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Capillary electrophoretic separation of the enantiomers of organophosphates with a phosphorus stereogenic center using the sodium salt of octakis(2,3-diacetyl-6-sulfo)-γ-cyclodextrin as resolving agent

Wenhong Zhu, Feiyue Wu, Frank M. Raushel, Gyula Vigh*

Department of Chemistry, Texas A and M University, College Station, Texas, TX 77842-3012, USA

Abstract

The sodium salt of the single-isomer, chiral resolving agent, octakis(2,3-diacetyl-6-sulfo)-γ-cyclodextrin (ODAS-γCD) has been used for the capillary electrophoretic separation of the enantiomers of alkylarylphosphates which carry a phosphorus-based stereogenic center. The effective mobilities and separation selectivities were measured at different ODAS-γCD and methanol concentrations to find the conditions under which the minor enantiomers could be adequately quantitated in samples obtained by chemical resolution of the racemic mixtures. This work extends the utility of ODAS-γCD to a hitherto unexplored field, the capillary electrophoretic separation of the enantiomers of organophosphorus compounds.
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Keywords: Enantiomer seperation; Background electrolyte composition; Organophosphorous compounds; Phosphates; Cyclodextrins

1. Introduction

As documented by a flurry of excellent, recent monographs, one of the most rapidly growing and most successful application areas of capillary electrophoresis (CE) in the analysis of small molecules is the separation of enantiomers using both noncharged and charged cyclodextrins (CDs) [1–4]. Most of the charged CDs used in the early work were complex mixtures of isomers which differed in their degree and loci of substitution [2]. Recently, single-isomer anionic [5–7] and cationic [8,9] β-cyclodextrins and anionic γ-cyclodextrins [10,11] were developed to eliminate the compositional variations that are typical of the randomly substituted charged CDs. The single-isomer sulfated CDs were successfully used in aqueous [5–7,10–13], hydroorganic [14] and nonaqueous [15–17] background electrolytes (BGEs) to separate the enantiomers of strong and weak acid, strong and weak base, and amphiprotic analytes that had carbon-based stereogenic centers. There is much less information available about the CE separation of enantiomers with stereogenic centers other than carbon. Valenzuela et al. reported the CE separation of different sulfonium ions [18], and in a follow-up publication demonstrated the separation of the enantiomers of several sulfonium ions using both neutral and randomly sulfated β-CD [19].

*Corresponding author. Tel.: +1-979-845-2456; fax: +1-979-845-4719.

E-mail address: vigh@mail.chem.tamu.edu (Gy. Vigh).

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The organophosphate triesters constitute one of the largest classes of agricultural insecticides used in the world today. They are highly toxic because of their inherent ability to covalently inactivate the enzyme acetylcholine esterase, an enzyme critical for nerve function [20]. For chiral organophosphates the degree of toxicity is dependent on the stereochemistry at the phosphorus center. The synthesis of chiral organophosphates at the phosphorus center is tedious since phosphotriesters are unknown as natural products. However, we are currently developing enzymatic methods for the rapid kinetic resolution of racemic mixtures of organophosphate triesters [21].

2. Experimantal

2.1. Reagents and chemicals

The structures of the compounds studied are shown in Fig. 1. The racemic phosphotriesters **1**–**3** were synthesized from *p*-nitrophenyl phosphorodichloridate and the corresponding alcohol by the general method of Steurbaut et al. [22]. The individual enantiomers of phosphotriesters **1**–**3** were synthesized using a modification of previously reported methods [21,23]. For compound **1**, a mixture of absolute ethanol and triethylamine in diethyl ether was added dropwise to a solution of *p*-nitrophenyl phosphorodichloridate under argon. After the mixture was refluxed for 3 h, a solution of L-proline methyl ester and triethylamine was added, and then refluxed overnight. The mixture was extracted with ether, and the combined etheral phases were washed with dilute sodium hydrogen carbonate and water, dried over anhydrous magnesium sulfate, filtered and concentrated to give a pale yellow oil. The diastereomers were separated by column chromatography on silica gel using ethyl acetate–hexane (15:85) as the eluent. Each diastereomer was refluxed with 1 *M* H_2SO_4–methanol for 4 h. After 10 ml of water was added and the excess methanol was removed, the mixture was extracted with ether. The combined etheral layers were washed with dilute sodium hydrogen carbonate and water, dried over anhydrous magnesium sulfate, filtered and concentrated to provide the enantiomeric (R_P)- and (S_P)-ethyl methyl *p*-nitrophenyl phosphate (**1**). The other two pairs of enantiomers (**2,3**) were also synthesized according to the method described above. For compound **2**, isopropanol was first reacted with *p*-nitrophenyl phosphoramidate. The two diastereomers were separated by column chromatography on silica gel. Each diastereomer of isopropyl-*p*-nitrophenyl phosphoramidate was refluxed with 1 *M* H_2SO_4–methanol to provide the enantiomeric (R_P)- and (S_P)-isopropylmethyl-*p*-nitrophenyl phosphate (**2**). For compound **3**, each diastereomer of isopropyl-*p*-nitrophenyl phosphoramidate was refluxed with 1 *M* H_2SO_4–ethanol to provide the enantiomeric (R_P)- and (S_P)-ethyl-isopropyl-*p*-nitrophenyl phosphate (**3**).

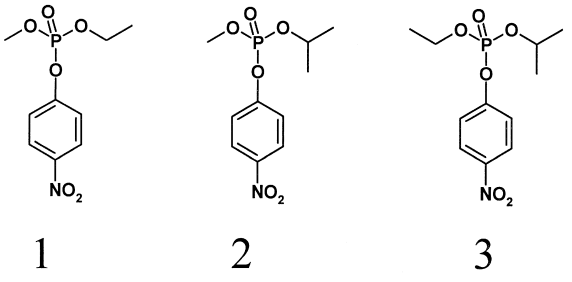

Fig. 1. Structure of the compounds studied.

All other chemicals were obtained from Aldrich (Milwaukee, WI, USA), except the sodium salt of octakis(2,3-diacetyl-6-sulfo)-γ-cyclodextrin (ODAS-γCD), which was synthesized and analytically characterized in our laboratory [10] and is now commercially available (J&W Scientific, Folsom, CA, USA).

2.2. Electrophoretic separations using ODAS -γCD

All CE separations were carried out on a P/ACE 2100 CE instrument (Beckman Instruments, Fullerton, CA, USA). The electropherograms were detected at 214 nm. The capillary cartridge coolant was thermostated at 20°C. The separations were carried out in 25 μm I.D. bare fused-silica capillaries (Polymicro Technologies, Phoenix, AZ, USA) with effective length 19.5 cm and total length 26.5 cm. Power dissipation was maintained between 500 and 700 mW/m by varying the applied potential between 10 kV and 20 kV. All samples were injected by 4 p.s.i. nitrogen (1 p.s.i.=6894.76 Pa) for 1 s.

Since the analytes were stable in the mid-pH range

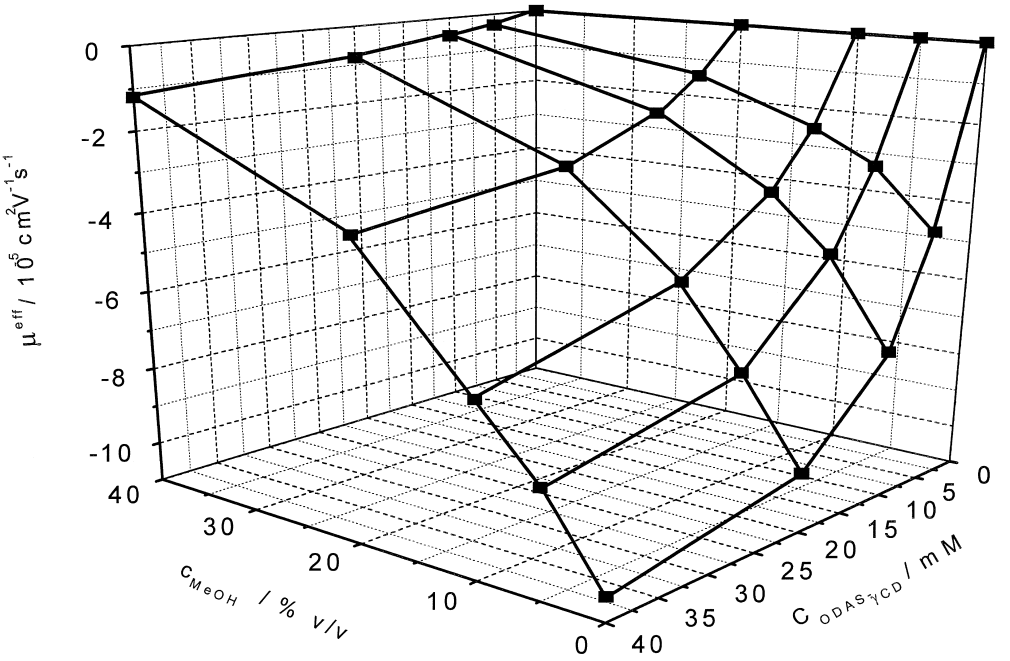

Fig. 2. Effective mobilities for the R_p enantiomer of **1** as a function of the ODAS-γCD and methanol concentration in the BGE. For conditions, see Experimental.

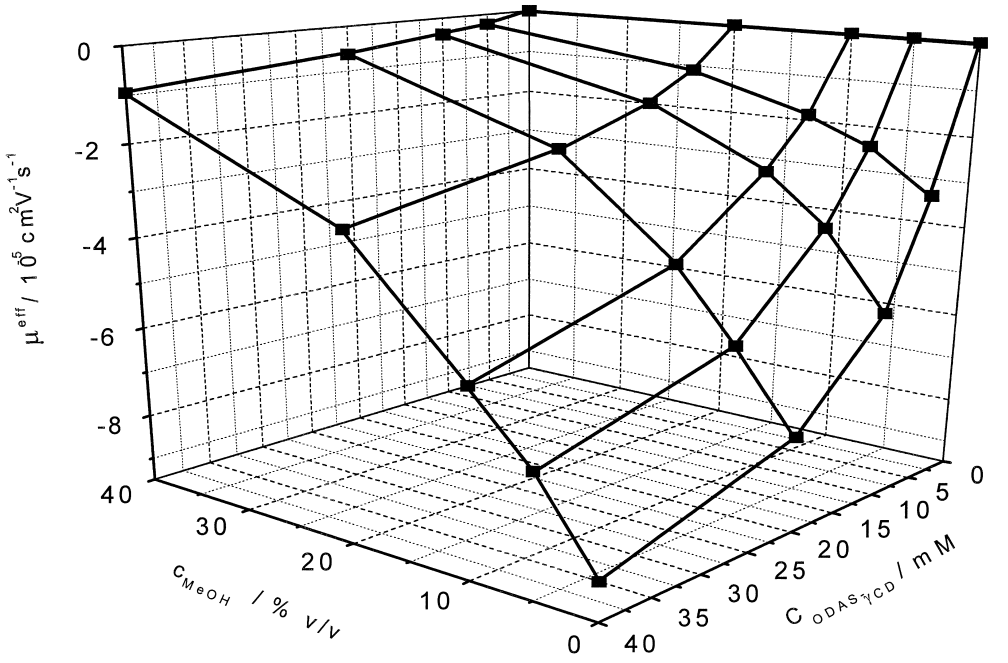

Fig. 3. Effective mobilities for the R_p enantiomer of **2** as a function of the ODAS-γCD and methanol concentration in the BGE. For conditions, see Experimental.

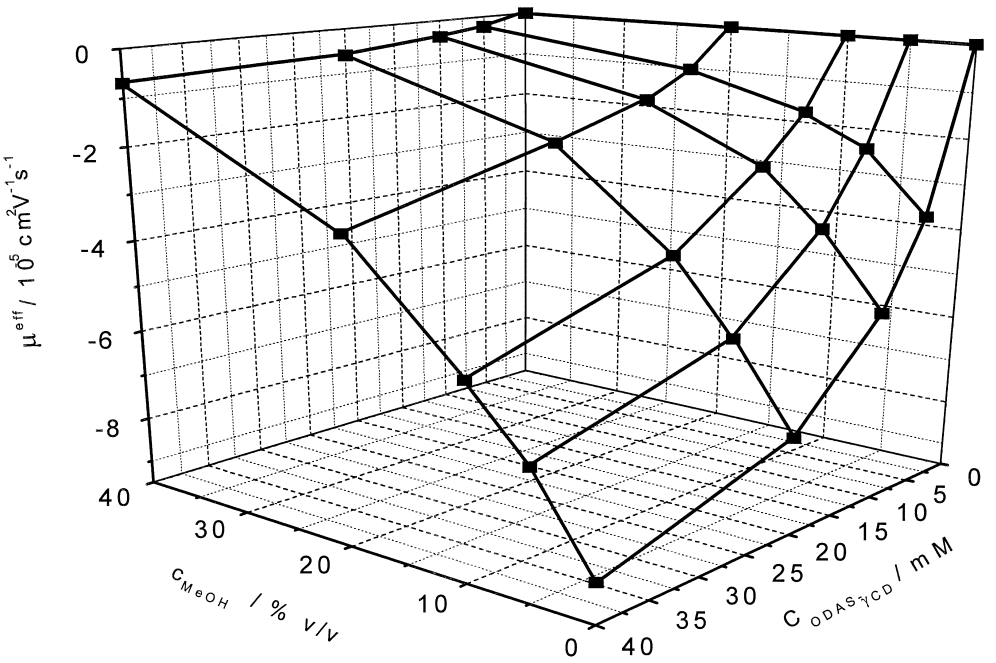

Fig. 4. Effective mobilities for the R_p enantiomer of **3** as a function of the ODAS-γCD and methanol concentration in the BGE. For conditions, see Experimental.

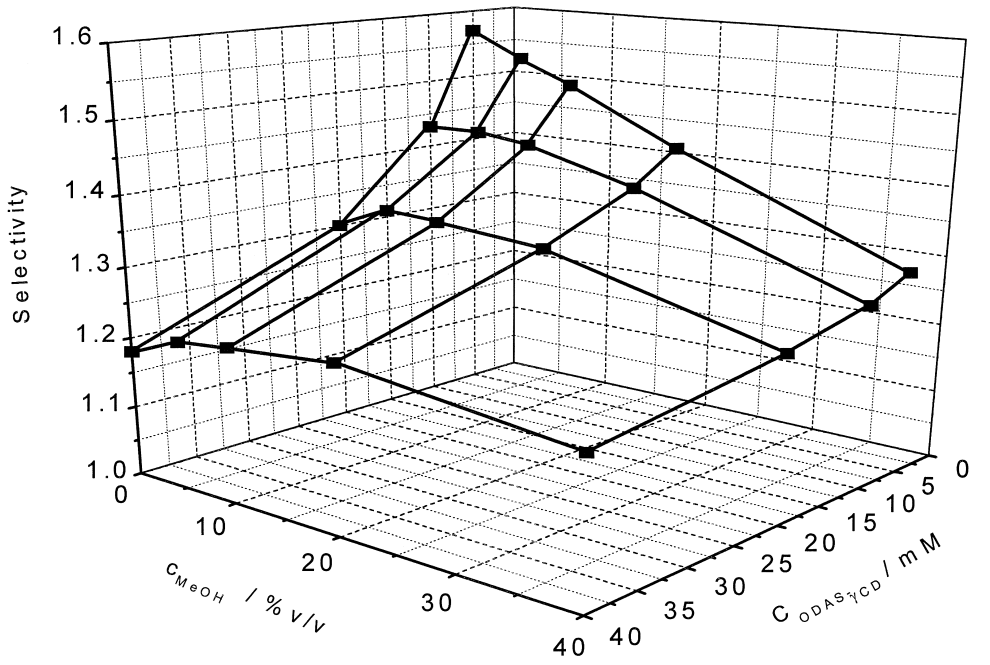

Fig. 5. Separation selectivities for **1** as a function of the ODAS-γCD and methanol concentration in the BGE. For conditions, see Experimental.

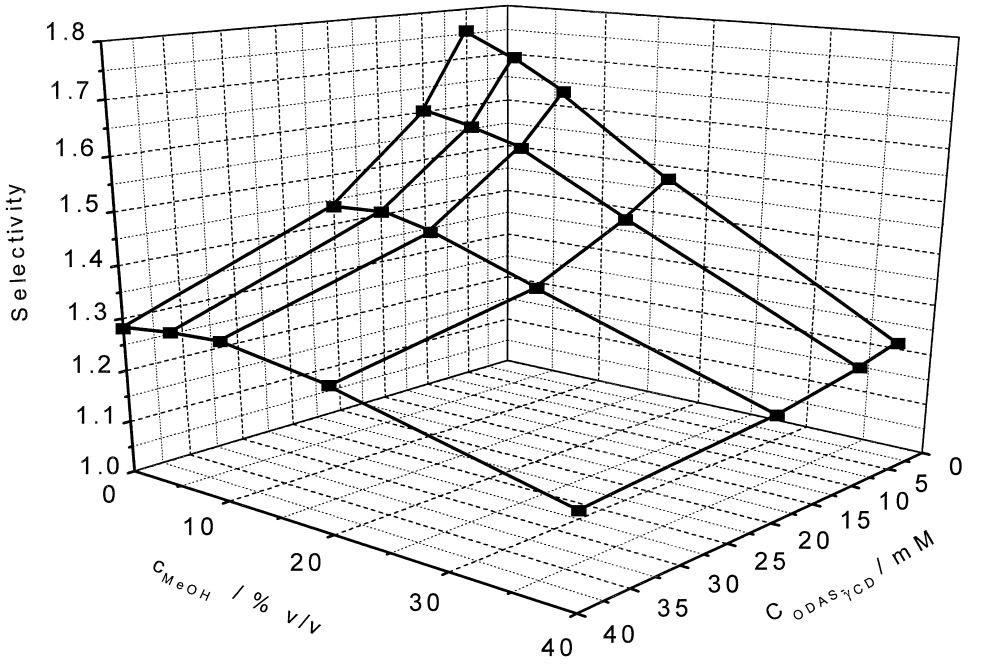

Fig. 6. Separation selectivities for **2** as a function of the ODAS-γCD and methanol concentration in the BE. For conditions, see Experimental.

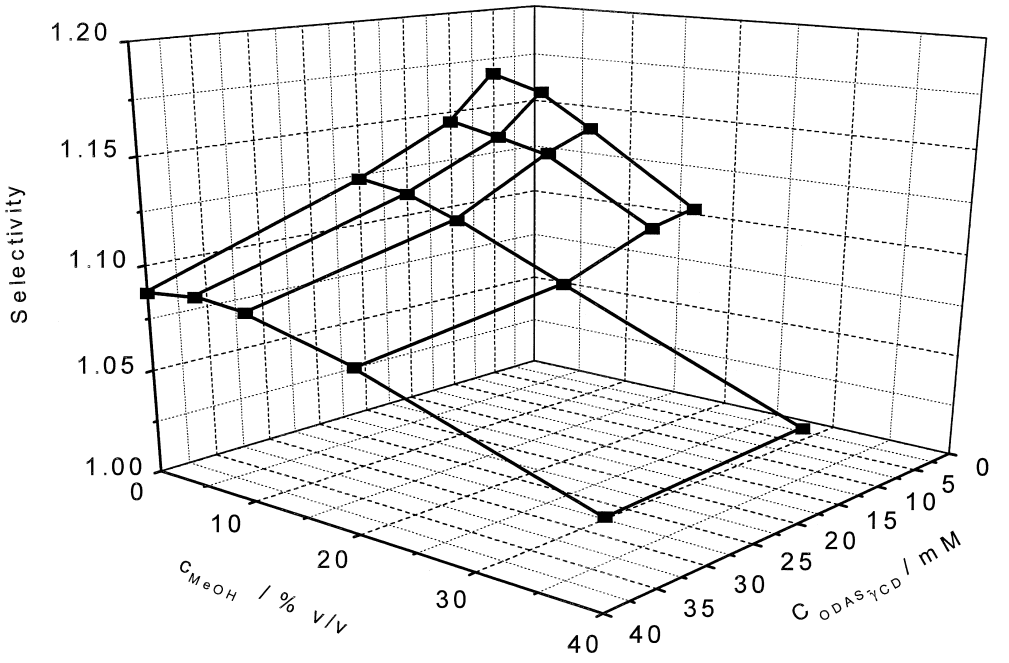

Fig. 7. Separation selectivities for **3** as a function of the ODAS-γCD and methanol concentration in the BE. For conditions, see Experimental.

but had limited aqueous solubility, aqueous methanol stock BGEs in the 0 to 40% (v/v) range were prepared by adding 25 mmol of 4-morpholinoethanesulfonic acid (MES), 12.5 mmol of sodium hydroxide and the calculated volume of methanol to a 1-l volumetric flask and filling the flask to the mark with deionized water obtained from a Milli-Q unit (Millipore, Walton, MA, USA). The 0, 5, 10, 20 and 40 m*M* ODAS-γCD BGEs were prepared freshly, immediately prior to use, by weighing out the required amounts of the sodium salt of ODAS-γCD into 25 ml volumetric flasks and bringing the volumes to mark with the respective aqueous methanolic stock BGE solution.

The samples were dissolved in the respective BGEs at an approximate concentration of 0.5 m*M*, immediately prior to analysis. The external electroosmotic flow (EOF) marker method [24] was used to ascertain that nitromethane did not complex appreciably with ODAS-γCD and could be used as primary electroosmotic flow marker. The effective mobilities of the enantiomers (μ_R^{eff} and μ_S^{eff}) were obtained as:

$$\mu_R^{eff} = \mu_R^{obs} - \mu_{EO}$$

Separation selectivity, α, was calculated as:

$$\alpha = \mu_R^{eff}/\mu_S^{eff}$$

3. Results and discussion

The effective mobilities of the R_P enantiomers of compounds **1**, **2** and **3** are shown in Figs. 2–4 as a function of the methanol and ODAS-γCD concentrations of the BGEs. At constant methanol concentration, the anionic effective mobilities of the analyte bands increase as the ODAS-γCD concentration is increased. At constant ODAS-γCD concentration, the anionic effective mobilities of the analyte bands decrease as the methanol concentration is increased. At identical ODAS-γCD and methanol concentrations, the anionic effective mobilities of the analyte bands decrease slightly in the **1**>**2**>**3** order as the size of the substituents around the phosphorus stereogenic center is increased.

The separation selectivities are shown in Figs. 5–7 for analytes **1**, **2** and **3**. In agreement with the predictions of the charged resolving agent migration model (CHARM model) for the CE separation of neutral enantiomers, separation selectivity decreases for all three analytes as the concentration of ODAS-γCD is increased [25]. This migration behavior is similar to what was found with heptakis(2,3-dimethyl-6-sulfo)-β-cyclodextrin as resolving agent for non-charged chiral analytes that contain carbon-based stereogenic centers [13]. At low (but non-zero) ODAS-γCD concentrations, separation selectivity decreases strongly and monotonously as the methanol concentration of the BGE is increased. This can be rationalized by noting that only a small fraction of

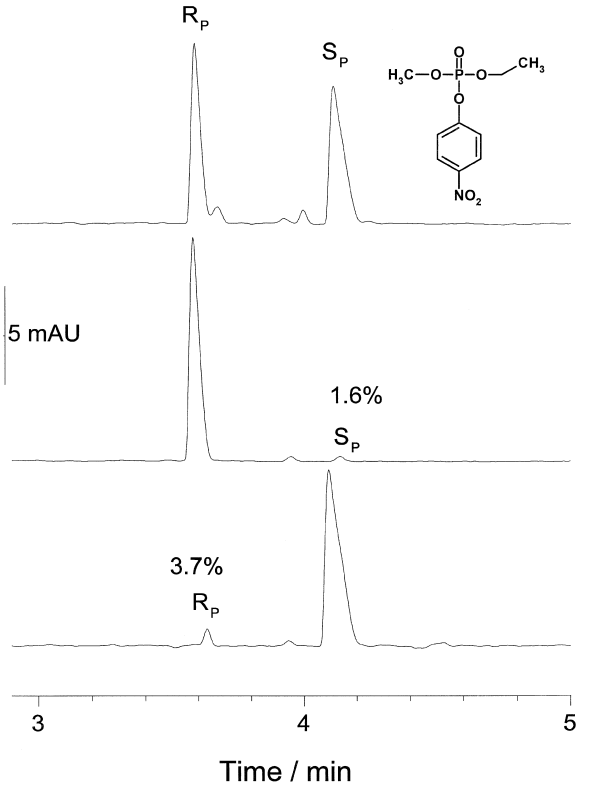

Fig. 8. Typical electropherograms of analyte **1** in the 10%, v/v, methanol, 20 m*M* ODAS-γCD BE. Top electropherogram: racemic mixture. Middle electropherogram: sample enriched in the R_P enantiomer. Bottom electropherogram: sample enriched in the S_P enantiomer. The numbers next to the peaks indicate the % of the minor enantiomer. Other conditions: see Experimental.

the enantiomers is present in the complexed form, even in pure water, thus methanol can compete for ODAS-γCD under very favorable conditions. At higher ODAS-γCD concentrations a greater portion of the enantiomers is complexed, so the same methanol concentration cannot provide as effective a competition as at lower ODAS-γCD concentrations. At identical methanol and ODAS-γCD concentrations (e.g., at 40%, v/v, and 40 m*M*), separation selectivity decreases in the **2**>**1**>**3** order, in accordance with the relative sizes of the alkyl substituents. Typical electropherograms of the racemic mixtures and chemically resolved samples of **1**, **2** and **3** are shown in Figs. 8–10. The separations are very fast (migration times less than 5 min), and the peak resolutions are large (R_s>2) allowing quantitation of

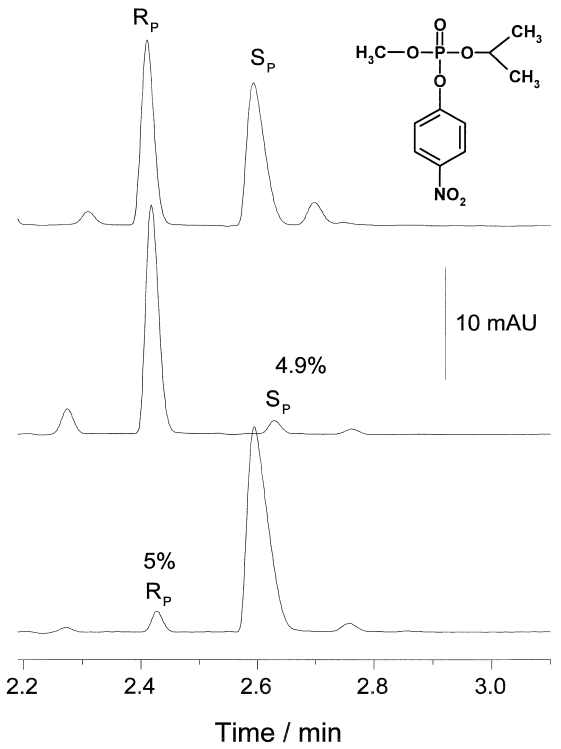

Fig. 9. Typical electropherograms of analyte **2** in the 10% v/v methanol, 7 m*M* ODAS-γCD BE. Top electropherogram: racemic mixture. Middle electropherogram: sample enriched in the R_P enantiomer. Bottom electropherogram: sample enriched in the S_P enantiomer. The numbers next to the peaks indicate the % of the minor enantiomer. Other conditions: see Experimental.

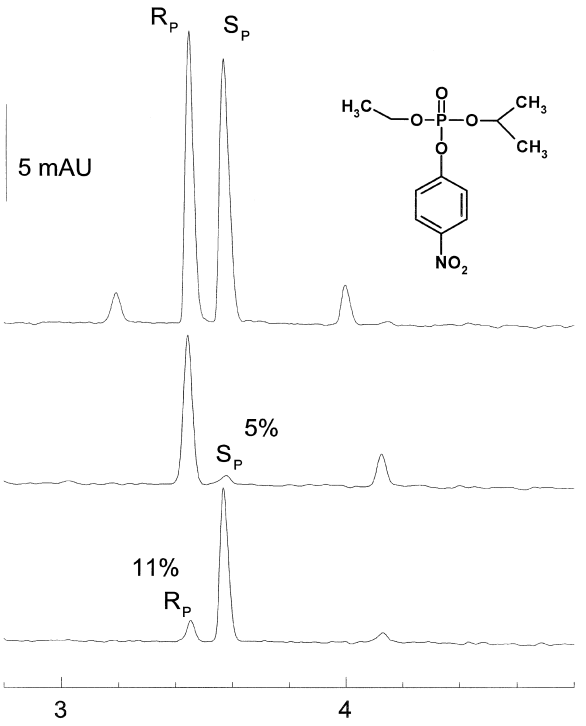

Fig. 10. Typical electropherograms of analyte **3** in the 10% v/v methanol, 20 m*M* ODAS-γCD BE. Top electropherogram: racemic mixture. Middle electropherogram: sample enriched in the R_P enantiomer. Bottom electropherogram: sample enriched in the S_P enantiomer. The numbers next to the peaks indicate the % of the minor enantiomer. Other conditions: see Experimental.

the minor enantiomer and optimization of the chemical resolution reaction [20–23].

4. Conclusions

The sodium salt of octakis(2,3-diacetyl-6-sulfo)-γ-cyclodextrin has been used for the first time CE separation of the enantiomers of organophosphates which contain a phosphorus stereogenic center. The methanol and the ODAS-γCD concentrations of the background electrolyte were varied to study the behavior of both their effective mobilities and separation selectivities. Both the effective mobilities and separation selectivities were found to be in agreement with the predictions of the CHARM model of CE enantiomer separations [25].

Acknowledgements

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