambda = 550$ nm is practically equal to zero [20, 21]. The same phenomena were observed for each of the synthesized sulfoalkyl ether β-cyclodextrins.

The elemental compositions are summarized in Table 1. The calculated data for each substitution in Table 1 have not included the water molecules that might exist in the sulfoalkyl ether β-CD crystal structure. As shown in Table 1, product **2** is a derivative having a relatively low average degrees of substitution and products **1** and **3** have relatively high average degrees of substitution. Since there are still many possible positional isomers, the products **1**, **2**, and **3** are mixtures of the CD derivatives with various degrees of substitution and positional isomers, as shown with the results of the NMR, MS spectra and DSC.

The average substitution degree was estimated from the elemental analysis. A nonlinear least regression program

was used to calculate the average degree of substitution. Because there might be some water molecules included inside the CD cavity or integrated in the crystal structure, the calculation for the average substitution degree from the elementary analysis has included a variable to estimate the number of water molecules in each sulfoalkyl ether β-CD. The results are listed in Table 3 for both average degree of substitution and number of water molecules per sulfoalkyl ether β-CD molecule. The calculated elemental compositions for each sulfoalkyl ether β-CD fit well with the elemental analysis results. However, it should be mentioned that the calculated water content in each sulfoalkyl ether CD should be confirmed by other experimental methods, such as thermal gravimetry and Karl–Fisher methods.

Mass spectrometry

By using negative ion fast-atom bombardment, well defined and clearly separated signals were obtained in the mass spectra of sulfoalkyl ether β-CDs. Each peak corresponds to monoanions formed by loss of one sodium ion from the sulfoalkyl ether β-cyclodextrin salt. Table 2 summarizes the mass spectral data for products **1**, **2**, and **3** including *m/z*, signal intensity, the corresponding structures and the theoretical mass for each structure. Product **1** has an average degree of substitution of 4.11 per cyclodextrin from the elemental analysis and the mass spectrum shows that the mixture is composed of the isomers with substitution degree ranging from 1 to 4 per cyclodextrin (Figure 2). The average degree of substitution estimated from the distribution of the mass spectrum peaks is 2.48, lower than that estimated from elemental analysis. Product **2** has an average degree of substitution of 1.79 from the elemental analysis and its mass spectra shows that it consists of isomers with substitution degree of 1 and 2, (Figure 3) and there are no isomers with substitution degree more than 2 in this product. For product **3**, the mass spectrum has shown that the substitution of sulfobutyl ranges from 1 to 4 (Figure 4), which is also consistent with the average degree of substitution of 2.57 obtained from the elemental analysis. The lower mass ions in the mass spectra of products **1**, **2**, and **3** also provide important confirmation of the substitution reaction. For example, a major fragment ion found in the mass spectrum of product **2** is at *m/z* = 283.1, which corresponds to the glucopyranose with a sulfopropyl group. Similar cases were also found in the mass spectra of products **1** and **3**.

Because there is no major peak in the mass spectra which corresponds to the structure of one glucopyranose with more than one sulfoalkyl substituent, it is very possible that no more than one substitution occurred on the same glucopyranose unit. This is not surprising because the average substitution degree is relatively low for each derivative and steric factors favor a low substitution degree on each glucopyranose unit. The mass spectra have been recorded to more than 2000 amu, and derivatives with substitution degrees greater than 5 have not been observed.

^1H NMR spectroscopy

In the NMR spectrum of sulfoethyl ether β-CD, there is a new peak at δ = 3.00 ppm (Figure 6) in addition to all the peaks present on β-CD's spectrum (Figure 5). This newly formed peak results from the 2 Hs of the —CH_2— group which bonds to the SO_3^- group. The peak of the 2 Hs of the other —CH_2— group of the sulfoethyl group is overlapped with the peaks of β-CD at δ = 3.7–3.8 ppm. This can be verified from the integration of the peaks at δ = 3.7– 3.8 ppm. The integration ratio between the peaks at δ = 3.7–3.8 ppm and the peak at δ = 4.85 ppm is 4 in β-CD's NMR, but is between 5 and 6 in sulfoethyl ether β-CD's NMR spectrum. Similar results are also observed in the NMR spectra of sulfopropyl and sulfobutyl ether β-CDs. In the case of sulfopropyl ether β-CD NMR spectrum (Figure 7), in addition to the peak at δ = 2.8 ppm, there is another new peak at δ = 1.8 ppm. This additional peak results from the 2 Hs of the central —CH_2— of the sulfopropyl group. The integration ratio between the peak at δ = 2.8 ppm and the peak at δ = 1.8 ppm is close to 1, which indicates that they belong to the same sulfopropyl group. The ^{1}H NMR spectrum of sulfobutyl β-CD (Figure 8) is similar to that of sulfopropyl ether β-CD except that the integration ratio between the peak at δ = 1.6 ppm and the peak at δ = 2.75 ppm is about 2. This suggests that the peak at δ = 1.6 ppm includes 4 Hs, which belong to the 2 central —CH_2— groups of the sulfobutyl group. From the ^{1}H NMR spectra, the average degree of substitution also can be qualitatively estimated by comparing the integration between the peak at δ = 4.85 ppm and the peak at δ = 2.8 ppm. The estimated degrees of substitution for the products **1**, **2**, and **3** are listed in Table 3. The data in Table 3 show that the estimated average substitution degrees for products **2** and **3** from the ^{1}H NMR spectra are fairly close to the substitution degree calculated from the elemental analysis data.

The ^{1}H NMR spectra may also provide information about the substitution position on the cyclodextrin ring [8]. In the NMR spectrum of cyclodextrin derivative **1**, the proton on C1 has been separated into two doublets. 2 of the 7 protons appear downfield at 4.93 ppm and the rest of the C1 protons are located at 4.85 ppm. It has been suggested that this split is caused by the modification of the secondary hydroxyl group on C2 [8]. This behavior may suggest that some of the substitutions of the sulfoethyl group occurred at the secondary hydroxyl group on C2. Considering that the average substitution degree is about 3.4 for this derivative, the majority of the substitutions of sulfoethyl group could occur on the secondary hydroxyl group on C2. However, in the NMR spectra of product **2** and **3**, almost all the 7 protons appear at 4.85 ppm as a single doublet, and no separation occurs. This could indicate that most of the substitution of sulfoalkyl groups occurs at the primary hydroxyl group on C6 and no major substitutions occur on the secondary hydroxyl group on C2. ^{13}C NMR should be used to confirm the observations from ^{1}H NMR and proposed substitution positions.

Table 1. Elemental analysis of sulfoalkyl ether β-cyclodextrins

Preparation Number	Name		Elemental C	Composition H	S
1	Sulfoethyl ether β-CD	Found	33.85%	4.74%	7.46%
		Cal.			
		for 4 sub.	36.25%	4.95%	7.76%
		for 5 sub.	34.95%	4.76%	8.99%
2	Sulfopropyl ether β-CD	Found	38.37%	5.95%	3.88%
		Cal.			
		for 1 sub.	42.22%	5.86%	2.51%
		for 2 sub.	40.5%	5.62%	4.51%
3	Sulfobutyl ether β-CD	Found	37.55%	5.79%	4.97%
		Cal.			
		for 2 sub.	41.35%	4.42%	5.79%
		for 3 sub.	40.27%	5.98%	5.66%

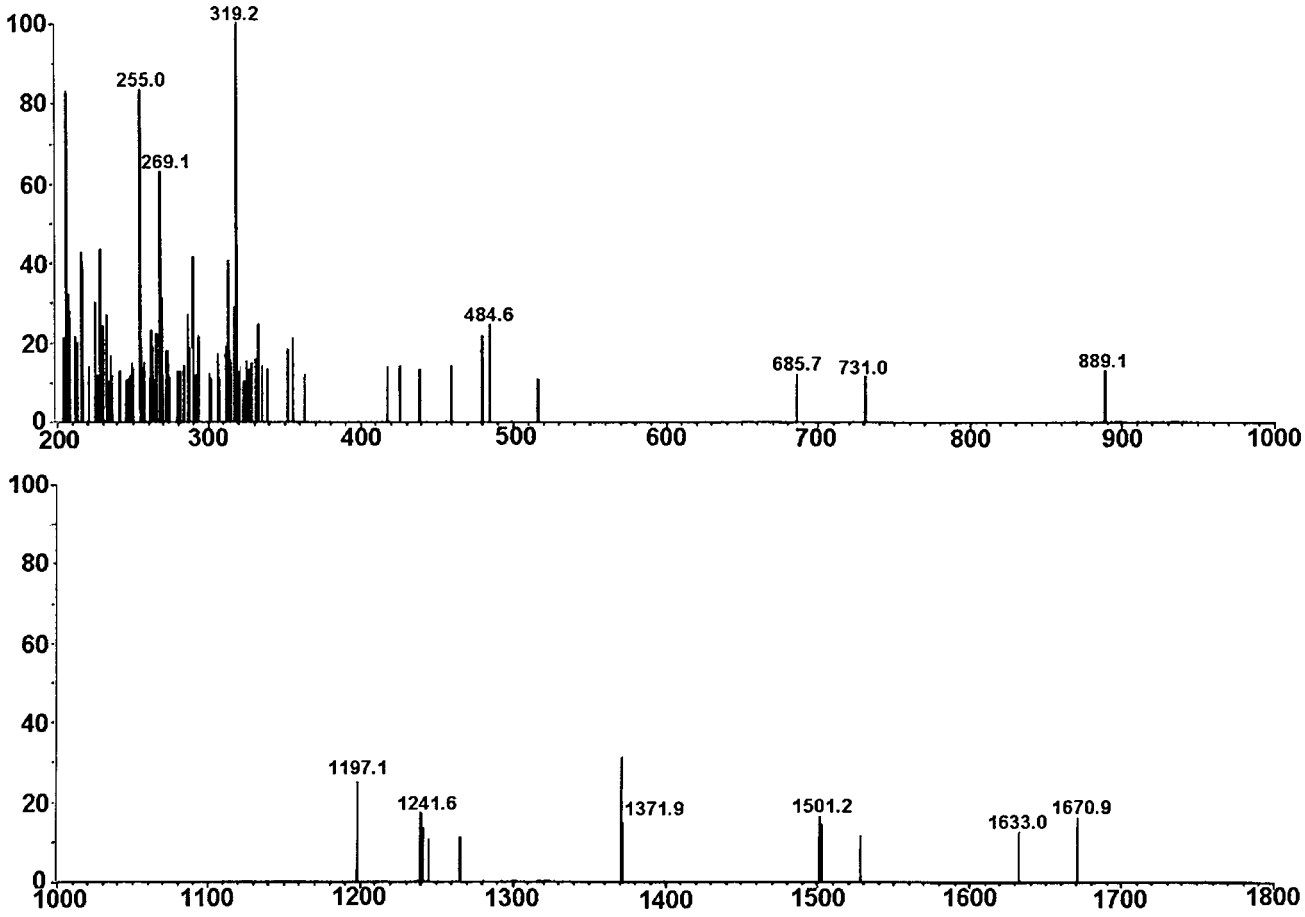


Figure 2. Mass spectrum of sulfoethyl ether β-CD.

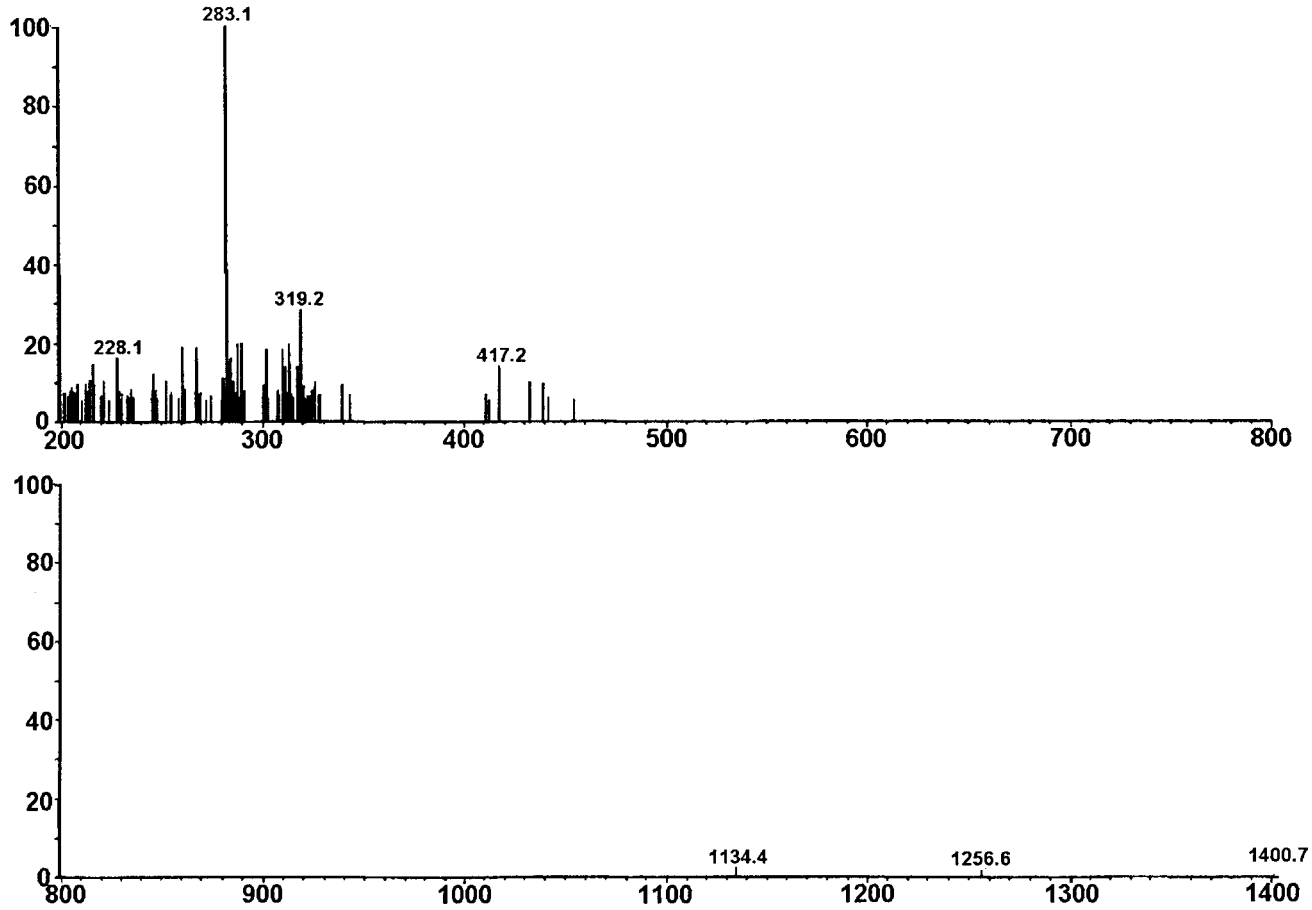
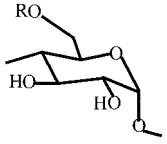


Figure 3. Mass spectrum of sulfopropyl ether β-CD.

Table 2. Summarized data from mass spectra of sulfoalkyl ether β-CDs

Products	*m/z*	Signal	M.W.	Structure
1	1241.6	13.2%	1242.2	$CD(CH_2CH_2SO_3)^-$
1	1371.9	14.4%	1372.4	$CD(CH_2CH_2SO_3)_2Na^-$
1	1501.2	16.2%	1501.6	$CD(CH_2CH_2SO_3)_3Na_2^-$
1	1633.0	12.1%	1631.8	$CD(CH_2CH_2SO_3)_4Na_3^-$
1	269.1	62.8%	269.3	Structure 1*
2	1134.4	2.0%	1134.1	CD^-
2	1256.6	1.2%	1256.3	$CD(CH_2CH_2CH_2SO_3)^-$
2	1400.7	0.3%	1400.4	$CD(CH_2CH_2CH_2SO_3)_2Na^-$
2	283.1	100%	283.3	Structure 2*
3	1133.6	2.0%	1134.1	CD^-
3	1270.0	3.2%	1270.3	$CD(CH_2CH_2CH_2CH_2SO_3)^-$
3	1428.4	1.0%	1428.5	$CD(CH_2CH_2CH_2CH_2SO_3)_2Na^-$
3	1586.5	2.1%	1586.6	$CD(CH_2CH_2CH_2CH_2SO_3)_3Na_2^-$
3	1745.3	1.1%	1744.8	$CD(CH_2CH_2CH_2CH_2SO_3)_4Na_3^-$
3	297.2	99.3%	297.3	Structure 3*



* Structure 1 R= $CH_2CH_2SO_3^-$
* Structure 2 R= $CH_2CH_2CH_2SO_3^-$
* Structure 3 R= $CH_2CH_2CH_2CH_2SO_3^-$

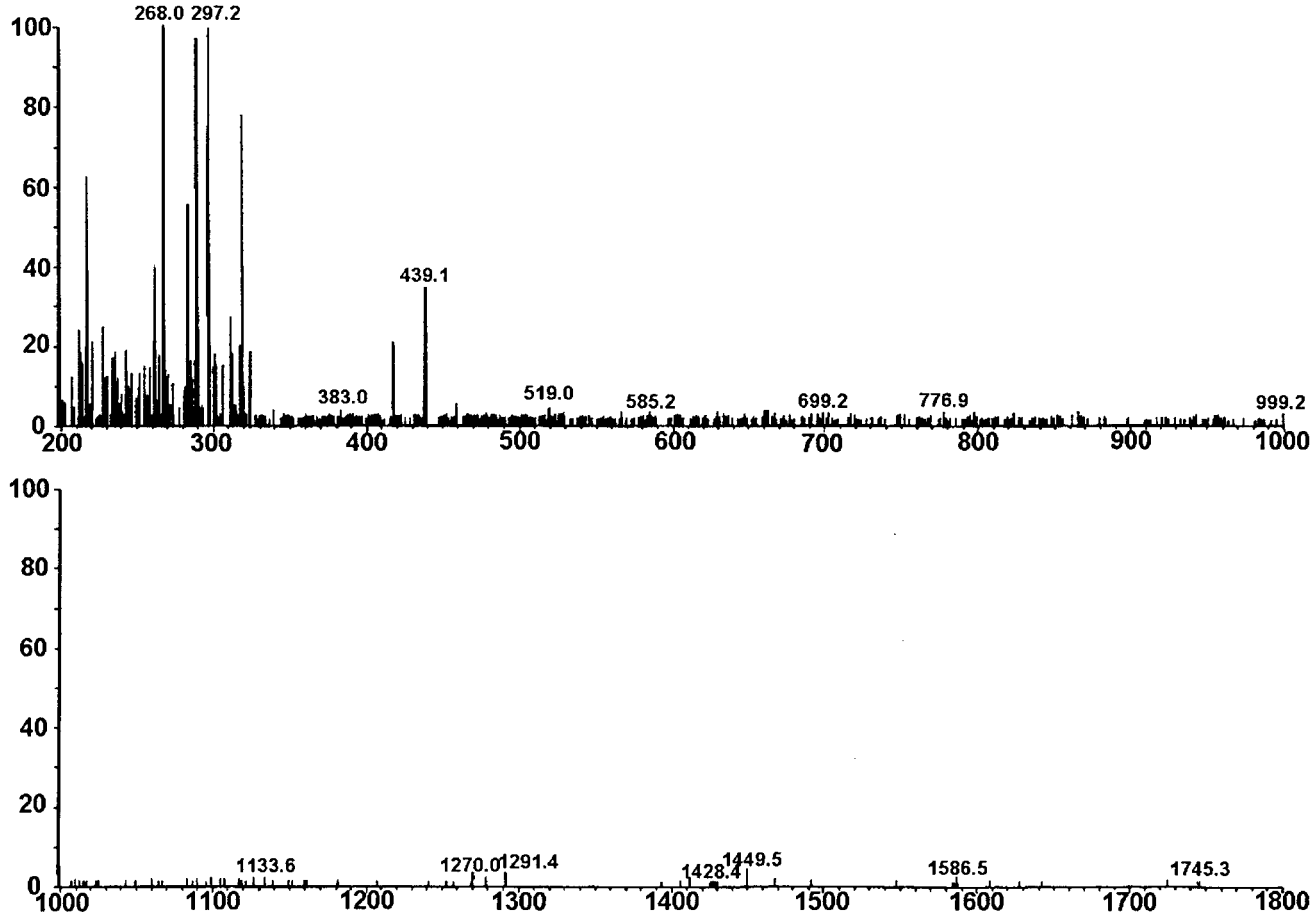


Figure 4. Mass spectrum of sulfobutyl ether β-CD.

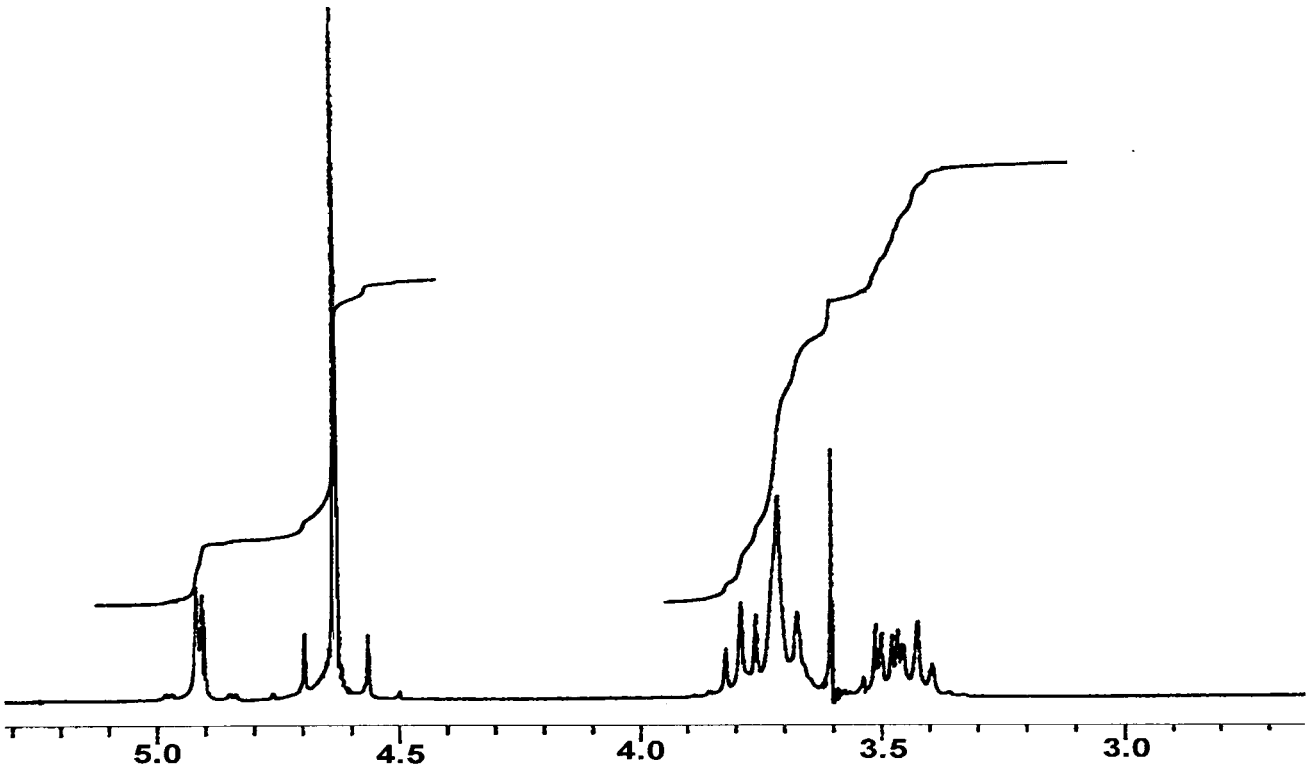


Figure 5. ^{1}H NMR of β-CD.

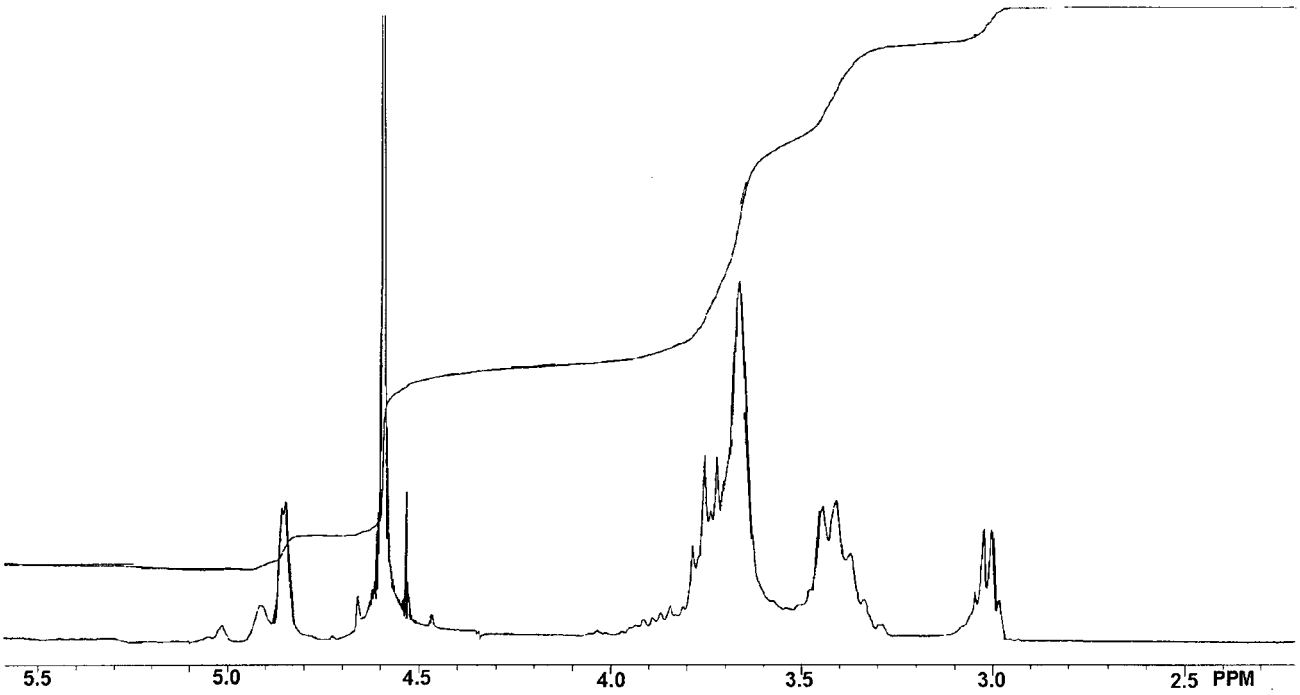


Figure 6. ^{1}H NMR of sulfoethyl ether β-CD.

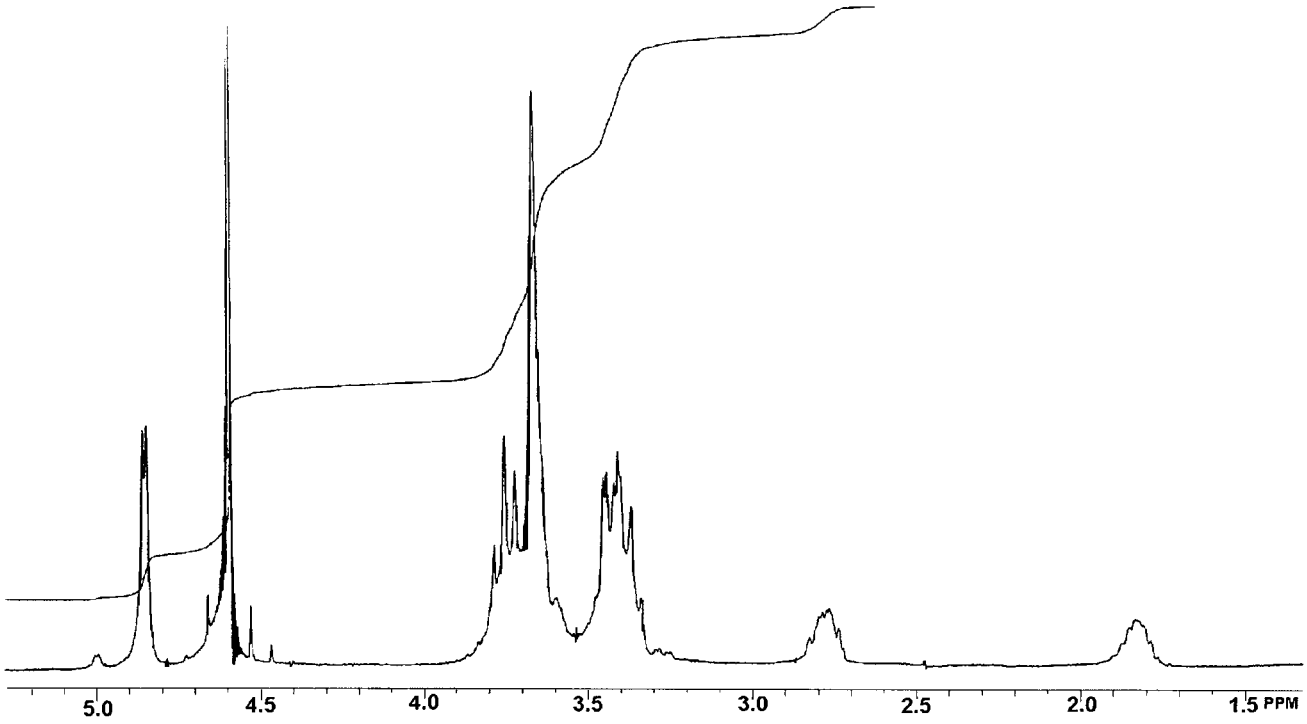


Figure 7. ^{1}H NMR of sulfopropyl ether β-CD.

DSC analysis

The sharp endothermic peak at 100 °C corresponds to the loss of water from β-cyclodextrin crystals (Figure 9). The change of the endothermic peaks corresponding to water evaporation indicates that the water content in the cyclodextrin derivatives **1**, **2**, and **3** is significantly different from that in β-cyclodextrin. The endothermic peak at 300 °C corresponds to the melting and decomposition of β-cyclodextrin. It was observed that a sharp endothermic peak occurs around 260 °C in each derivative's DSC analysis, but no peaks appear around 300 °C. Another DSC analysis for sulfobutyl ether β-CD is reproduced in Figure 10. In this test, the first run was programmed from 50 °C to 280 °C with a heating rate of 10 °C/min. and then the sample was cooled to 50 °C and the second run was performed from 50 °C

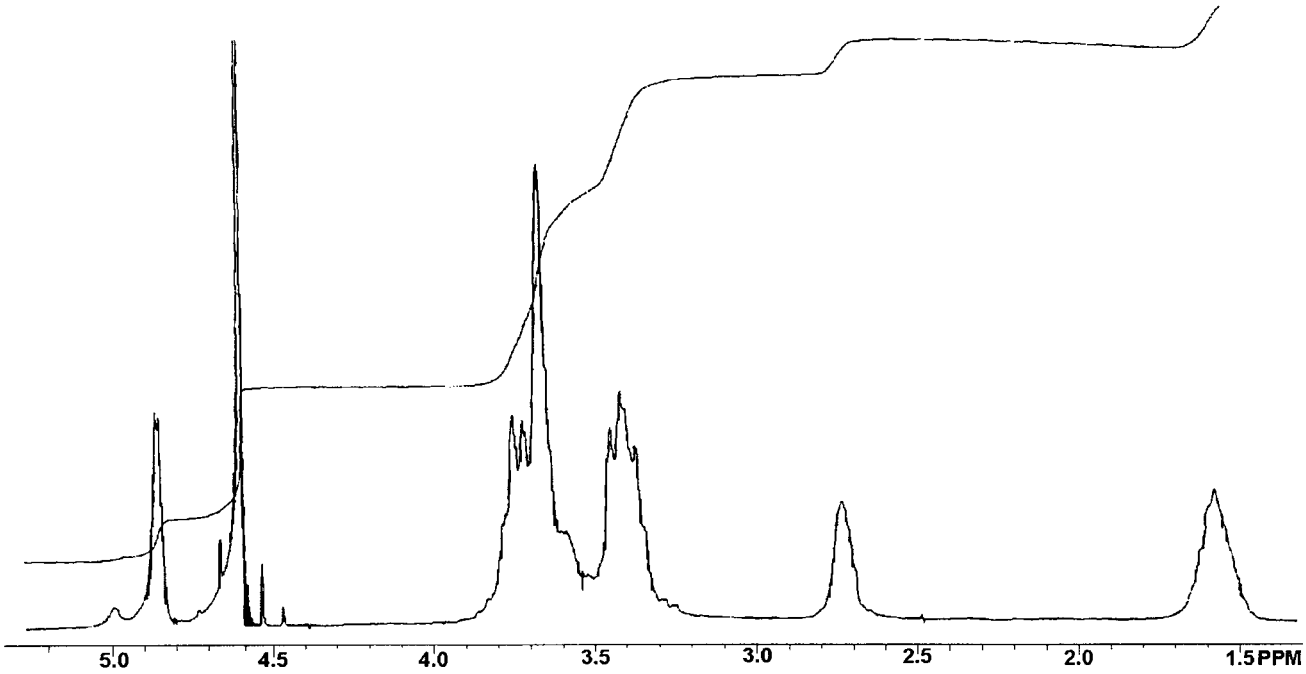


Figure 8. ^{1}H NMR of sulfobutyl ether β-CD.

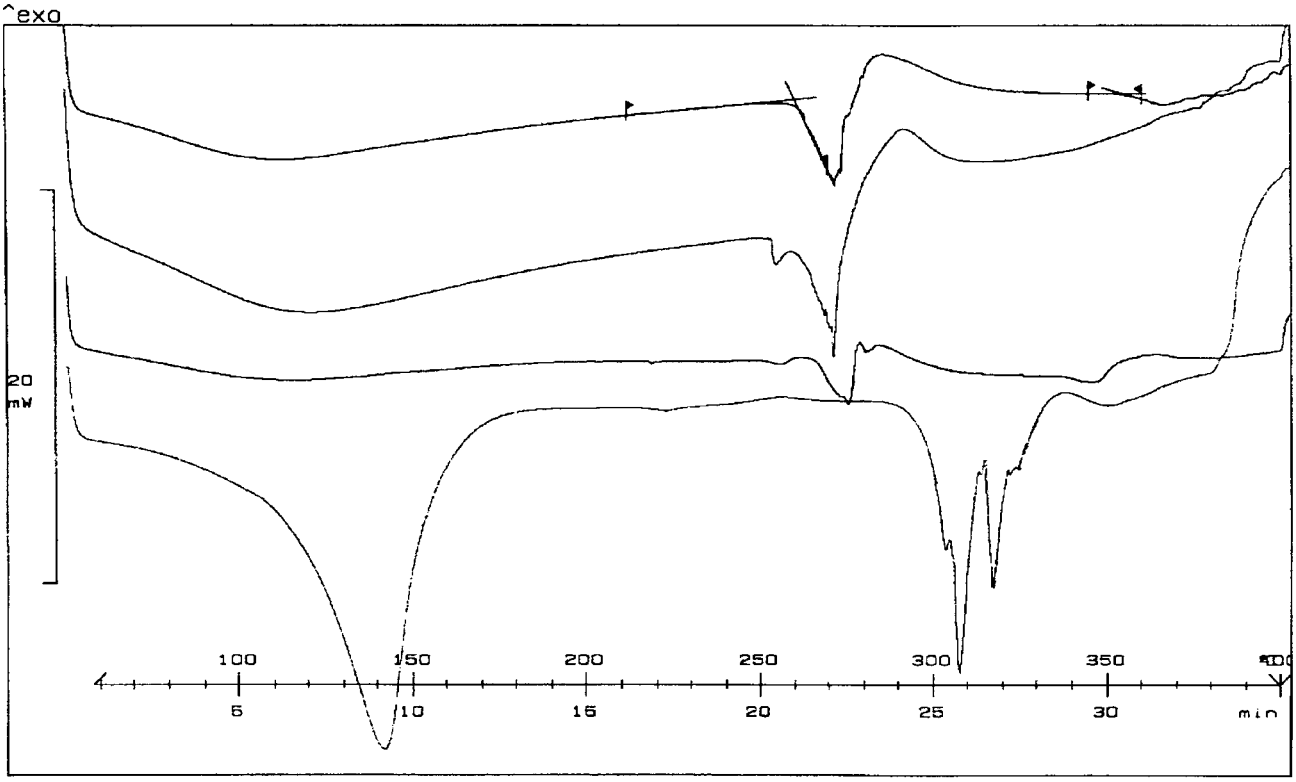


Figure 9. DSC analysis of sulfoalkyl ether β-cyclodextrins and β-cyclodextrins.

Table 3. Average degree of substitution (DS) from elemental analysis, MS, and NMR

	Name	Average DS from elemental analysis	Average number of waters per CD	Average DS from MS	Average DS from NMR
1	Sulfoethyl ether β-CD	4.11	1.19	2.48	3.5
2	Sulfopropyl ether β-CD	1.79	4.62	1.2	1.8
3	Sulfobutyl ether β-CD	2.57	4.53	2.2	2.6

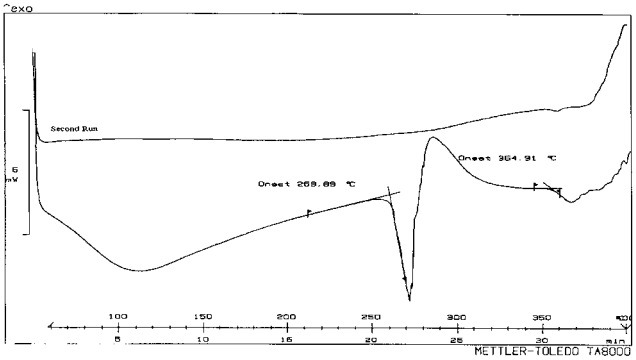


Figure 10. Repeated DSC analysis of sulfobutyl β-CD.

to 400 °C, again with heating rate of 10 °C/min. There is no peak during the second run, which indicates that the endothermic peaks around 260 °C should correspond to the decomposition of the cyclodextrin derivatives. Apparently, the derivatives have lower melting and decomposition temperatures (∼260 °C) than β-cyclodextrin (∼300 °C).

Conclusions

A family of sulfoalkyl ether β-cyclodextrins was synthesized and characterized and dramatical increase of water solubility was observed. The accurate characterization of sulfoalkyl ether β-cyclodextrins is very challenging due to the number of the possible positional isomers and the difficulty of controlling reaction selectivity. The average degree of substitution of the derivatives has been estimated from elemental analysis, negative FAB mass spectroscopy, and ^{1}H NMR spectroscopy. The results from different methods are in agreement with each other (Table 3).

Mass spectrometry has been used to determine the individual substitution degree and the structure of the derivatives; however, the reliability of the average substitution degree is questionable. We have found the average substitution degree estimated from this method is lower than that obtained from other methods.

^{1}H NMR spectroscopy was used to characterize the structure of sulfoalkyl ether cyclodextrins in this work. The separation of ^{1}H NMR peaks of the C7 protons may provide additional information about whether the substitution occurred on a primary hydroxyl group or on a secondary hydroxyl group. However, ^{13}C NMR should be employed to confirm the observations from ^{1}H NMR.

DSC analysis clearly shows that the sulfoalkyl ether β-cyclodextrins have lower melting and decomposition temperatures than β-cyclodextrin. DSC analysis also provides useful information about the water content in the samples. From elemental analysis, the sulfoalkyl ether β-cyclodextrins have much less water (less than 5 molecules of water per CD) than β-cyclodextrin itself (about 10 molecules of water per CD). Other methods, such as thermal gravimetry, could be used to experimentally determine the water content. We have concluded that DSC analysis cannot estimate individual and average substitution degrees of these β-cyclodextrin derivatives.

In this work the sodium hydroxide concentration was high and main substitutions are on the primary OH groups on C6 as indicated by ^{1}H NMR spectra. Only sulfoethyl ether β-CD has major substitutions on the secondary OH on C2. This may be attributed to the size of the substituent. Because bulky substituents prefer to react with the primary hydroxyl groups on C6, sulfopropyl and sulfobutyl favor reaction with the primary OH on C6, while sulfoethyl is more reactive with the secondary OH on C2.

References

1. A.C.R. Villiers: *Hebd. Seances Acad. Sci.* **112**, 536 (1891).
2. J. Szejtli: *Medicine Res. Rev.* **14**, 353 (1994).
3. J. Szejtli and K.-H. Fromming: *Cyclodextrins in Pharmacy*, Kluwer Academic Publishers, the Netherlands (1993).
4. J. Szejtli: *Cyclodextrin Technology*, Kluwer Academic Publishers, Dordrecht (1988).
5. K. Uekama, F. Hirayama and T. Irie: *Drug Targeting Delivery* **3**, 411 (1994).
6. F.M. Menger and M.A. Dulany: *Tetrahedron Lett.* **26**, 267 (1985).
7. G. Wenze: *Angew. Chem. Int. Ed. Engl.* **33**, 803 (1994).
8. C.T. Rao, B. Linderg, J. Linderg and J. Pitha: *J. Org. Chem.* **56**, 1327 1991.
9. J. Pitha, L.M. Mallis, D.J. Lamb, T. Irie and K. Uekama, *Pharm. Res.* **8**, 1151 (1991).
10. Y.G. Gao, L.H. Tyong, Y. Inoue and A. Tai: *Synth. Commun.* **25**, 703 (1995).
11. J. Jindrich, J. Pitha, B. Lindberg, P. Seffers and K. Harata: *Carbohydr. Res.* **266**, 75 (1995).
12. R.J. Tait, D.J. Skanchy, D.P. Thompson, N.C. Chetwyn, D.A. Dunshee, R.A. Rajewski, V.J. Stella and J.F. Stobaugh: *J. Pharm. Biomed. Anal.* **1**, 615 (1992).
13. Cs. Novak, G. Pokol, J. Sztatisz, L. Szente and J. Szejtli: *Anal. Chim. Acta* **282**, 313 (1993).
14. K. Shiotni, K. Uehata, T. Irie, K. Uekama, O.T. Diane and V.J. Stella: *Pharm. Res.* **12**, 78 (1995).
15. *Chem. Abstr.* 115: P189787u (1991).
16. T. Rao, J. Pitha, B. Lindberg and J. Lindberg: *Carbohydr. Res.* **223**, 99 (1992).
17. B. Lindberg, J. Lindberg, J. Pitha, T. Rao and K. Harata: *Carbohydr. Res.* **222**, 113 (1991).
18. D. Rong and V. D'souza *Tetrahedron Lett.* **31**, 4275 (1990).
19. R.C. Van Etten, J.F. Sebastian, G.A. Clowes and M.L. Bender: *J. Am. Chem. Soc.* **69**, 3242 (1967).
20. J.K. Sasaki, S.D. Christian and E.E. Tucker: *J. Colloid Interface Sci.* **134**, 412 (1990).
21. J.K. Sasaki, S.D. Christian and E.E. Tucker: *Fluid Phase Equilibria* **49**, 281 (1989).