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Evaluation of the utility of capillary electrophoresis for the analysis of sulfobutyl ether β-cyclodextrin mixtures

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Abstract

A capillary electrophoresis (CE) method for the analysis of a sulfobutyl ether β-cyclodextrin (SBE-β-CD) mixture is described. The SBE-β-CD has been prepared as a parenterally safe solubilizing agent and has historically been characterized by elemental analysis and nuclear magnetic resonance spectroscopy. While these methods provide gross values for the degree of substitution, the CE method described resolves the mixture of positional and regional isomers based on the degree of SBE substitution. The method uses benzoic acid in the running buffer and detects the CD by a decrease in background absorbance of the benzoic acid due to complexation. The necessity of a defined column wash sequence between injections was evaluated. The reproducibility of migration times and peak areas/heights for 10 components of the mixture was determined. The modular CE system gave a relative standard deviation of 2.5% ($n = 3$) for six of the 10 peaks. Further refinements (pH buffer effects) were explored to improve the reproducibility with remaining components. The method was used to evaluate the reproducibility of the synthesis (21 different lots) and the effect of reaction variables (time, temperature and base) on the composition pattern of the modified CD.

Keywords: Anionic cyclodextrin; Capillary electrophoresis; Sulfobutyl ether cyclodexrin

1. Introduction

The objective of this work was to evaluate utility of a capillary electrophoresis (CE) method developed in these laboratories for the analysis of derivatized cyclodextrins (CDs). The sulfobutyl ether derivatives (SBE-β-CD) studied are a mixture of positional and regional isomers containing from one to as many as 10 SBE groups per CD molecule. The effect of reaction conditions during the synthesis of the SBE-CDs on the relative population of isomers in any one substitution band was unknown. Therefore, it was necessary

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to develop a method which resolves the components of the mixture based on the degree of substitution (DS) and allows for relative quantitation of the respective bands.

CDs have been widely investigated for use in pharmaceutical formulations because they have the capacity to alter the physical, chemical, and biological properties of guest molecules through formation of inclusion complexes [1]. However, natural CDs (α-, β-, and γ-CD) have relatively low solubility, both in water and organic solvents, which limits their applications in the pharmaceutical field. The apparent CDs are known to interact with, and extract, cholesterol and other membrane components, particularly upon accumulation in the kidney tubule cells [2]. This toxic effect is particularly pronounced in the case of the β-CD. To overcome these limitations the natural CDs have to be chemically modified [1–4].

The recently patented sodium salts of SBE-β-CD [3] have the same or better binding capacity for most drugs as the parent CD with no apparent toxicity and very high water solubility [3–12]. The SBE-β-CD derivatives were designed based on the current understanding of the toxicity of the parent cyclodextrins. The anionic sulfobutyl ether functional group was intentionally introduced to capitalize on the kidney's ability to rapidly excrete ionic compounds.

The SBE-β-CDs are generally prepared by adding 1,4-butane sultone to an alkaline solution of β-CD and stirring the resulting mixture at 70°C for 24 h [1]. A generalized structure of the substituted SBE-β-CD is shown in Fig. 1. The material includes regional and positional isomers distributed over a range of substitution levels from mono- to deca-substituted product. Initial analytical information (elemental analysis and NMR) on batches prepared under identical conditions suggests that the materials generated possess equivalent DSs. However, mass spectral analysis of these anionic CDs has shown these derivatives to be heterogeneous mixtures with an average molar DS ranging from 1–7 depending upon the synthetic preparation.

A CE method has been previously reported [13] that provides a "picture" of the composition pattern of the CD preparation. This method utilized

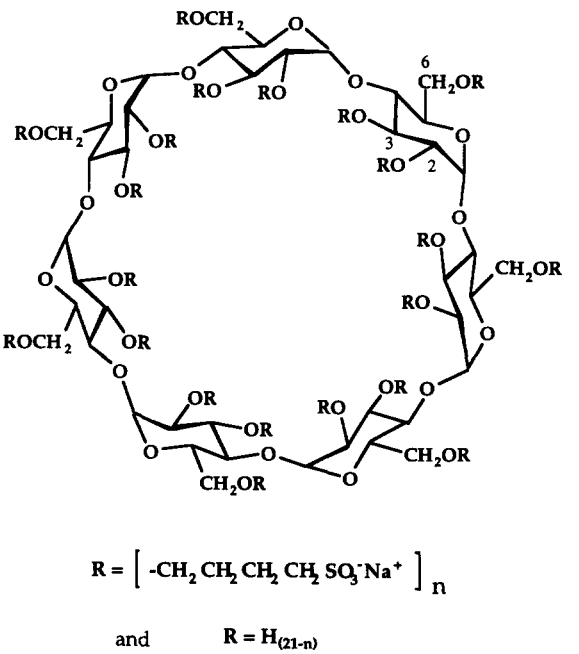

Fig. 1. Generalized structure of the SBE-β-CD, where n is the average DS.

benzoic acid in the running buffer and detects the CD by a decrease in the background absorbance due to complexation. The CE profile shows complete resolution of mono- to deca-derivatized β-CD as shown in Fig. 2. The electropherogram depicted corresponds to a mixture of SBE-β-CD with an average DS of 4. However, the initial conditions have shown erratic migration times

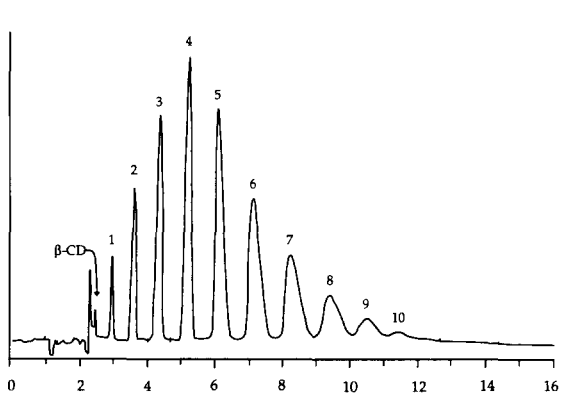

Fig. 2. Electropherogram of the SBE4-β-CD mixture obtained on the modular electropherogram under the conditions described in Section 2.

and the method was limited in its ability to evaluate differences between preparations.

The present work describes the optimization of the CE conditions and the use of this method to evaluate the reproducibility of the synthesis and the effect of reaction variables (time, temperature and base) on the composition pattern of the modified CD.

2. Experimental

2.1. Materials

All Chemicals used were of at least analytical-reagent-grade purity. Benzoic acid and tris(hydroxymethyl)aminomethane (Tris) were obtained from Sigma Chemical Co. (St. Louis, MO). The SBE-β-CD preparations analyzed were synthesized previously in these laboratories according to the general procedure of Stella and Rajewski [4].

2.2. Equipment

All CE analyses except temperature studies were performed on the modular electropherograph previously described [13]. The system consisted of a Spellman Model CZE 1000R high voltage power supply (Plainview, NY), an ISCO Model CV4 UV–Vis detector (Lincoln, NE) and a Plexiglas enclosure with an interlock system to protect the operator from electrical discharge. The column was a PolyMicro Technologies Inc. 365 μm o.d., 50 μm i.d. and 60 cm long uncoated capillary (Phoenix, AZ) with an on-column detection window positioned 30 cm from the injection end of the capillary. The detection window was prepared by burning the polyimide coating and then wiping off the exposed fused silica with acetone. Pressure injections were preformed with high purity nitrogen gas actuated with a Mac 200 series pneumatic valve (Wixom, MI) with time actuation controlled by a Potter and Brumfield CNT series electronic relay (Princeton, IN). Data acquisition was performed on a PC compatible using Gilson 712 HPLC software.

The CE analysis with temperature control was performed on a P/ACE 2210 electrophoresis system (Beckman) using a 37 cm (30 cm effective length), 365 μm o.d. and 50 μm i.d. uncoated capillary.

2.3. Preparation of solutions

All solutions were prepared in freshly double-distilled water and filtered through a 0.45 μm nylon membrane before injection. Sodium hydroxide (0.1 N) was prepared and stored in a glass bottle.

2.3.1. Running buffer preparation

Benzoic acid (3.0 mmol, 366 mg) was suspended in 80 ml of double-distilled water, small portions of 0.1 M Tris base were added while monitoring the pH until dissolution of the solid was complete. The pH was then adjusted to 6.0 with 0.1 M Tris base, the solution transferred to a 100 ml volumetric flask and made up to volume with water. The solution was filtered through a 0.45 μm filter to remove particulates and sonicated for 5 min to complete the degassing.

2.3.2. Sample preparation

The samples were prepared by dissolving the corresponding amount of solid in the running buffer and filtering the solution through a 0.45 μm filter prior to injection.

2.4. CE methods

2.4.1. Capillary conditioning

New columns were conditioned by filling the capillary with 0.1 N sodium hydroxide, soaking for 20 min, and rinsing with 5 volume exchanges (VEs) of water.

2.4.2. Conditions for the modular electropherograph

The capillary was treated with the following wash sequence before each injection: 7 VEs water, 5 VEs 0.01 N sodium hydroxide, 3 VEs water, 5 VEs running buffer. The column was equilibrated for 10 min with the voltage applied. The running buffer was 30 mM benzoic acid–Tris pH 6.0. The field strength was 417 V cm^{-1} and the current was 25–30 μA. The separations were conducted

at room temperature. The buffer at both ends of the capillary was replaced every three runs. Samples were introduced by pressure injections: 1 s at 5 psi.

2.4.3. Conditions for P/ACE 2210 electropherograph

In the P/ACE 2210 the washing sequence prior to the injection was as follows at high pressure, (20 psi): 2 min 0.1 N sodium hydroxide, 0.5 min water and 2 min running buffer. The buffer at both ends of the capillary was replaced every three runs. Samples were introduced by pressure injections: 5 s at 0.5 psi.

2.4.4. Reproducibility studies

The experiments to establish the conditions for a reproducible analysis were conducted on the modular electropherograph in triplicate under the general conditions described above. The SBE-β-CD concentration was 16 mg ml^{-1} and the detection was at 254 nm. The peak areas for the respective substitution bands were averaged and the relative standard deviation (RSD) for the respective peaks was calculated as RSD = standard deviation × 100/average peak area percent. The results are shown in Fig. 3. Analysis of the data by percent total peak area and peak height normalization (i.e. percent total peak area/migration time and percent total peak height/migration time) did not improve results.

2.4.5. pH studies

The effect of pH on resolution was studied on the modular electropherograph. The running buffer was prepared following the general procedure described above except the pH was adjusted to 7.5 and 9.0 before dilution. The SBE-β-CD sample concentration was 8 mg ml^{-1} in buffer pH 6.0. The detection was at 230 nm. Results are shown in Fig. 5.

2.4.6. Temperature studies

The effect of temperature on resolution was conducted on the P/ACE 2210 system. The conditions were as described above. The capillary was equilibrated for at least 1 h at the desired temperature prior to the injection with detection at 230 nm. The sample concentration was 8 mg ml^{-1}. The CE profiles obtained are shown in Fig. 6.

2.4.7. Field strength studies

The effect of field strength on the separation was determined in the P/ACE 2210 CE system at 50°C and at two different applied voltages (20 and 30 kV). The detection was at 230 nm and the sample concentration was 10 mg ml^{-1}. The results are presented in Fig. 7.

2.4.8. Effect of the counter ion of the running buffer

Experiments to explore the effect of cation species in the running buffer on the separations were performed on the modular electropherograph at room temperature and under the general conditions described. The running buffer was prepared following the general procedure but using 0.1 N sodium hydroxide instead of Tris base to adjust the pH. The sample concentration was 11 mg ml^{-1} and detection was at 254 nm. Results are shown in Fig. 8.

2.4.9. Synthetic lot evaluation

SBE-β-CD lots synthesized previously in these laboratories were analyzed in the modular electropherograph according to the conditions described in Section 2.4. The sample concentration range was 14–18 mg ml^{-1}. The detection was at 254 nm. The parameter used for comparison was percent total peak area which represents the percent contribution of each peak to the total peak area of a given sample. The values for the respective substitution bands were averaged and the SD and RSD for the respective peaks were calculated as before. The results are presented in Figs. 9 and 10.

3. Results and discussion

Historically, the SBE-β-CD preparations have been characterized for the molar DS, as determined by NMR or elemental analysis, the molar ratio of sodium to sulphur, and the residual β-CD content. However, these data did not demonstrate any appreciable differences in the material prepared in different batches or under different reaction conditions.

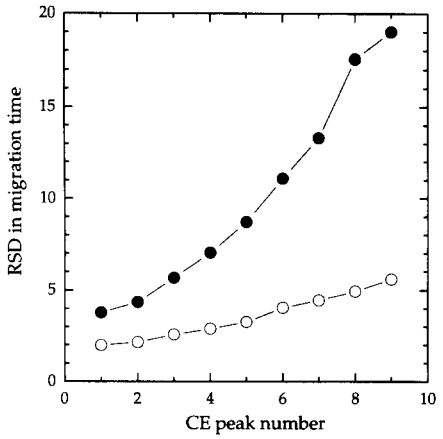

Fig. 3. Reproducibility of the migration times for three consecutive injections of a SBE4-β-CD mixture: (○) with washing sequence; (●) without washing sequence.

The preliminary CE method [13] provided a way to fingerprint the composition pattern, but lacked the reproducibility to explore variations in the preparations of the SBE-β-CDs. Therefore, it was necessary to develop a method which resolves these isomers based on the DS and allows for relative quantitation of the respective bands.

3.1. Reproducibility of the CE method

The previously described method [13] exhibited a lack of reproducible migration times as shown in Fig. 3 for three consecutive injections of the same sample when no washing sequence was used between injections. A plot of the RSD in the observed migration time for the nine peaks detected in the electropherogram versus the CE elution order (CE peak number) of every substitution level shows a steady increase from 4% to greater then 18% when a washing sequence was not used.

The development of a reproducible CE separation was dependent on the utilization of a washing sequence between injections. Fig. 3 shows the improvement in the reproducibility of the migration times when the wash sequence was utilized. The use of capillary preconditioning resulted in migration times with RSDs < 5% for all peaks analyzed. The wash sequence removed residual interferences from the column, thus providing a consistent conditioning of the capillary. This was found to be essential for the reproducibility of the method.

The reproducibility of the quantitation of the nine peaks detected in the CE analysis can be evaluated by comparing the RSD in the area percents or height percents determined for each peak in the electropherogram. Fig. 4 shows the RSDs of peak area and peak height percent obtained for a set of three injections with preconditioning of the capillary before every run. The first seven peaks in the analysis show RSDs of 2–5% which are considered good for a manual injection system without temperature control. The variability of the last two peaks is most likely due to their broadness. The results show that similar precision was obtained for both peak area and peak height. Additionally, studies in these laboratories suggest that further improvements in the reproducibility of migration times and quantitation can be obtained with an automated system and with improvements in the resolution of the separation (data not shown).

3.2. Improvements in the CE method

The current CE method is satisfactory for the analysis of the SBE4-β-CD. However, the late-eluting peaks with DS > 7 exhibit more variability

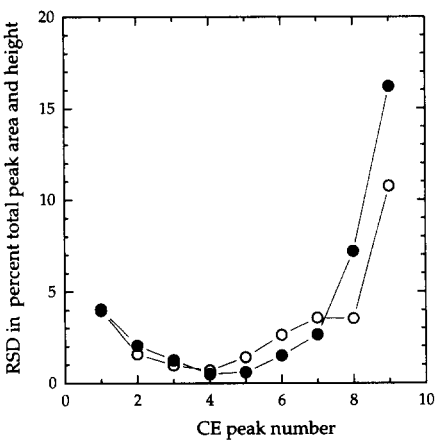

Fig. 4. Reproducibility of the total percent peak area and total percent peak height for the SBE4-β-CD mixture ($n = 3$ injections); (○) percent total peak area; (●) percent total peak height.

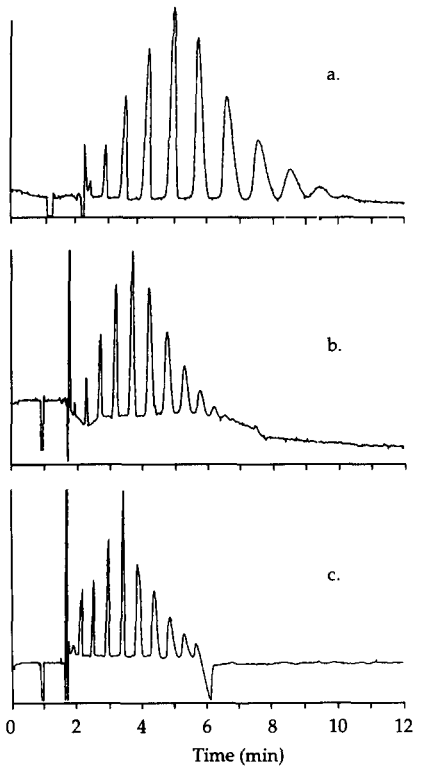

Fig. 5. Electropherograms of a SBE4-β-CD sample obtained with 30 mM benzoic acid–Tris buffer at three different pHs: (a) 6.0; (b) 7.5; (c) 9.0.

in quantitation than desirable (RSD ≈ 7–17%). This is particularly important for the analysis of SBE-CDs with average DSs of 7 or higher. To further improve the peak area precision, an attempt was made to look at the factors that affect the electroosmotic flow (EOF), since this is a fundamental constituent of the CE operation. Changes in the buffer pH, temperature of the separation, voltage applied and buffer cation were explored in order to modify the EOF and improve the peak shape of the late-eluting components of a SBE-β-CD mixture. The results are shown in Figs. 5–8.

3.2.1. Effect of pH

Practically, the most dramatic changes in EOF can be made simply by altering the pH of the running buffer, particularly in the pH region 5–8 [14]. An increase in the pH from 6.0 to 9.0 would increase the ionization of the silanol groups in the capillary wall, changing the zeta potential, and thus increasing the rate of the EOF. Consequently, a decrease in the migration time of all the components of the mixture was observed. A sharpening of all the peaks was obtained without loss of resolution, as shown in Fig. 5. At the highest pH a negative system peak interferes with the detection of the SBE-β-CD with higher DS.

3.2.2. Effect of temperature

It is known that an increase in the temperature reduces buffer viscosity, thereby increasing the EOF [14]. A change from 25°C to 50°C in the temperature of the separation (see Fig. 6) shortened the analysis time to approximately one-half with an improvement in the peak shape of all the components of the mixture without loss of resolution. Temperature proved to be a useful and practical variable to modify since it is easily controlled instrumentally.

3.2.3. Effect of field strength

Fig. 7 shows the effect of the field strength on the separation of the SBE-β-CD mixture. When

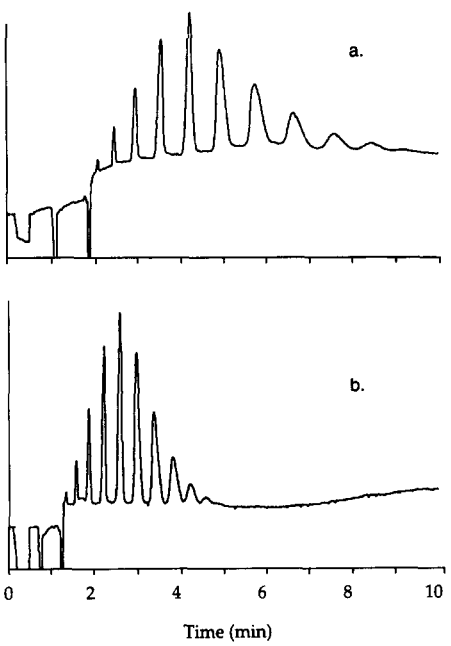

Fig. 6. Electropherograms of a SBE4-β-CD sample obtained with 30 mM benzoic acid–Tris buffer at two different temperatures: (a) 25°C; (b) 50°C.

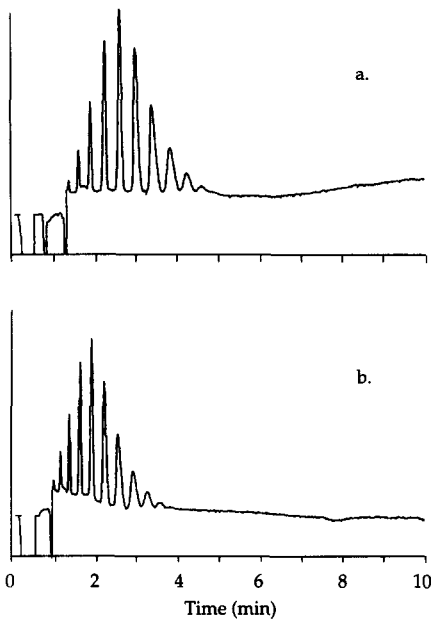

Fig. 7. Electropherograms of a SBE4-β-CD sample obtained with 30 mM benzoic acid–Tris buffer at two different field strengths: (a) 541 V cm^{-1}; (b) 811 V cm^{-1}.

the voltage applied across the capillary was increased (increasing the field strength), a proportional increase in the EOF occurred due to an increase in the mobility of the cations. A shorter total analysis time was observed. Field strength is also a useful variable since it is instrumentally controlled.

3.2.4. *Effect of the cation*

The use of sodium instead of Tris as a counter ion in the running buffer also affected the separation, decreasing the migration time of all the components as can be observed in Fig. 8. The observed current reading was double that with the buffer having Tris as the counterion. This effect can probably be attributed to changes in the conductivity of the solution. An increase in the current may generate a temperature gradient due to joule heating with a resulting change in the viscosity of the buffer. A loss of resolution of the mixture and a noisy baseline were observed with a consequent loss of sensitivity (only eight peaks were detected).

3.3. *Utility of the CE analysis*

Once the conditions for a reproducible analysis of SBE4-β-CDs were established, the CE method was used to evaluate the synthetic reproducibility and the effect of changes in the reaction conditions on the pattern of distribution of the mixture of products.

3.3.1. *Batch-to-batch variability*

The CE method was used to obtain the composition pattern of the material generated in 21 different preparations of SBE4-β-CD (Fig. 9) made at different synthetic scales (10–150 g batches) and by three different experimentalists. The results show close agreement in the distribution patterns over all 21 preparations as shown by the SD at each level of substitution. This indicates a consistent preparation of material no matter the scale of the reaction or the experimentalist.

3.3.2. *Reaction variables analysis*

Fig. 10 shows the use of the CE method to evaluate the effect of reaction variables on the

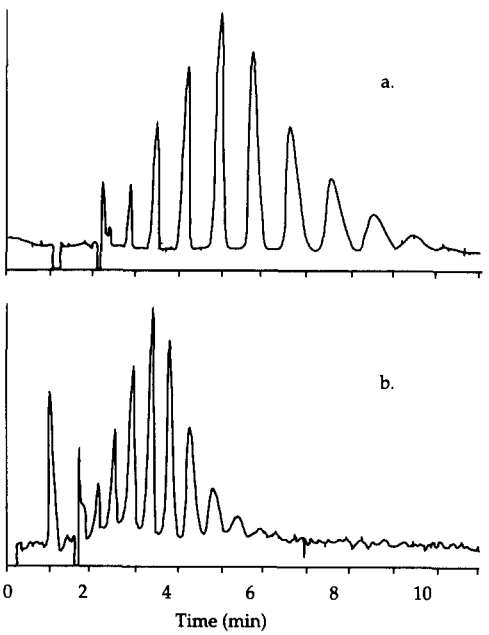

Fig. 8. Electropherograms of a SBE4-β-CD sample obtained with two different running buffers: (a) 30 mM benzoic acid–Tris pH 6.0; (b) 30 mM benzoic acid–NaOH pH 6.0.

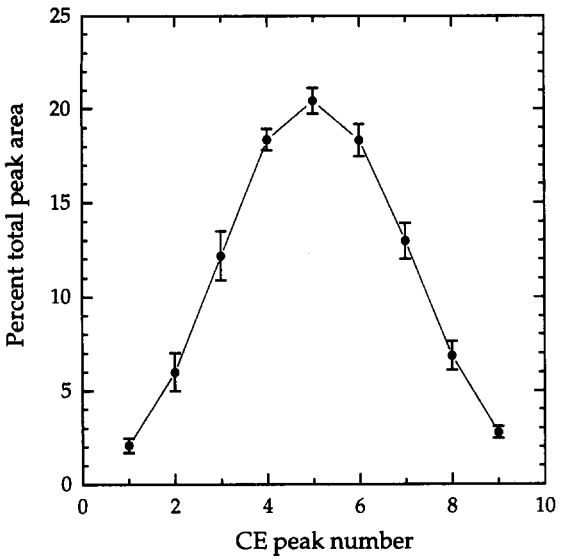

Fig. 9. Reproducibility of the SBE4-β-CD preparations. Each point is an average of 21 different preparations at five different scales (10–150 g) and by three different experimentalists.

composition pattern. The variables modified systematically were time of the reaction (1–24 h), temperature (30, 50 and 70°C) and base equivalents (sodium hydroxide, 6 to 14 equivalents) added. Neither temperature nor reaction time appeared to affect the distribution of the product mixture (Fig. 10a and Fig. 10b). However, changes in the amount of base caused a change in the composition pattern (Fig. 10c). The use of 7–12 equivalents of base produced a fairly narrow distribution pattern, but a drop to 6 equivalents of base caused a shift in the distribution from the lower level of substitution to a higher population of intermediate substituted materials. A change in the distribution pattern was also observed with the use of 14 and 21 equivalents of base. A high base concentration produced a broader distribution of materials with a shift of the maximum to a slightly lower level of substitution. This CE method is the first analytical technique which has provided a measure of the difference between various preparations. The method has been used with equivalent success for other sulfoalkyl CD derivatives.

4. Conclusions

This study clearly shows that CE is a useful, rugged, and reproducible analytical technique for the determination of the pattern of composition for a mixture of mono- to deca-substituted anionically charged CDs, the SBE-β-Cds. To provide

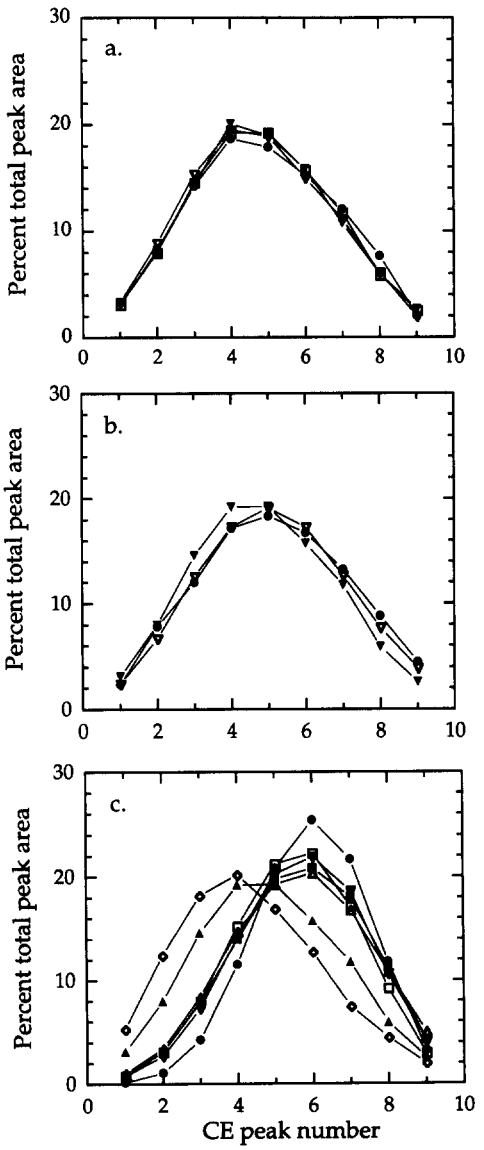

Fig. 10. Effect of changes in the reaction variables on the pattern of distribution of a SBE4-β-CD. (a) Reaction time (h): (●) 1; (▽) 6; (▼) 12; (□) 24. (b) Reaction temperature (°C): (●) 30; (▽) 50; (▼) 70. (c) Equivalents of base added: (●) 6; (▼) 7; (□) 8; (■) 10; (△) 12; (▲) 14; (◇) 21.

consistent analysis a wash sequence between injections was found to be essential. Although the initial CE method provided a reproducible analysis method for the determination of the fingerprint of the pattern of composition, the separation can be improved by changing the pH, temperature and/or field strength for the separation. Studies continue to determine the best combination of these variables.

Acknowledgements

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References

[1] K. Uekama, F. Hirayama and T. Irie, Pharmaceutical uses of cyclodextrin derivatives, in M. Szicher (Ed.), High Performance Biomaterials, Technomic Publishers, Lancaster, PA, 1991, Chapter 45, pp. 789–806.

[2] O. Bekers, E.V. Uijtendaal, J.H. Beijnen, A. Bult and W.J.M. Underberg, Drug Dev. Ind. Pharm., 17 (1991) 1503–1549.

[3] R.A. Rajewski, Development and Evaluation of the Usefulness and Parenteral Safety of Modified Cyclodextrins, Ph.D. Dissertation, University of Kansas, Lawrence, KS, 1990.

[4] V.J. Stella and R.A. Rajewski, Derivatives of Cyclodextrins Exhibiting Enhanced Aqueous Solubility and Pharmaceutical Uses Thereof, U.S. Pat. 5, 134, 127, 1992.

[5] R.A. Rajewski, G. Traiger, J. Bresnahan, P. Jaberaboansari, V.J. Stella and D.O. Thompson, J. Pharm. Sci., 85 (1995) 927–932.

[6] M.D. Johnson, B.L. Hoesterey and B.D. Anderson, J. Pharm. Sci., 83 (1994) 1142–1146.

[7] V.J. Stella, H.Y. Lee and D.O. Thompson, Int. J. Pharm., 120 (1995) 189–195.

[8] V.J. Stella, H.Y. Lee and D.O. Thompson, Int. J. Pharm., 120 (1995) 197–204.

[9] T. Järvinen, K. Järvinen, D.O. Thompson and V.J. Stella, Curr. Eye Res., 13 (1994) 897–905.

[10] T. Järvinen, K. Järvinen, A.K. Urtti, D.O. Thompson and V.J. Stella, J. Ocul. Pharmacol., 11 (1994) 95–106.

[11] B.A. Gorecka, D.S. Bindra, Y.D. Sanzgiri and V.J. Stella, Int. J. Pharm., 125 (1995) 55–61.

[12] T. Järvinen, K. Järvinen, N. Schwarting and V.J. Stella, J. Pharm. Sci., 84 (1995) 295–299..

[13] R.J. Tait, D.J. Skanchy, D.O. Thompson, N.C. Chetwyn, D.A. Dunshee, R.A. Rajewski, V.J. Stella and J.F. Stobaugh, J. Pharm. Biomed. Anal., 10 (1992) 615–622.

[14] D.N. Heiger, in High Performance Capillary Electrophoresis—An Introduction, Hewlett-Packard, France, 1992.