Continuous Tank Reactor Synthesis of Highly Substituted Sulphobutylether β-Cyclodextrins

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ABSTRACT

Batch synthesis of Sulphobutyl ether β-cyclodextrin (also known as SBE-β-CD or SBECD) is a process effectively divided into three main stages, i.e. initial reagent dissolution, a sulphoalkylation reaction and final reaction quenching. This reaction is followed by downstream processing and purification, and ultimate isolation of the solid SBECD material. However, a feature associated with using this synthetic method is that a high proportion of lower substituted SBECD is observed. There is therefore a need to provide an improved synthetic method for producing higher substituted cyclodextrins.

The authors here present a Continuous Tank Reactor (CTR) method for preparing sulphobutyl ether-cyclodextrins. The method comprises first contacting cyclodextrin with a base to form activated cyclodextrin. The method then involves separately contacting the activated cyclodextrin with an 1,4-butane sultone to form sulphoalkyl ether-cyclodextrin.

The activation reaction is carried out in batch synthesis mode and the sulphoalkylation reaction is carried out under continuous flow conditions resulting in a novel method for the synthesis of highly derivatised cyclodextrins.

The work is particularly concerned with producing controlled substitution in sulphobutyl ether β-cyclodextrins and novel compositions of highly substituted sulphoalkyl ether β-cyclodextrins are described.

Abbreviations

ADS	Average Degree of Substitution
β-CD	β-Cyclodextrin
BS	1,4-butane sultone
CD	Cyclodextrin
CD-Screen-DAP	HPLC Stationary Phase for Analysis of Cyclodextrin-Derivatives
CTR	Continuous Tank Reactor
ELSD	Evaporative Light Scattering Detection
HPLC	High performance liquid chromatography
IDS	Individual Degree of Substitution
MPA	Mobile phase A
MPB	Mobile phase B
PTFE	Polytetrafluoroethylene
SBE-β-CD	Sulphobutyl ether β-cyclodextrin
SBECD	Sulphobutyl ether β-cyclodextrin
USP35/NF30	United States Pharmacopeia 35 and National Formulary 30
US FDA	US Food and Drug Administration

Introduction

Sulphobutyl ether β-cyclodextrin (SBECD) is one of a class of polyanionic, hydrophilic water soluble cyclodextrin derivatives. The parent β-cyclodextrin can form an inclusion complex with certain active pharmaceutical ingredients (API) with two benefits, the apparent aqueous solubility of the API increases and, if labile functional groups are included, chemical stability is improved. However, the parent β-cyclodextrin suffers from two problems, including lower aqueous solubility and nephrotoxicity when given via injection, e.g. the intravenous route. Derivatisation of β-cyclodextrin (and its variants α and γ-cyclodextrin) has been shown to be beneficial with respect to both of these two defects. The first derivatised cyclodextrin was the hydroxypropyl derivative, which was later followed by sulphobutyl ether (see Figure 1). These two derivatised cyclodextrins are the most commercially significant.

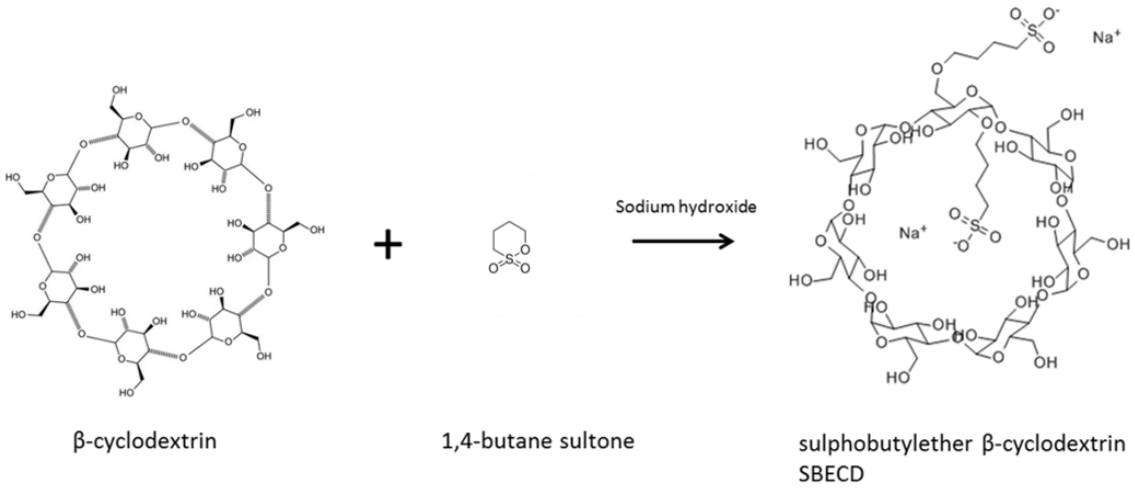

Figure 1: A general scheme for the synthesis of SBECD from the reagents β-cyclodextrin (β-CD) and 1, 4-butane sultone (BS).

SBECD is currently used as an effective pharmaceutical excipient, and has been given the registered trade name Captisol. To date, there are five US FDA-approved, Sulphobutyl ether β-cyclodextrin enabled drug products on the market: Nexterone (Baxter International); Geodon and Cerenia (Pfizer); Kyprolis (Onyx); Abilify (Bristol Myers Squibb).

Shah *et al* (1) has previously described a batch synthesis of SBECD, the process being effectively divided into three main stages, i.e. initial reagent dissolution, a sulphoalkylation reaction and final reaction quenching. The reaction is then followed by downstream processing and purification, and ultimate isolation of the solid SBECD material. However, a feature associated with using this synthetic method is that a high proportion of lower substituted SBECD is observed. Antle (2) has also described a continuous manufacturing process. However, there are significant conceptual differences between our approach and that of Antle in that our approach requires lower temperatures and operates at ambient pressure, and also allows for controlled substitution in sulphobutyl ether β-cyclodextrins and the production of novel compositions of highly substituted sulphoalkyl ether β-cyclodextrins.

It has been reported that the method of preparation of a cyclodextrin derivative can have an impact upon the final structure (3). Previous studies have demonstrated that, of the three types of hydroxyl groups present in CDs, those at the six position (C6, primary hydroxyl) are the most nucleophilic, those at the two position (C2) are the most acidic, and those at the three position (C3) are the most inaccessible(4,5). It has also been reported that at high alkali concentration the primary hydroxyls have higher reactivity than the secondary hydroxyls on C2 (6). Additionally, bulky substituents prefer to react with the primary hydroxyl on C6 (6).

Methods

The Continuous Tank Reactor (CTR) based Manufacturing Process.

The continuous flow experiments consisted of two Masterflex pumps connected to a glass double 10 ml jacketed Continuous Tank Reactor (CTR). The two pumps were connected to the CTR holding chamber via a three-way connector and PTFE tubing. Non-return valves were fitted in line in the vicinity of the three-way connector to prevent the reagent stream reverse flow as a result of the differential flow pressure in either of the feed lines. The PTFE tubing was put in a water bath to maintain temperature at approximately 60 ℃. In a typical experiment, a round bottom flask containing a stock solution of β-cyclodextrin in NaOH solution was first prepared as follows: 15 g of β-CD (1.32×10^{-2} mole) was added with stirring to an aqueous solution composed of 6 g of NaOH in 30 ml water. This solution was maintained at

60 ℃ with a hotplate stirrer . The first pump (Figure 2) was then used to deliver stock β-CD solution into the CTR where the substitution reaction takes place via the three way connector, while the second pump was used to deliver neat 1,4-butane sultone also held at 60 ℃ through the three way connector. An internal vortex circulation was generated with the continuous flowing reaction stream and the reaction proceeded in a continuous manner, i.e. once the pumps started they were not switched off until completion of the reaction. The crude product was harvested in a 20 ml sample bottle.

Analytical Methodology for the Analysis of High Substituted SBECD Species

High performance liquid chromatography with evaporative light scattering detection (ELSD) was used for the separation of sulphobutylether β-cyclodextrin into its substituted constituents in order to determine the average degree of substitution. Identification of each substituted cyclodextrin was determined by comparing the retention times with materials produced by the method of Shah (1).

The chromatographic conditions are summarised as follows:

Instrument:	Agilent 1100 series
Software:	OpenLAB
Column:	CD-Screen-DAP, 3 µm, 150 × 4.0 mm, (CD-Screen -DAP-1504-03)
Column temperature:	25°C. ± 1°C.
Mobile phase A (MPA):	0.5% triethylamine-acetic acid buffer, pH = 5
Mobile phase B (MPB):	acetonitrile, HPLC grade
Flow rate:	1.0 ml/min

Gradient Ratio	Time (min)	0	6	15
	MPA (%)	100	50	50
	MPB (%)	0	50	50

Detection:	ELSD
Injection volume:	5 µl
Concentration:	10 mg/ml
Acquisition time:	15 minutes with post-time of 5 minutes
Needle wash:	none

ELSD Conditions

Instrument:	Alltech ELSD 2000
Tube temperature:	*115°C.*

Gas flow (nitrogen):	3.2 L/min
Gain:	2
Impactor:	Off

Results and Discussion

The authors carefully studied the batch sulphoalkyl ether β-cyclodextrin production method that was described by Shah *et al* (1), and have then devised a Continuous Tank Reactor Synthesis (CTR) method for producing SBECD and experimented with the stoichiometry of the reaction. A significant modification to existing methods comprised contacting cyclodextrin with a base to form activated cyclodextrin and separately reacting the activated cyclodextrin with an 1,4-butane sultone to form sulphoalkyl ether β-cyclodextrin. In our method the sulphoalkylation reaction is carried out under continuous flow conditions. The resultant substituted sulphoalkyl ether β-cyclodextrin is novel as it exhibits a higher degree of substitution, for a lower input of 1,4-butane sultone and base than that which is produced using the known batch process. The higher Average Degree of Substitution arises from the presence of highly substituted species with an Individual Degree of Substitution in excess of 10.

By comparison, the batch method of preparing substituted sulphoalkyl ether β-cyclodextrin produced a higher concentration of lower degrees of sulphoalkyl ether β-cyclodextrin substitution than that produced using a Continuous Tank Reactor method**.** Furthermore, it can be seen that material produced by the process described in US 6,153,746 (1) has a range of substitution from 2 to 10, while material produced in accordance with CTR processing has a range of substitution from 3 to 13. In addition the method does not produce any detectable di-substituted sulphobutylether β-cyclodextrin and produces significant quantities of degree of substitution of 11-13 not detected in the US 6,153,746 (1) material.

As described in the Method Section, the set-up for the continuous flow experiments consisted of two pumps connected to a double Continuous Tank Reactor (CTR) acting as a holding chamber/sight glass. The two pumps were connected to the CTR holding chamber via a three-way connector. In a separate round bottom flask, a stock solution of β-cyclodextrin in NaOH solution was first prepared and this solution was maintained at 60 ℃ with a hotplate stirrer. The sodium hydroxide was present

in an amount which was stoichiometrically controlled, relative to the amount of cyclodextrin, to achieve a desired degree of substitution.

As β-cyclodextrin was added to the sodium hydroxide solution, a three stage 'activation' process occurred. Firstly, it takes a finite time to add the β-cyclodextrin into the reservoir vessel containing aqueous sodium hydroxide. Next, the β-cyclodextrin dissolves in the sodium hydroxide solution. Finally and more significantly, an initial solution straw colouration progressively 'deepens' (the activation process has typically taken 30 minutes) which is considered to be a visual sign of reaction of the β-cyclodextrin by sodium hydroxide. With the deep colouration present, and with both reagents at the specified temperature, mixing then proceeded (see Figure 2).

Pump (1) was first turned on to feed β-CD until it reached the first chamber of the CTR (4), after which pump (2) was turned on to feed heated BS into the CTR (4). An internal vortex circulation was generated with the continuous flowing reaction stream which ensured rapid mixing. It is important that both the aqueous, basic β-cyclodextrin solution and the neat 1,4-butane sultone were heated within the range 50-60 ℃ prior to mixing.

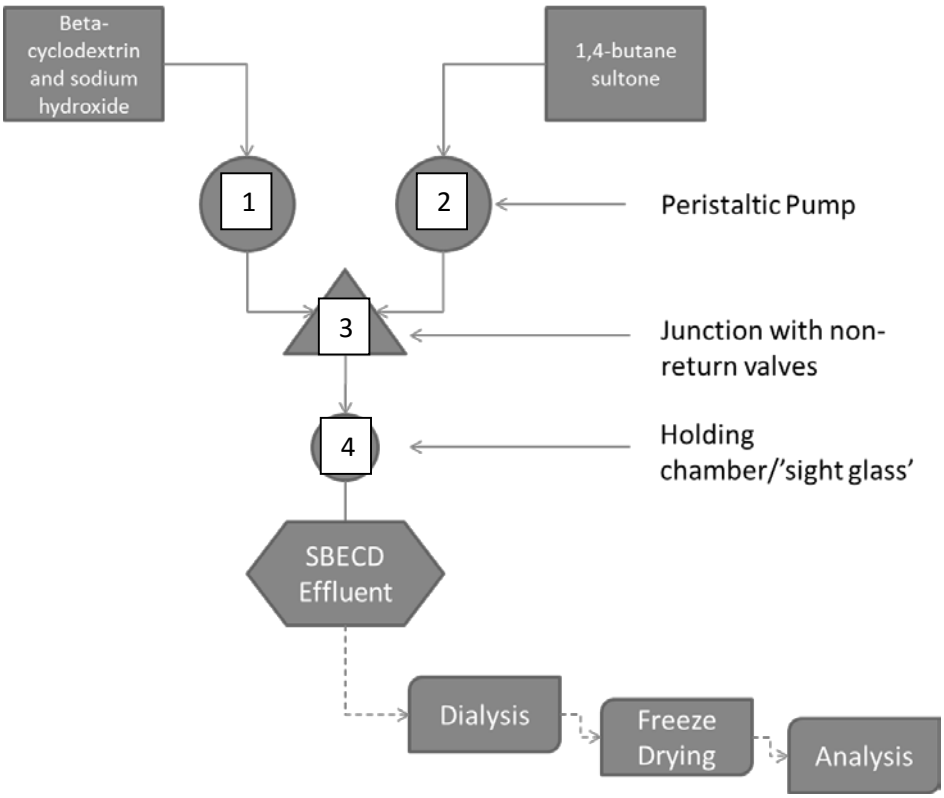

Figure 2: Continuous Tank Reactor Synthesis (CTR) method for producing SBECD

The total amount of 1,4-butane sultone was reacted to the extent that less than 0.1% by weight, of unreacted cyclodextrin was left. The entire initial charge of cyclodextrin is thus reacted by being partially substituted. Residual cyclodextrin can be monitored throughout this initial phase, for example by HPLC as described below, until a desired endpoint of less than 0.1%, of residual cyclodextrin starting material, has been achieved.

Typical Flow rates and cyclodextrin to 1,4-butane sultone ratios are shown in Table 1.

Table 1: The relationship between pump drive speed and flow rate giving rise to different butane sultone-β-cyclodextrin molar ratios – constant 1,4-butane sultone flow rate.

	CD		BS
Drive speed(rpm)	11	15	5
Flow rate(ml/min)	0.99	1.35	0.45
Concentration Mol.min $x10^{-4}$	4.36	5.94	4.4×10^{-3}
[BS:CD] Mole ratio	10:1	7:1	—

The reaction proceeds in a continuous manner, i.e. once the pumps have started they are not switched off until completion of the reaction. The reaction takes place in a temperature range of 50-60 ℃, in contrast to Antle (2) where high temperatures and pressures were used. The CTR process handles the β-cyclodextrin-sodium hydroxide solutions and 1,4-butane sultone as an immiscible, two phase system. We have calculated that Antle's conditions, on the other hand, seem to create the conditions where 1,4-butane sultone and the aqueous β-cyclodextrin-sodium hydroxide streams become miscible, an enabler of flow chemistry processing. Judging by the average degree of substitution achieved by Antle, the goal of miscibility appears to have been achieved at the expense of 1,4-butane sultone stability leading to very low degrees of substitution.

The crude product was harvested in a 20 ml sample bottle. Reaction products were dialysed and lyophilized to obtain the sulphobutyl ether of β-CD as a white solid. The

product was initially analysed using capillary electrophoresis as described by United States Pharmacopoeia 35/National Formulary 30 (7), in order to show the degree of substitution. Mass spectroscopy was then carried out to show the absence of unreacted β-CD and levels of 1, 4-butane sultone were analysed by gas chromatography as described by Shah. The lyophilised product was weighed to give the yield.

The electropherogram in Figure 3 compares SBECD manufactured using our flow synthesis process and a standard sample manufactured using the batch manufacture method according to Shah (1).

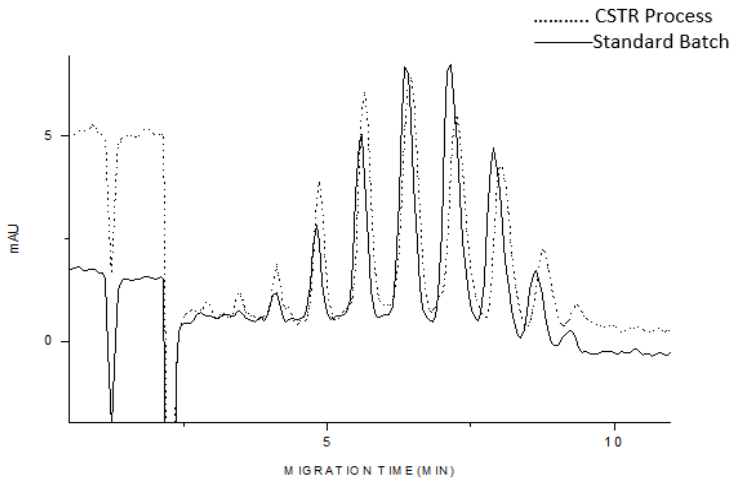

Figure 3: Electropherogram showing a standard sample of batch-produced SBECD according to Shah, (solid line) and an SBECD Sample produced by a Flow Synthesis Process (dotted line).

Coincidence of the two electropherograms indicates an equivalent 'substitution envelope', however it is remarkable that the flow synthesis process only requires 50 % of the sodium hydroxide used in the batch process and a 7:1 molar ratio of 1,4-butane sultone to β-cyclodextrin instead of 10:1 used in the batch process. This finding was unexpected as our entering bias was equivalent synthetic efficiency. It would appear that the shielding of sodium hydroxide from 1,4-butane sultone up to

the point where the reactions streams mix and the reaction takes place allows for an efficient activation of β-cyclodextrin hydroxyl groups at the point of the reaction with minimal degradation of 1,4-butane sultone to low molecular weight by-products. In short, more 1,4-butane sultone can react with β-cyclodextrin more efficiently to generate higher degrees of substitution resulting in more efficient use of the starting materials.

To test this hypothesis further, we attempted to increase the ratio of sodium hydroxide to β-cyclodextrin ratio as outlined in Table 2. In a batch process, according to Shah, this would have no beneficial effect on the degree of substitution, i.e. a change in the substitution envelope, because the sodium hydroxide would simply destroy the 1,4-butane sultone before reaction with cyclodextrin could take place. In essence there is a kinetic limit to the degree of substitution under batch processing conditions. Shah exploits this to reduce the residual concentration of reactants upon batch reaction completion.

Table 2: Composition of sulphobutylether β-cyclodextrin determined by different reagent stoichiometries produced by the CTR method.

1,4-butane sultone to β-CD molar ratio	**NaOH to β-CD molar ratio**	**NaOH relative to Stella(11)**	**Average Degree of Substitution**	**IDS_n present in Shaw(1) and not in the CTR-produced SBECD**	**IDS_n present in the CTR-produced SBECD and not in Shaw(1)**
7:1	9:1	-25%	6.9	None	IDS_1, IDS_{11}
7:1	11:1	0%	8.7	None	IDS_{11} – IDS_{13}
7:1	14:1	+25%	12.1	IDS_2 – IDS_6	IDS_{11}-IDS_{14}
10:1	6:1	-50%	6.0	None	None
10:1	9:1	-25%	6.8	None	IDS_1, IDS_{11}
10:1	11:1	0%	8.4	None	IDS_1, IDS_{11}, IDS_{12}
10:1	14:1	+25%	10.4	IDS_2	IDS_{11} – IDS_{13}

It can be seen from Table 2 that in general, an increase in the content of sodium hydroxide will increase the Average Degree of Substitution of sulphobutylether β-cyclodextrin. This observation is in agreement with an earlier report of batch type systhesis by Stella (11). However, the CTR reactions produce material with an Average Degree of Substitution at levels not previously seen using batch or continuous flow reactions. The higher Average Degree of Substitution arises from

the presence of highly substituted species with an Individual Degree of Substitution in excess of 10.

The process used to produce the material in Figure 4 requires 25% more sodium hydroxide than the batch process with an increase in the molar ratio of 1,4-butane sultone to β-cyclodextrin from 7:1 to 10:1. This material produced by CTR synthesis is unprecedented and demonstrates a positive skew in the substitution envelope with a smaller population of lower degrees of substitution (electropherogram migration time range 2-7 minutes) and an increase in higher degree of substitution products with migration times ranging from 6 minutes to 9 minutes. It is concluded that an increase in efficiency (more efficient activation of β-cyclodextrin hydroxyl groups by sodium hydroxide and less consumption of 1,4-butane sultone resulted in a higher degree of substitution. It is not possible to produce highly substituted SBECD using the batch process. Figure 4 shows an electropherogram of this procedure versus standard batch SBECD (1).

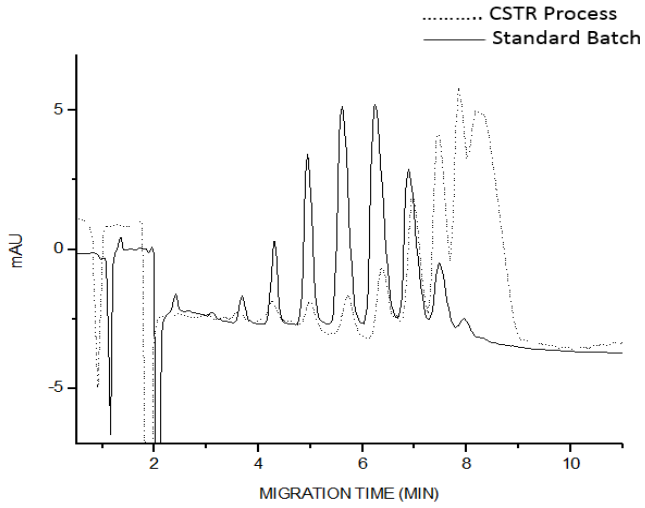

Figure 4: Electropherogram Showing a SBECD Sample produced by a Flow Synthesis Process (dotted line) compared to a Standard Sample of Batch-Produced SBECD according to Shah (solid line).

This method of carefully reacting sodium hydroxide with β-cyclodextrin to activate it in advance of a two-phase continuous flow reaction seems to be the key to creating a highly efficient reaction and a controllable average degree of substitution. The activation process must be conducted at controlled temperature and for a specified time after the β-cyclodextrin has dissolved in the aqueous sodium hydroxide solution. The activation process has typically taken 30 minutes at this scale; the major indicator of completion is the colour change which could be measured colorimetrically but we have not verified this experimentally.

Initial pH control assures the reduction of certain by-products. It is noted that acid is produced as a result of the sulphoalkylation and that the pH tends to decrease as the reaction proceeds. The reaction must be maintained in basic conditions because if the reaction medium is allowed to become too acidic the reaction will stop and so it is important to maintain the pH of the reaction medium at a level of at least 8 by adding aqueous hydroxide as needed. If the pH is allowed to exceed pH 11, then the reaction starts to produce a high level of the by-products 4-hydroxyalkylsulphonate and bis-sulphoalkyl ether, thus consuming 1,4-butane sultone. By initially monitoring pH and maintaining it within the range of 8 to 11, as opposed to simply providing the full charge of hydroxide at the start of the reaction, the reaction proceeds while producing a relatively low level of by-products. The total amount of hydroxide added throughout the reaction was typically on the order of the amount stoichiometrically required plus a 10-20 % molar excess relative to the amount of 1,4-butane sultone employed. Once the sulphoalkylation reaction was complete and the low residual cyclodextrin end point reached, additional hydroxide can be added to destroy any residual sultone.

Although the recommended method for determination of substitution SBECD species is based on a capillary electrophoresis method (Figures 3 and 4), it can be seen that whilst a qualitative idea of the substitution pattern is possible, it is difficult to integrate the areas under the peaks reliably due to the shifting baseline. It is also evident from Figure 4 that peak resolution deteriorates with increasing substitution. Peaks appear to merge after approximately 8 minutes resulting in an inability to quantify the pattern of substitution. It was therefore concluded that it would not be possible to quantify

the degree of substitution for the new CTR process using the USP35/NF30 capillary electrophoresis method.

Alternative methods have been proposed for the analysis of cyclodextrin derivatives using high performance liquid chromatography by Szeman (8). This has been recently applied to sulphobutylether β-cyclodextrin (9). The method is based on a specialized ion-exchange HPLC column, CD-Screen-DAP, using a bonded dimethyl amino phenyl function to improve the selectivity of the analytical method. The analysis of sulfobutyl ether-beta-cyclodextrin mixtures by ion-spray mass spectrometry and liquid chromatography-ion-spray mass spectrometry has also been reported by Grard et al (10).

High performance liquid chromatography with evaporative light scattering detection (ELSD) was used for the detection of the separation of sulphobutylether β-cyclodextrin into its substituted constituents in order to determine the average degree of substitution. Identification of each substituted cyclodextrin was determined by comparing the retention times of the standard, produced by the method described in US 6,153,746 (1), with that of a material produced using our CTR processing method. A typical chromatogram for the standard material is given in Figure 5:

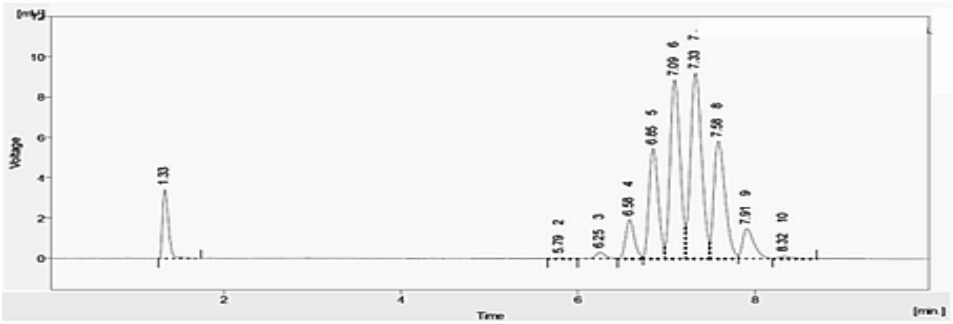

Figure 5: Chromatogram of sulphobutylether β-cyclodextrin produced by the method described in US 6,153,746 (Shah, 2000). HPLC conditions are based on a gradient separation with a CD-Screen-DAP column and ELSD detection.

The chromatogram for the material corresponding to CTR processing with ratios of 1,4-butane sultone to β-cyclodextrin of 10:1 are shown in Figure 6.

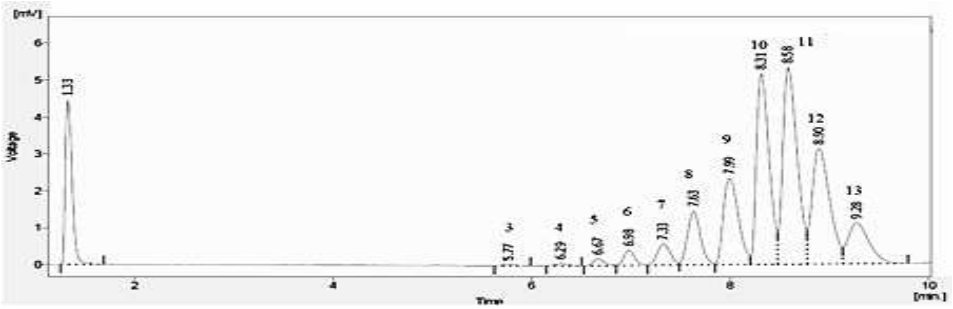

Figure 6: Chromatogram of sulphobutylether β-cyclodextrin produced by the CTR method.

Upon further examination of Figure 5, it can be seen that material produced by the process described in US 6,153,746 (1) has a range of substitution from 2 to 10. Figure 6 indicates that material produced in accordance with CTR processing has a range of substitution from 3 to 13. In addition the method does not produce any detectable di-substituted sulphobutylether β-cyclodextrin and produces significant quantities of degree of substitution of 11-13 not detected in the US 6,153,746 (1) material.

From the chromatographic data, an Individual Degree of Substitution (IDS_n) can be calculated using the following formula:

$$IDS_n = (PA_n/\sum PA) \times 100 \qquad \text{Equation (1)}$$

$$\text{where } \sum PA = \sum PA_L + PA_{L+1} \ldots PA_H \qquad \text{Equation (2)}$$

n = Substitution Number

PA = Peak area

PA_L = Peak area corresponding to lowest degree of substitution seen on the chromatogram

PA_H = Peak area corresponding to highest degree of substitution seen on the chormatogram

These data can be used to describe an 'Envelope of Substitution' which is used as the basis of a specification element in USP30/NF30 (7), where each IDS_n should fall within a Proven Acceptable Range.

Table 3 shows calculated data based on the chromatogram shown in Figure 6. This was processed using Equations 1-2 to give an Individual Degree of Substitution (IDS_n).

Table 3: Calculated Individual Degree of Substitution (IDS_n).

Substitution Number:	Retention Time:	Peak Area:	IDSn
3	5.77	0.271	0.133
4	6.29	0.507	0.248
5	6.67	1.455	0.712
6	6.98	3.142	1.537
7	7.33	5.221	2.553
8	7.63	13.283	6.496
9	7.99	24.842	12.148
10	8.31	46.056	22.528
11	8.58	53.920	26.368
12	8.90	39.220	19.180
13	9.28	16.570	8.103

The Individual Degree of Substitution metrics are then used to calculate the Average Degree of Substitution as follows:

$$ADS = \sum(IDS_n \times n)/ 100 \qquad \text{Equation (3)}$$

The material described in Figure 6 has an average degree of substitution (ADS) of 10.4 which is substantially higher than material produced by batch manufacture (1) or Antle's flow process (2) which typically results in ADS values of 6 to 7.

Conclusions

The Continuous Tank Reactor method of synthesis resulted in a lower concentration of lower substituted sulphoalkyl ether β-cyclodextrin (i.e. a degree of substitution value of 1-3) and surprisingly much higher concentrations of the higher substituted sulphoalkyl ether β- cyclodextrin (i.e. an average degree of substitution value of 3-13) than reported using more standard batch or flow techniques. The CTR process depends upon pre-activation of the β-cyclodextrin feedstock by sodium hydroxide where the extent of activation determines Average Degree of Substitution. The process allows greater control of the Average Degree of Substitution by varying sodium hydroxide concentration. The process can be used to produce material with a high Average Degree of Substitution, the utility of which is currently under investigation (12). It should also be possible to manufacture material compliant with the USP35/NF30 specification for sulphobutylether β-cyclodextrin, the current article of commerce, if that is desired. Using an improved HPLC analytical method, we have been able to validate these general observations. The technique has allowed us to produce descriptive statistics for highly substituted materials. The composition, produced by the CTR process, is novel in two respects: an unprecedented high average degree of substitution and the existence of highly substituted species with IDS_n values higher than 10.

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