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1 **Continuous Tank Reactor Synthesis of Highly Substituted Sulphobutylether  $\beta$ -**  
2 **Cyclodextrins**

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15 Key Words: cyclodextrin; Sulphobutyl ether  $\beta$ -cyclodextrin; Continuous Tank

16 Reactor; SBE- $\beta$ -CD, SBECD; CD-Screen-DAP, Evaporative Light Scattering

17 Detection

18

19 ABSTRACT

20

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

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

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

37 The work is particularly concerned with producing controlled substitution in  
38 sulphobutyl ether  $\beta$ -cyclodextrins and novel compositions of highly substituted  
39 sulfoalkyl ether  $\beta$ -cyclodextrins are described.

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50 **Abbreviations**

51	ADS	Average Degree of Substitution
52	$\beta$ -CD	$\beta$ -Cyclodextrin
53	BS	1,4-butane sultone
54	CD	Cyclodextrin
55	CD-Screen-DAP	HPLC Stationary Phase for Analysis of Cyclodextrin-Derivatives
56	CTR	Continuous Tank Reactor
57	ELSD	Evaporative Light Scattering Detection
58	HPLC	High performance liquid chromatography
59	IDS	Individual Degree of Substitution
60	MPA	Mobile phase A
61	MPB	Mobile phase B
62	PTFE	Polytetrafluoroethylene
63	SBE- $\beta$ -CD	Sulphobutyl ether $\beta$ -cyclodextrin
64	SBECD	Sulphobutyl ether $\beta$ -cyclodextrin
65	USP35/NF30	United States Pharmacopeia 35 and National Formulary 30
66	US FDA	US Food and Drug Administration

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74 **Introduction**

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

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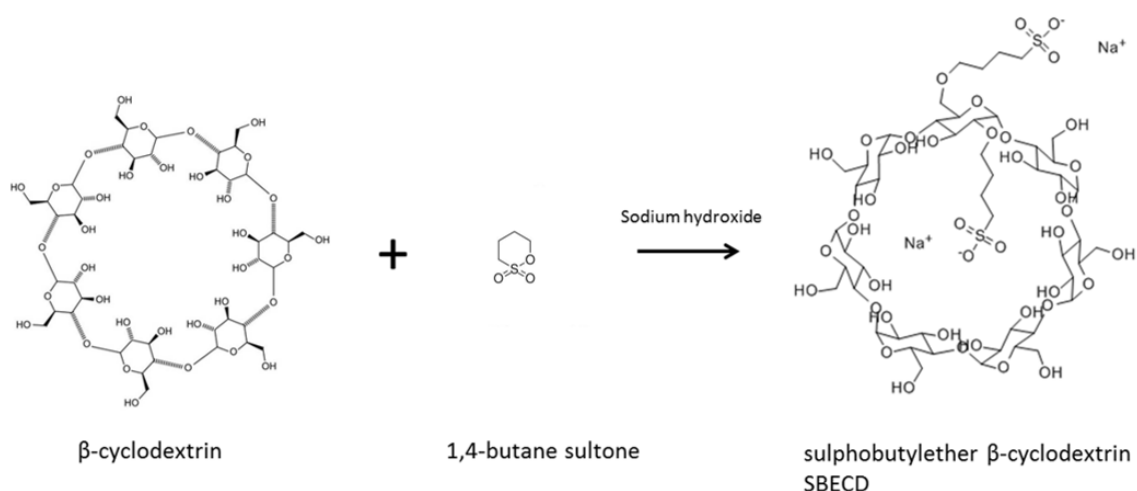
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94 Figure 1: A general scheme for the synthesis of SBECD from the reagents  $\beta$ -cyclodextrin ( $\beta$ -CD)  
95 and 1, 4-butane sultone (BS).

96

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

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

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

121

## 122 **Methods**

### 123 The Continuous Tank Reactor (CTR) based Manufacturing Process.

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

134 60 °C with a hotplate stirrer. The first pump (Figure 2) was then used to deliver stock  
135  $\beta$ -CD solution into the CTR where the substitution reaction takes place via the three  
136 way connector, while the second pump was used to deliver neat 1,4-butane sultone  
137 also held at 60 °C through the three way connector. An internal vortex circulation  
138 was generated with the continuous flowing reaction stream and the reaction  
139 proceeded in a continuous manner, i.e. once the pumps started they were not  
140 switched off until completion of the reaction. The crude product was harvested in a  
141 20 ml sample bottle.

#### 142 Analytical Methodology for the Analysis of High Substituted SBECD Species

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

148 The chromatographic conditions are summarised as follows:

Instrument: Agilent 1100 series  
Software: OpenLAB  
Column: CD-Screen-DAP, 3  $\mu$ m, 150  $\times$  4.0 mm,  
(CD-Screen -DAP-1504-03)  
Column temperature: 25° C.  $\pm$  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  $\mu$ l  
Concentration: 10 mg/ml  
Acquisition time: 15 minutes with post-time of 5 minutes  
Needle wash: none

#### 149 ELSD Conditions

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

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

151

## 152 **Results and Discussion**

153 The authors carefully studied the batch sulphoalkyl ether  $\beta$ -cyclodextrin production  
154 method that was described by Shah *et al* (1), and have then devised a Continuous  
155 Tank Reactor Synthesis (CTR) method for producing SBECD and experimented with  
156 the stoichiometry of the reaction. A significant modification to existing methods  
157 comprised contacting cyclodextrin with a base to form activated cyclodextrin and  
158 separately reacting the activated cyclodextrin with an 1,4-butane sultone to form  
159 sulphoalkyl ether  $\beta$ -cyclodextrin. In our method the sulphoalkylation reaction is  
160 carried out under continuous flow conditions. The resultant substituted sulphoalkyl  
161 ether  $\beta$ -cyclodextrin is novel as it exhibits a higher degree of substitution, for a lower  
162 input of 1,4-butane sultone and base than that which is produced using the known  
163 batch process. The higher Average Degree of Substitution arises from the presence  
164 of highly substituted species with an Individual Degree of Substitution in excess of  
165 10.

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

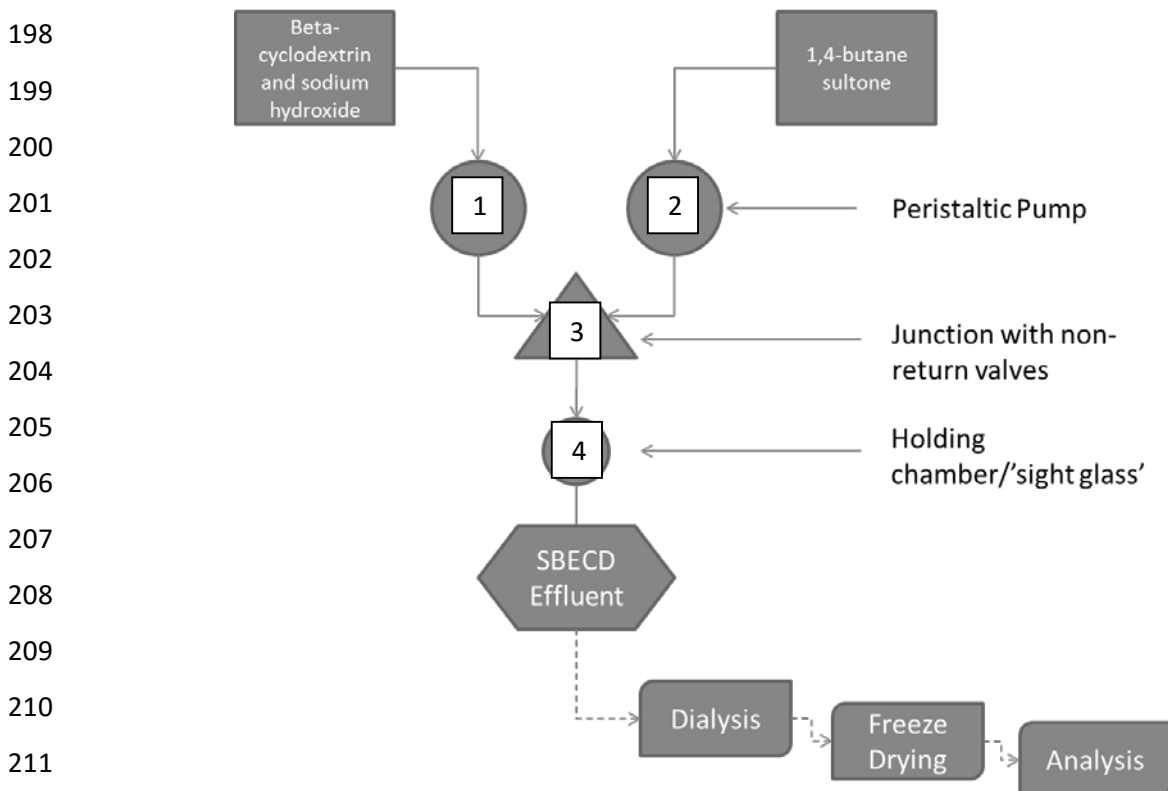
175 As described in the Method Section, the set-up for the continuous flow experiments  
176 consisted of two pumps connected to a double Continuous Tank Reactor (CTR)  
177 acting as a holding chamber/sight glass. The two pumps were connected to the CTR  
178 holding chamber via a three-way connector.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



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

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

192 Pump (1) was first turned on to feed  $\beta$ -CD until it reached the first chamber of the  
193 CTR (4), after which pump (2) was turned on to feed heated BS into the CTR (4). An  
194 internal vortex circulation was generated with the continuous flowing reaction stream  
195 which ensured rapid mixing. It is important that both the aqueous, basic  $\beta$ -  
196 cyclodextrin solution and the neat 1,4-butane sultone were heated within the range  
197 50-60 °C prior to mixing.



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

213 The total amount of 1,4-butane sultone was reacted to the extent that less than  
214 0.1% by weight, of unreacted cyclodextrin was left. The entire initial charge of  
215 cyclodextrin is thus reacted by being partially substituted. Residual cyclodextrin  
216 can be monitored throughout this initial phase, for example by HPLC as  
217 described below, until a desired endpoint of less than 0.1%, of residual  
218 cyclodextrin starting material, has been achieved.

219

220

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

223

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

226

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

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

243

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

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

252

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

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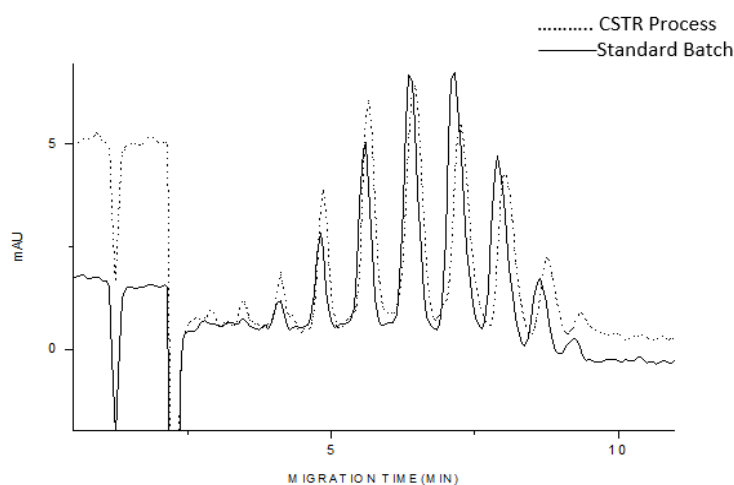
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267 Figure 3: Electropherogram showing a standard sample of batch-produced SBECD according to Shah,  
268 (solid line) and an SBECD Sample produced by a Flow Synthesis Process (dotted line).

269 Coincidence of the two electropherograms indicates an equivalent 'substitution  
270 envelope', however it is remarkable that the flow synthesis process only requires 50  
271 % of the sodium hydroxide used in the batch process and a 7:1 molar ratio of 1,4-  
272 butane sultone to  $\beta$ -cyclodextrin instead of 10:1 used in the batch process. This  
273 finding was unexpected as our entering bias was equivalent synthetic efficiency. It  
274 would appear that the shielding of sodium hydroxide from 1,4-butane sultone up to

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

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

289

290 Table 2: Composition of sulphobutylether  $\beta$ -cyclodextrin determined by different reagent stoichiometries  
 291 produced by the CTR method.

1,4-butane sultone to $\beta$ -CD molar ratio	NaOH to $\beta$ -CD molar ratio	NaOH relative to Stella(11)	Average Degree of Substitution	IDS <sub>n</sub> present in Shaw(1) and not in the CTR-produced SBECD	IDS <sub>n</sub> present in the CTR-produced SBECD and not in Shaw(1)
7:1	9:1	-25%	6.9	None	IDS <sub>1</sub> , IDS <sub>11</sub>
7:1	11:1	0%	8.7	None	IDS <sub>11</sub> – IDS <sub>13</sub>
7:1	14:1	+25%	12.1	IDS <sub>2</sub> – IDS <sub>6</sub>	IDS <sub>11</sub> -IDS <sub>14</sub>
10:1	6:1	-50%	6.0	None	None
10:1	9:1	-25%	6.8	None	IDS <sub>1</sub> , IDS <sub>11</sub>
10:1	11:1	0%	8.4	None	IDS <sub>1</sub> , IDS <sub>11</sub> , IDS <sub>12</sub>
10:1	14:1	+25%	10.4	IDS <sub>2</sub>	IDS <sub>11</sub> – IDS <sub>13</sub>

292

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

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

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

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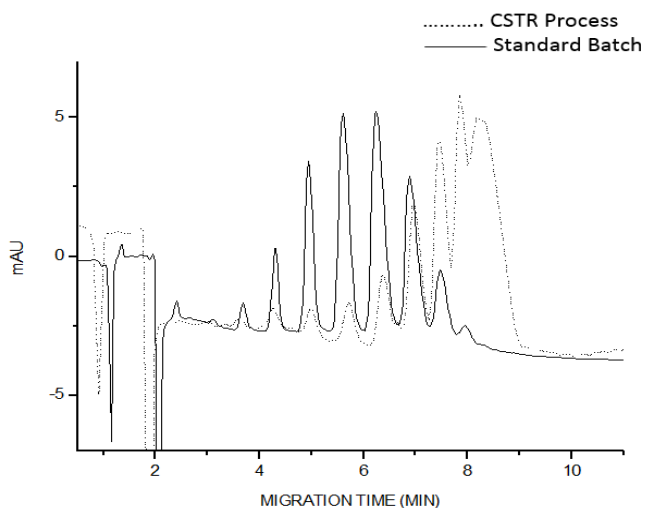
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324 Figure 4: Electropherogram Showing a SBECD Sample produced by a Flow Synthesis Process (dotted  
325 line) compared to a Standard Sample of Batch-Produced SBECD according to Shah (solid line).

326

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

335 Initial pH control assures the reduction of certain by-products. It is noted that  
336 acid is produced as a result of the sulphoalkylation and that the pH tends to  
337 decrease as the reaction proceeds. The reaction must be maintained in basic  
338 conditions because if the reaction medium is allowed to become too acidic the  
339 reaction will stop and so it is important to maintain the pH of the reaction medium  
340 at a level of at least 8 by adding aqueous hydroxide as needed. If the pH is  
341 allowed to exceed pH 11, then the reaction starts to produce a high level of the  
342 by-products 4-hydroxyalkylsulphonate and bis-sulphoalkyl ether, thus consuming  
343 1,4-butane sultone. By initially monitoring pH and maintaining it within the range  
344 of 8 to 11, as opposed to simply providing the full charge of hydroxide at the start  
345 of the reaction, the reaction proceeds while producing a relatively low level of by-  
346 products. The total amount of hydroxide added throughout the reaction was typically  
347 on the order of the amount stoichiometrically required plus a 10-20 % molar excess  
348 relative to the amount of 1,4-butane sultone employed. Once the sulphoalkylation  
349 reaction was complete and the low residual cyclodextrin end point reached,  
350 additional hydroxide can be added to destroy any residual sultone.

351

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

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

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

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

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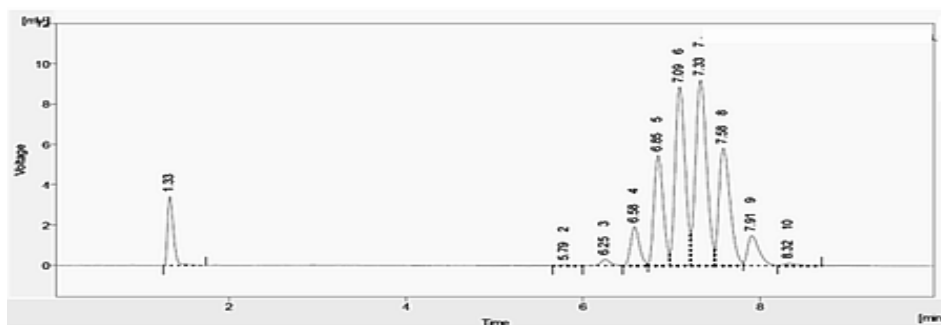
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382 Figure 5: Chromatogram of sulphobutylether  $\beta$ -cyclodextrin produced by the method described in  
383 US 6,153,746 (Shah, 2000). HPLC conditions are based on a gradient separation with a CD-  
384 Screen-DAP column and ELSD detection.

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389 The chromatogram for the material corresponding to CTR processing with ratios of  
 390 1,4-butane sultone to  $\beta$ -cyclodextrin of 10:1 are shown in Figure 6.

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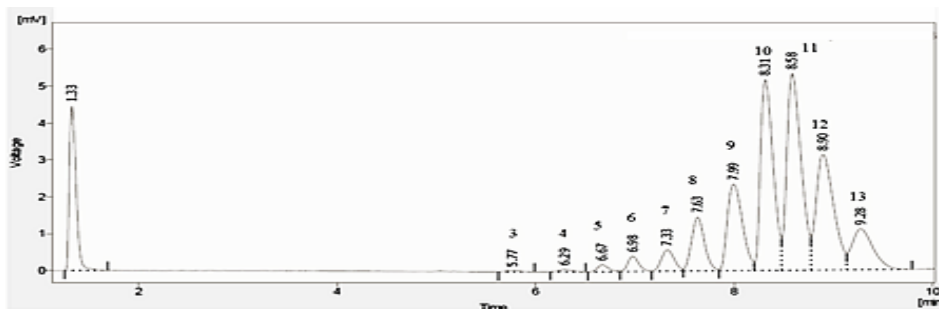
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397 Figure 6: Chromatogram of sulphobutylether  $\beta$ -cyclodextrin produced by the CTR method.

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399

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

407

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

410 
$$IDS_n = (PA_n / \sum PA) \times 100 \quad \text{Equation (1)}$$

411

412 
$$\text{where } \sum PA = \sum PA_L + PA_{L+1} \dots PA_H \quad \text{Equation (2)}$$

413

414 n = Substitution Number

415 PA = Peak area

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

418  $PA_H$  = Peak area corresponding to highest degree of substitution seen on  
 419 the chromatogram



420

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

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

427

428

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

Substitution Number:	Retention Time:	Peak Area:	$IDS_n$
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

430

431

432

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

435

436 
$$ADS = \sum(IDS_n \times n) / 100 \quad \text{Equation (3)}$$

437

438 The material described in Figure 6 has an average degree of substitution (ADS) of  
439 10.4 which is substantially higher than material produced by batch manufacture (1)  
440 or Antle’s flow process (2) which typically results in ADS values of 6 to 7.

441

442

443

444

445 Conclusions

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

## REFERENCES

1. Shah, B.K., Sklavounos, C., 2000, Process for Making a Cyclodextrin. U.S. Patent 6,153,746.
2. (a) Antle, V., 2009, Sulfoalkyl Ether Cyclodextrin Compositions. US Patent 7,635,733 B2.  
(b) Matos, J. R., Antle V. D., 2013, A method of producing cyclodextrin derivatives, WO 2013/123254A1.
3. Tongiani, S., Vander Velde, D., Ozeki, T., Stella, V.J., Sulfoalkyl ether-alkyl ether cyclodextrin derivatives, their synthesis, NMR characterization, and binding of 6 $\alpha$ -methylprednisolone, 2005, *Journal of Pharmaceutical Sciences*, V 94, Issue 11, 2380–2392.
4. Rong D, D'Souza VT., 1990, A convenient method for functionalization of the second position of cyclodextrins. *Tetrahedron Lett* 31: 4275–4278.
5. Brewster M, Huang MJ, Pop E, Pitha J, Dewar MJS, Kaminski JJ, Bodor N. 1993. An AM1 molecular orbital study of  $\alpha$ -D-glucose and B-maltose: Evaluation and implications. *Carbohydr Res* 242: 53–67.
6. Jindrich J, Pitha J, Lindberg B, Seffers P, Harata K. 1995, Regioselectivity of alkylation of cyclomaltoheptaose ( $\beta$ -Cyclodextrin) and synthesis of its mono-2-O-methyl, -ethyl, -allyl, and -propyl derivatives. *Carbohydr Res* 266(1): 75–80.
7. United States Pharmacopoeia 35/National Formulary 30 (USP35/NF30). ISBN: 9781936424009
8. Szeman, J., Csabai, K., Kekesi, K., Szente, L., Varga, G., 2006, Novel stationary phases for high-performance liquid chromatography. *Journal of Chromatography A*, 76-82.
9. Szeman, J., Sohajda, T., Olah, E., Varga, E., Csabai, K., Varga, G., Szente, L., 2012, Characterization of Randomly Substituted Anionic Cyclodextrin Derivatives with Different Analytical Methods. *16th International Cyclodextrin Symposium*. Tianjin, China.

10. Grard S, Elfakir C, Dreux M., 2001, Analysis of sulfobutyl ether-beta-cyclodextrin mixtures by ion-spray mass spectrometry and liquid chromatography-ion-spray mass spectrometry.  
*J Chromatogr A.*, 3;925(1-2):79-87.
11. Stella, V. J. , Rajewski, R., 1994, Derivatives of cyclodextrins exhibiting enhanced aqueous solubility and the use thereof US 5,376,645, US 5376645 A
12. Savage, T., Wicks, S ., Mitchell, J ., 2014, Patent CYCLODEXTRIN 20150025023, US Patent App. 14/333,417.