

# Analyzing the average degree of substitution in betadex sulfobutyl ether sodium using capillary electrophoresis

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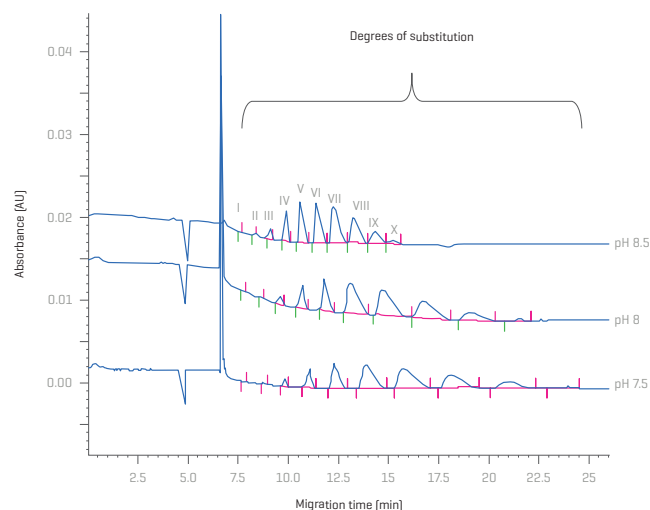
Sulfobutyl-beta-cyclodextrin [SBE- $\beta$ -CD], also known as betadex sulfobutyl ether sodium, is an anionic, highly water-soluble beta-cyclodextrin derivative commonly used in drug formulations with limited solubility in water. It can effectively enhance the bioavailability of such drugs. Since the first FDA-approved product containing Betadex sulfobutyl ether sodium, this derivative has been widely used in drug formulations. Therefore, it is essential to establish a robust assay to ensure the product quality for safety and batch-to-batch consistency.

In this technical note, a series of background electrolytes were evaluated for the best performance for analyzing betadex sulfobutyl ether sodium – using system suitability parameters set by the United States Pharmacopeia [USP], with the PA 800 plus system.

Betadex sulfobutyl ether sodium is prepared by alkylation of betadex using 1,4 butane sultone in a basic environment, yielding a product with several degrees of substitution that must be adequately characterized and quantified.

USP specifies the use of capillary electrophoresis [CE] for the analysis of the average degree of substitution [ADS] of sulfobutyl- $\beta$ -cyclodextrin<sup>1</sup>, providing detailed specifications for the CE instrument conditions, capillary treatment and separation parameters. However, because the USP method is instrument agnostic, the capillary length may vary from platform to platform. Therefore, the method does not specify the pH value of the background electrolyte [BGE].

This work studied 3 different pHs for the background electrolyte and the results were evaluated according to the system suitability criteria established by USP.



**Figure 1. Separation of Betadex using the USP method as a function of pH.** The peaks labelled with Roman numerals represent the 10 degrees of substitution.

## Key features

- 3 different pH values of background electrolyte [BGE] were investigated to analyze the ADS of sulfobutyl- $\beta$ -cyclodextrin standard solution according to USP criteria for Normalized corrected peak area and degree of substitution [Figure 1].
- The results show that the calculated average degree of substitution of the standard solution under pH 7.5–8.5 conditions meets the system suitability requirements of the USP Pharmacopoeia.
- Under pH 8.5 conditions, the peak elutes faster and has a better peak shape. Under pH 8.0 conditions, the relative migration times of the standard solution peaks [I–X] are closest to the reference values in the USP Pharmacopoeia and can be used for peak identification.
- The reproducibility of migration time using BGE with pH 8 for the 10 degrees of substitution was as low as 0.7% for peak II and 3.14% for peak X [Figure 2].

## Methods

**Sample:** sulfobutyl- $\beta$ -cyclodextrin standard - [ P/N 1065550] was purchased from USP (Rockville, MD)

**Sample preparation:** sulfobutyl- $\beta$ -cyclodextrin standard was used without any purification. 10 mg cyclodextrin standard was diluted in 1 mL of ddi water to produce a 10mg/mL solution.

**Reagents:** benzoic acid [P/N 242381], Tris buffer, 0.1 M [P/N 648315] and 0.1 M sodium hydroxide solution [P/N 43617] were purchased from Sigma-Aldrich [St. Louis, MO].

**Background electrolyte preparation (BGE):** 30 mM benzoic acid was prepared with ddi water. The pH was adjusted with 100 mM Tris buffer solution.

**Instrument:** PA 800 Plus system was equipped with a UV detector. The detection type was indirect at 200 nm. The separation was carried out in a bare fused silica capillary, 50  $\mu$ m inner diameter [P/N 338451], with 40/50 cm [effective/total length] and an aperture of 200  $\mu$ m. All these parts were from SCIEX [Framingham, MA].

**New capillary treatment:** before first use, the capillary was treated with a 1 M NaOH rinse for 1 hr followed by ddi water rinse for 2 hrs at 20 psi.

**Capillary conditioning method:** at the beginning of each day, the capillary was rinsed with 0.1 M NaOH for 30 min followed by a ddi Water rinse for 2 hrs and then background electrolyte for 1 hr at 20 psi.

**Separation method:** The method consists of a series of rinses with 0.1 M NaOH [2 min], ddi water [3 min], and background electrolyte [3 min] at 20 psi. Next, the sample is injected at 0.5 psi for 10 s and then separated at 30 kV for 30 minutes with a ramp time of 10 minutes.

The temperature for the capillary cartridge and the sample compartment was maintained at 25 °C at all times.

**Software:** Instrument control, creation of methods and sequences and data analysis were done using 32 Karat, Version 10.

## Results and discussion

### Investigation of pH for the BGE

Due to the variation between capillary conditions used in different platforms, USP does not specify the pH of the background electrolyte. Therefore, it is necessary to investigate the best pH to detect and resolve the 10 peaks corresponding to the 10 degrees of substitution found in betadex sulfobutyl ether sodium.

Figure 1 shows an overlay of 3 electropherograms of the various degrees of substitution of betadex sulfobutyl ether sodium using BGE with pH 7.5 [bottom], pH 8 [middle] and pH 8.5 [top]. The separation pattern clearly shows the higher the BGE's pH, the faster the separation. Additionally, the peaks become sharper compared to the shape of the peaks separated at pH 7.5 or 8.

### Analysis of System Suitability Requirements

The USP establishes the following requirements for system suitability:

1. Identify 10 peaks corresponding to the 10 degrees of substitution
2. The resolution between peaks IX and X of betadex sulfobutyl ether sodium may not be less than 0.9.
3. The average degree of substitution must be between 6.2 and 6.9.

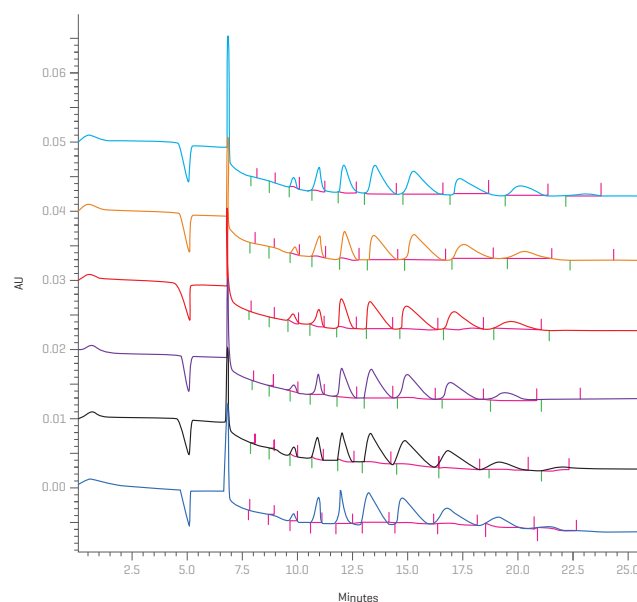


Figure 2. Reproducibility of peak migration times for sulfobutyl- $\beta$ -cyclodextrin standard at pH 8.0 [n=6]

### Criterion 1. Identifying the degrees of substitution peaks

USP established relative migration time [RMT] values for the 10 peaks to facilitate the correct peak assignment. It is important to note that The RMT is provided for reference use only and should not be considered a system suitability parameter. RMT also plays an important role in determining the proper pH of the background electrolyte. Table 1 shows the USP RMT of the 10 peaks for reference, and the RMT values were obtained using BGE with pH 7.5, 8.0, and 8.5. Based on these results, results from pH 8 showed the closest values to the RMT guidelines established by USP.

Table 1 A comparison between a reference value established by USP and the relative migration time values determined under pH 7.5, pH 8 and pH 8.5

Peak number	Relative migration time [min]			
	USP reference values	pH 7.5	pH 8	pH 8.5
I	0.58	0.50	0.57	0.62
II	0.63	0.56	0.63	0.67
III	0.69	0.63	0.69	0.75
IV	0.77	0.71	0.76	0.81
V	0.83	0.79	0.83	0.87
VI	0.91	0.88	0.91	0.93
VII	1.00	1.00	1.00	1.00
VIII	1.10	1.15	1.11	1.08
IX	1.20	1.33	1.23	1.17
X	1.30	1.52	1.34	1.24

**Criterion 2: Resolution between Peaks IX and X may not be less than 0.9**

Table 2 demonstrates the calculated resolution of between peak IX and X under all 3 pHs all meet the USP criteria greater than 0.9.

**Criterion 3. The average degree of substitution must be between 6.2 and 6.9**

The synthesis of Betadex sulfobutyl ether sodium yields the formation of a product with various substitution degrees. The system suitability must show distinct 10 peaks and an average degree of substitution between 6.2 and 6.9.

The degree of substitution in percentage is calculated by following the steps below:

- $A_i$  represents the velocity-corrected peak area for each peak

$$A_i = P A \quad \times \quad \frac{\text{capillary effective length [cm]}}{\text{migration time [min]}}$$

- $NA_i$  represents the normalized corrected peak area

$$NA_i = \frac{A_i}{\sum_{i=1}^n A_i} \times 100$$

- The average degree of substitution [ADS] is calculated by:

$$ADS = \frac{\sum_{i=1}^n (\text{Level of substitution for a peak}) \times NA_i}{100}$$

The degree of substitution for each peak is as follows: peak I has a degree of substitution of 1, peak II has a degree of substitution of 2, and so on, up to Peak X, which has a degree of substitution of 10.

USP has established the range for the normalized corrected peak area % for each 10 peaks [I, II, through X]. Table 2 shows the USP range for  $NA_i$  for each of the 10 peaks, and the  $NA_i$  shown was obtained using BGEs with pH 7.5, 8, and 8.5. Table 2 also shows the calculated ADS.

These results clearly show that regardless of the pH of the BGE, all 3 pH yielded results meeting USP criteria.

Table 2. Comparison of peak area percentages, separation degree, and average substitution degree of standards at different pH levels.

	Peak number	Range of USP $NA_i$ %	pH 7.5	pH 8	pH 8.5
Range of Normalized corrected peak area [ $NA_i$ ]	I	0 - 0.3	0.02	0.12	0.08
	II	0 - 0.9	0.35	0.42	0.43
	III	0.5 - 5.0	1.79	2.06	2.31
	IV	2.0 - 10.0	5.64	6.12	6.58
	V	10.0 - 20.0	12.86	13.44	14.67
	VI	15.0 - 25.0	21.41	21.27	22.43
	VII	20.0 - 30.0	24.59	25.54	25.19
	VIII	10.0 - 25.0	20.60	19.15	18.56
	IX	2.0 - 12.0	9.54	8.97	8.02
	X	0 - 4.0	3.20	2.89	1.71
ADS		<b>6.2 - 6.9</b>	<b>6.45</b>	<b>6.44</b>	<b>6.39</b>
Resolution between peaks IX and X		<b>≥0.9</b>	<b>1.349</b>	<b>1.510</b>	<b>1.351</b>

**Method reproducibility**

Figure 2 shows an overlay of 6 runs of betadex sulfobutyl ether sodium at pH 8, and Table 3 shows the reproducibility of the migration time for 6 replicate runs for each of the 10 peaks corresponding to the 10 degrees of substitution. Even though USP does not specify a requirement for reproducibility of migration time, the %RSD values obtained at pH 8 were acceptable.

Peak #	Migration time (min, n=6)						RSD[%]
	Run 1	Run 2	Run 3	Run 4	Run 5	Run 6	
I	7.850	7.917	8.008	7.846	7.921	7.933	0.76
II	8.808	8.821	8.829	8.829	8.846	8.858	0.20
III	9.875	9.887	9.900	9.904	9.917	9.946	0.25
IV	10.963	10.983	11.004	11.021	11.063	11.079	0.41
V	12.008	12.042	12.075	12.100	12.192	12.192	0.63
VI	13.342	13.375	13.433	13.496	13.65	13.629	0.96
VII	14.863	14.967	15.025	15.108	15.458	15.413	1.61
VIII	16.854	17.021	17.125	17.208	17.642	17.546	1.77
IX	19.212	19.375	19.454	19.667	20.425	20.433	2.72
X	21.7	21.9	21.967	22.775	23.429	23.029	3.14

## Conclusions

- Separation performance was investigated as a function of BGE under different pH values, 7.5, 8 and 8.5. The results indicated a faster separation and better peak shape when using a BGE with pH 8.5.
- Regardless of the pH difference of the BGE, all 3 methods passed the requirements for system suitability: normalized corrected peak area % [NAi], resolution between peaks IX and X, and the ADS
- This study established that for a PA 800 plus system, a BGE with pH 8 should be considered the starting point and further adjusted with a 0.1M Tris solution according to the passing criteria specified by USP.

## References

1. Betadex / Official Monographs, The United States Pharmacopeia

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