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Allylamine–β-cyclodextrin copolymer A novel chiral selector for capillary electrophoresis

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Abstract

A novel, positively charged, copolymer of allylamine and 2-hydroxy-3-methacryloyl-β-cyclodextrin was synthesized to be used as a chiral selector in capillary electrophoresis. In the copolymer, cyclodextrin molecules are spaced from the backbone though a spacer arm which prevents sterical hindrance of the CD cavity. The self-mobility of the CD polymer in its charged form, opposite to the analytes, is the cause for the enhanced separation factor provided by this selector. Moreover, the positive charged polymer induces a reversal of electroosmotic flow which is beneficial in enantioseparations of acidic compounds as it reduces analysis time and increases peak efficiency. The ability of this copolymer to act as a CE chiral selector in the separation of 2,4-dinitrophenylamino acid enantiomers was investigated in coated and uncoated capillaries and its performance was much better then that of native β-cyclodextrin.

Keywords: Chiral selectors; Background electrolyte composition; Enantiomer separation; Amino acids; Cyclodextrins

1. Introduction

Chiral capillary electrophoresis has recently become a routine method for the analysis of racemic compounds. The success of the technique lies in its versatility, high efficiency and low cost as well as short analysis time. In fact, CE combines features of liquid chromatography with speed and simplicity of electrokinetic techniques and is nowadays considered an important technique, complementary to liquid chromatography and capillary gas chromatography.

Cyclodextrins (CDs) are the most popular additives used in CE as isomer selectors as documented by the number of publications appeared in literature on this topic in the last decade [1–3] and countless compounds of practical interest were separated by these selectors. However, selectivity problems are quite common in chiral CE and an intense research activity is currently devoted to improve the enatioselectivity of the various selectors. The capability of cyclodextrins to discriminate racemic compounds, for instance, can be expanded by introducing uncharged or ionizable groups on the rim of the smaller opening of the cavity. Neutral groups like methyl, hydroxyethyl, hydroxypropyl, carboxymethyl or acetyl functions introduce extra interaction points in the molecule [4–7] as well as ionizable groups as carboxyl [8], phospate [9] or alkylsulfate [10] that are capable of coulombic interactions. Also the problem represented by the poor solubility of cyclodextrins in water can be

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relieved by using polymeric cyclodextrins which have solubility one order of magnitude greater than that of native compounds [11–13]. Our group has recently introduced a new polymeric neutral β-cyclodextrin consisting of an alkyl backbone with pendant β-cyclodextrin units, obtained by radical co-polymerization of vinylpyrolidone and 2-hydroxy-3-methacryloyl β-cyclodextrin [14]. In polymeric cyclodextrins, the access to the cavity and thus the ability to discriminate differences in the sterical structure of the various racemates, may be modified by the introduction of a binding agent on the CD molecule. Therefore, any chemical derivatization of the cavity rim can alter the CD enantioselective capability. This is particularly important for polymeric cyclodextrins in which the access to the cavity can be hindered by the formation of a polymeric network.

The present work reports on the synthesis of a novel polymer containing β-cyclodextrins [poly(allylamine–co - 2 - hydroxy - 3 - methacryloyl - β - cyclodextrin) (PAA-β-CD)], obtained by radical co-polymerization of allylamine with a new cyclodextrin derivative monomer called 2-hydroxy-3-methacryloyl-β-cyclodextrin (Fig. 1). This monomer offers the advantage of being readily polymerizable, allowing introduction of β-CD into a polymer without cross-linking of β-CD. Interesting features of this polymer are: (i) the spacer arm between the polymer backbone and the cyclodextrin which prevents sterical hindrance of the CD cavity and (ii) the positive charge on the backbone which improves the separation of enantiomers of opposite charge. The ability of this copolymer to act as a CE chiral selector was investigated in the separation of 2,4 dinitrophenyl (DNP)-amino acid derivatives in coated and uncoated capillaries.

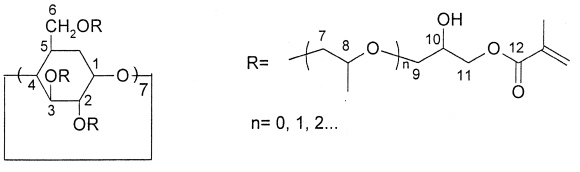

Fig. 1. Structure of 2-hydroxy-3-methacryloyl-β-cyclodextrin monomer, (average number of double bonds per cyclodextrin unit: 2.5).

2. Experimental

2.1. Materials

Allylamine and 2,4-dinitrofluorobenzene (DNFB) were from Aldrich Chemie (Steinheim, Germany). 2-Hydroxy-3-methacryloyl-β-cyclodextrin (BETAW-7MAHP) was a gift from Wacher Chemie. Acrylamide, and *N*,*N*,*N*′,*N*′-tetramethylenediamine (TEMED) were from Bio-Rad Labs (Hercules, CA, USA). Potassium peroxydisulfate was from Janssen Chimica, Beerse, Belgium. β-Cyclodextrin was from Hewlett-Packard (Wilmington, DE, USA). D,L-Amino acids were from Sigma (St. Louis, MO, USA). Fused silica capillaries, of 50 μm in I.D. were purchased from Polymicro Technologies (Phoenix, AZ, USA). Polyacrylamide-coated capillaries were prepared according to [15]. Glacial acetic acid was from Carlo Erba (Milan, Italy). All chemicals and water were of analytical grade.

2.2. Apparatus

CE separations were carried out in a SpectraPhoresis 1000 capillary system (Thermo Separation Products, Freemont, CA, USA). Data collection was peformed on a personal computer using a SW-Phoresis 1000 software. Detection wavelength was 254 nm. Electroosmotic flow and viscosity measurements were done in a Waters Quanta 4000 capillary electrophoresis system (Millipore, Milford, MA, USA) equipped with a laboratory-made device to control the capillary temperature. Data were collected on a personal computer using SW-Phoresis 1000 software.

2.3. Synthesis of PAA-β-CD polymer

The new copolymer of allylamine and 2-hydroxy-3-methacryloyl-β-cyclodextrin (Fig. 2) was obtained by radical copolymerization in water using potassium peroxydisulfate as initiator according to [16]. Some minor modifications were introduced in order to obtain soluble polymers containing high amounts of β-CDs. A typical polymerization reaction was carried out as follows: 4 g of BETAW7MAHP and 1 g of allylamine were dissolved in 10 ml of water in a

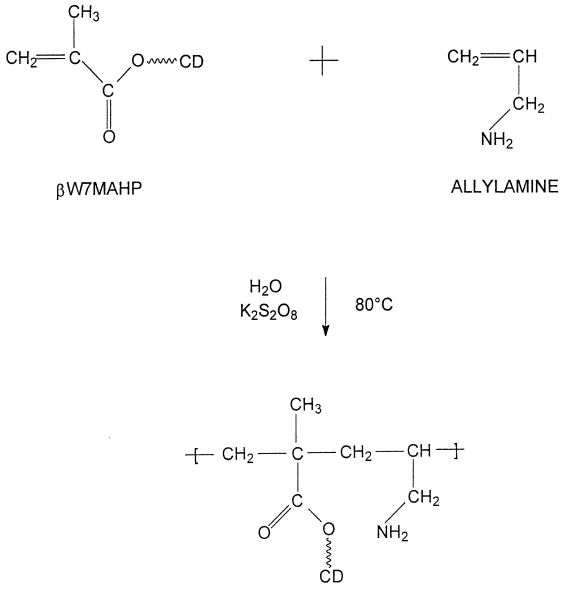

Fig. 2. Scheme of the radical copolymerization of allylamine and 2-hydroxy-3-methacryloyl-β-cyclodextrin monomer.

two-necked, round-bottomed flask equipped with a condenser and a nitrogen inlet. The solution was degassed under vacuum for 30 min, before the introduction of 0.05 g of initiator. The mixture was kept at 50°C for 24 h. After cooling, the solution was dialysed against water during one week, using a dialysis membrane with an M_r cut-off of 12 000 from Sigma, and was recovered by lyophilization. The copolymer used in this work contained 600 μmol of β-CD per g copolymer, as determined by using blue tetrazolium method [17]. Bulk viscosities of the background electrolytes (BGEs) with different concentrations of PAA-β-CD were measured by low-pressure displacement through the capillary with a second injection of 1% dimethyl sulfoxide (DMSO) in water as a marker.

2.4. Synthesis of DNP-amino acids

To a suspension of 0.5 mmol of amino acid in 10 ml of $NaHCO_3$, 11 ml of a solution obtained by dissolving 0.25 ml of 2,4 dinitrofuorobenzene (DNFB) in 49 ml of acetone were added. The reaction mixture was stirred for 2 h at 30–40°C and the progress of the reaction evaluated by RP–HPLC. After the reaction was completed, 0.5 ml of 2 *M* HCl were added and the solvent evaporated under vacuum. The reaction product was purified by semi-preparative RP–HPLC and quantified by UV spectroscopy at 362 nm (ϵ=18 180 l mol^{-1} cm^{-1}) [18].

2.5. Procedures

DNP-Amino acids were separated in a 40 cm long capillary (32 cm to the window) at pH 4.0 in 50 m*M* acetic acid titrated to pH 4.0 with sodium hydroxide, by applying a negative polarity (20 kV) at the inlet. Before each run, the 40 cm long capillary (32 cm to the optical window) was rinsed with the BGE and then filled with the BGE, containing different amounts of the PAA-β-CD copolymer by applying a pressure of $1.03 \cdot 10^{-1}$ MPa for 3 min. The samples (0.2 m*M* water solutions) were loaded electrophoretically by applying 25 V/cm for 1 s.

The viscosities of BGE with different amounts of PAA-β-CD were determined in a Quanta 4000 instrument, equipped with a laboratory-made device to control the temperature, at 25°C by low pressure displacement in 50 m*M* sodium acetate buffer at pH 4.0, using a 40 cm (effective length 32 mm)×50 μm capillary.

EOF was measured according to a method reported by Williams and Vigh [19] in 50 m*M* sodium acetate buffer at pH 4.0 containing various amounts of PAA-β-CD.

3. Results and discussion

PAA-β-CD, with a relative molecular mass of 12 000, was obtained in water by means of a radical polymerization process (Fig. 2). The cavity of the cyclodextrin, linked to the polymer backbone through a hydrophilic spacer arm of at least 10 atoms, can freely be approached by the analytes. Besides cyclodextrins, the polymer backbone bears primary amino groups that are fully protonated at acidic or neutral pH so that the polymer migrates toward the cathode. An important feature of PAA-β-CD is its ability to reverse the direction of the

Table 1
EOF and viscosity of pH 4.0 BGE containing various amounts of PAA-β-CD, see Section 2.5

Polymer concentration (w/v)	CD concentration (mM)	Viscosity relative to water at 25°C (Kg m^{-1} s^{-1})	EOF (10^8 m^2/V s)
1.6	1	0.0089	0.7
6.4	4	0.0094	0.7
16	10	0.0094	0.71
32	20	0.0099	0.88

electroosmotic flow. Table 1 reports EOF values measured according to [19] in uncoated capillaries filled with 50 m*M* sodium acetate at pH 4.0, containing increasing amounts of PAA-β-CD polymer. At a polymer concentration of 1.6% (w/v), corresponding to a CD concentration of 1 m*M*, a weak anodal electroosmotic flow was observed. The EOF value reached a value of $0.88 \cdot 10^{-8}$ m^2/V s for a 32% (w/v) polymer concentration. Table 1 also reports the dependence of buffer viscosity on polymer concentration. The viscosity was similar to that of plain buffer at a 1.6% polymer concentration whereas a slight increase was observed for a polymer concentration of 32% (w/v).

A set of seven DNP-amino acid enantiomers were separated using PAA-β-CD as the chiral selector by applying a counter-current CE technique which was previously proposed for charged CD-type chiral selectors [20]. The technique consists of filling the capillary with the running buffer containing the CD polymer whereas the buffer reservoirs are filled with pure buffer. When a potential is applied across the capillary, PAA-β-CD, being positively charged at the operative pH migrates, toward the cathode creating a counter-current process in which the chiral selector and the analytes migrate in opposite directions (Fig. 3). This technique has been extensively used with UV absorbing chiral selectors such as macrocyclic antibiotics to detect analytes in a selector free detector cell, thus preventing loss of sensitivity resulting from background absorption of the chiral additives. In our particular case, the counter-current technique was used to reduce the consumption of the chiral selector. One of the problems with this technique is the re-mixing of the two discrete zones of enantiomers that may occur if the resolved analyte zones migrate through a too long section of a selector-free capillary. In a first set of experiments a 50 m*M* sodium acetate buffer at pH 4.0 was used as the background electrolyte. The presence of a moderate EOF at this pH accelerates the migration of the analytes towards the detector and slows down the migration of the CD-polymer towards the cathode. As a result the time spent by the analyte in the capillary section not containing the chiral selector is reduced in a favorable way. Fig. 4 shows the separation of DNP-alanine in both coated and in uncoated capillaries filled with acetate buffer containing 16% (w/v) CD polymer corresponding to 10 m*M* cyclodextrin concentration. In both cases, a baseline resolution was obtained but the migration

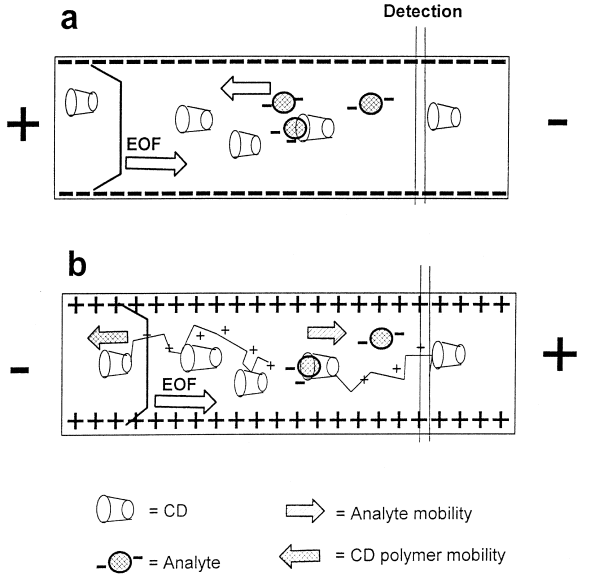

Fig. 3. Schematic representation of a typical chiral analysis with cyclodextrins (a) and polymeric cyclodextrins (b). As shown by the scheme in (b), the EOF is directed towards the anode; electrophoretic and electroosmotic mobility of the analytes are towards the anode whereas polymer chains bearing cyclodextrins migrate in the opposite direction.

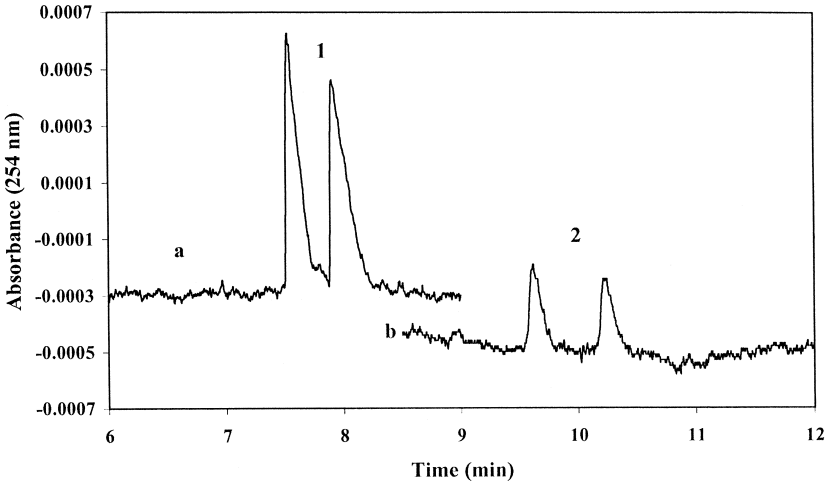

Fig. 4. Electopherogram of D,L-DNP-alanine in (a) uncoated and (b) polyacrylamide coated capillaries filled with the BGE containing 1.6% (w/v) CD polymer corresponding to 10 m*M* cyclodextrin concentration. Separation conditions: 40 cm long capillary (32 cm to the window), running buffer, 50 m*M* acetic acid titrated to pH 4.0 with sodium hydroxide, applied voltage, 20 kV, negative polarity at the inlet. Before each run the capillaries were rinsed with the BGE and then filled with the BGE containing PAA-β-CD copolymer by applying a pressure of $1.03 \cdot 10^{-1}$ MPa for 3 min. The samples (0.2 m*M* water solutions) were loaded electrophoretically by applying 25 V/cm for 1 s.

time was shorter in the uncoated capillary due to the presence of a reversed EOF. Transit times, selectivity and efficiency for the test analytes in coated and uncoated capillaries are compared in Tables 2 and 3. In general, the analyte elution order was different in the two capillaries but selectivity and efficiency were comparable in the two cases. The effect of PAA-β-CD concentration on the separation of various racemates was investigated using the same BGE. As an example, the separation of DNP-norvaline at three different CD concentrations is reported in Fig. 5. Increasing the CD polymer concentration, from 4 to 20 m*M*, resulted in a significant increase of transit times and selectivity but also in a significant reduction of peak efficiency with only a moderate gain in resolution. Similar resolution values were obtained for seven test racemates in uncoated and polyacrylamide coated capillaries as shown in Figs. 6 and

Table 2
Selectivity, efficiency and transit times for DNP-amino acid enantiomers separated in an uncoated capillary

Analyte	PAA-β-CD concentration											
	4 Nm				10 mM				20 mM			
	t_1[a]	t_2[b]	α[c]	N/m[d] ($\times 10^3$)	t_1	t_2	α	N/m ($\times 10^3$)	t_1	t_2	α	N/m ($\times 10^3$)
DNP-asp	5.0	5.1	1.02	–	5.3	5.5	1.03	–	8.6	8.9	1.03	63
DNA-ala	6.7	7.03	1.04	40	7.5	7.9	1.05	24	13.2	14.2	1.08	12
DNP-gln	7.0	7.2	1.02	–	7.6	7.9	0.96	17	8.2	8.9	1.08	15
DNP-*h*-ser	7.10	7.4	1.04	94	7.8	8.3	1.06	81	9.0	9.9	1.08	40
DNP-pro	7.4	8.00	1.06	24	8.5	9.3	1.09	19	10.2	10.4	1.02	13
DNP-*n*-val	8.2	8.7	1.06	102	8.7	9.58	1.09	84	10.0	10.4	1.04	42
DMP-glu	12.6	12.9	1.02	–	13.5	13.8	1.02	67	14.0	14.5	1.04	21

Conditions: 50 mM acetate buffer, pH 4.0, all other run conditions as given in Fig. 4.
[a,b] Migration times of the first and second peak (min).
[c] Selectivity $= \mu_1 / \mu_2$.
[d] Number of theoretical plates per meter.

Table 3
Selectivity, efficiency and transit times for DNP-amino acid enantiomers separated in a polyacrylamide coated capillary

Analyte	PAA-β-CD concentration											
	4 Nm				10 mM				20 mM			
	t_1	t_2	α	N/m ($\times 10^3$)	t_1	t_2	α	N/m ($\times 10^3$)	t_1	t_2	α	N/m ($\times 10^3$)
DNP-asp	5.2	5.3	1.01	–	6.06	6.5	0	–	6.23	6.6	1.06	15
DNP-glu	6.6	6.9	1.04	55	8.18	8.3	1.02	33	9.04	9.69	1.05	24
DNP-*h*-ser	6.7	6.9	1.02	12	8.82	9.4	1.07	11	13.42	14.6	1.09	9.0
DNP-pro	7.0	7.4	1.05	10	10.09	11.1	1.10	96	16.81	18.8	1.12	7.4
DNP-gln	7.1	7.3	1.02	–	9.28	9.4	1.02	24	13.24	14.3	1.08	21
DNA-ala	7.3	7.6	1.04	30	9.61	10.1	1.06	23	14.33	15.4	1.08	12
DNP-*n*-val	7.9	8.4	1.06	11	11.20	11.6	1.04	9.8	15.94	17.5	1.10	10

Conditions: 50 mM acetate buffer, pH 4.0, all other run conditions as given in Fig. 4.

7 that report resolution at various CD concentrations. Anodal migration of the analytes in uncoated capillaries confirms the suppression of cathodal electroosmotic flow resulting from surface coating properties of PAA-β-CD.

The same set of samples was analyzed by adding increasing amounts of native β-cyclodextrin to the same BGE. In the absence of the dynamic wall coating, most of the analytes did not emerge from the uncoated column since these species migrated against the EOF with a velocity comparable to or even greater then that of electroosmotic flow. The analytes required infinite amounts of time to emerge from the column (data not shown) their apparent mobility being close to zero if not negative. In a polyacrylamide coated capillary using native β-CD as the chiral selector the analytes migrated, as expected, towards the anode. However, a poor resolution was observed for all the samples and none of the racemates were separated with 4 m*M* native β-CD, a concentration at which polymeric CD was able to resolve the majority of the analytes (Fig. 8). Only four of the seven test analytes were separated in a buffer solution saturated with β-CD whereas all

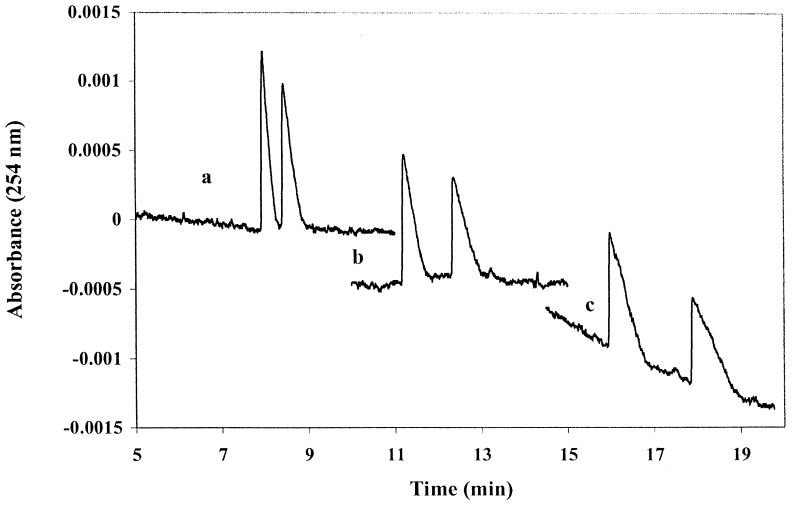

Fig. 5. Separation of D,L-DNP-norvaline at three different CD polymer concentrations, (a) 4 m*M*, (b) 10 m*M*, (c) 20 m*M*. Separation conditions as in Fig. 4.

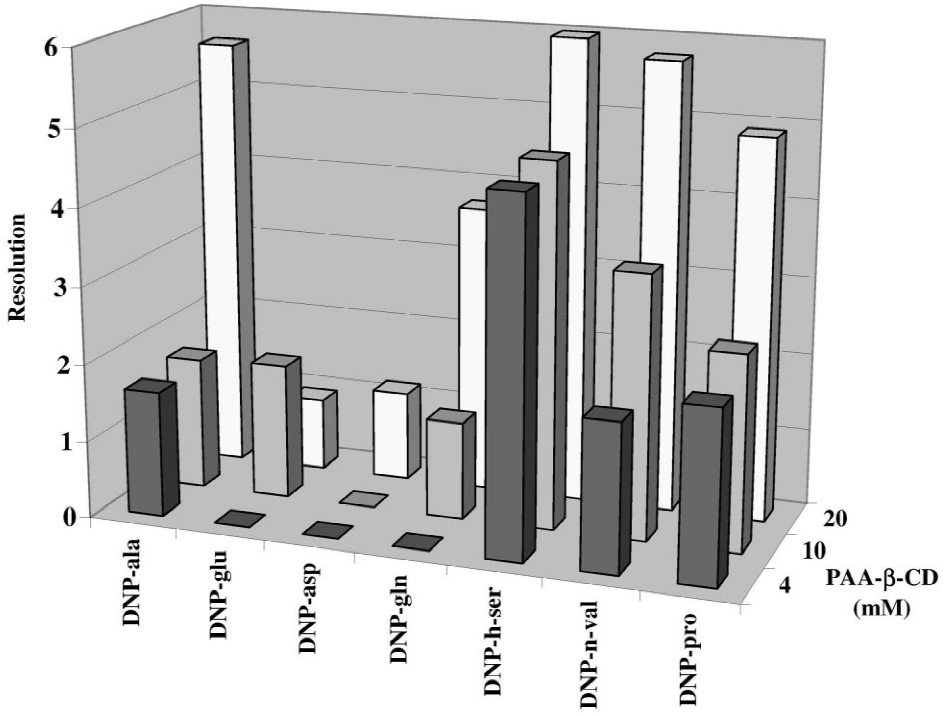

Fig. 6. Resolution of D,L-DNP amino acids as a function of CD polymer concentration in an uncoated capillary. Separation conditions as in Fig. 4.

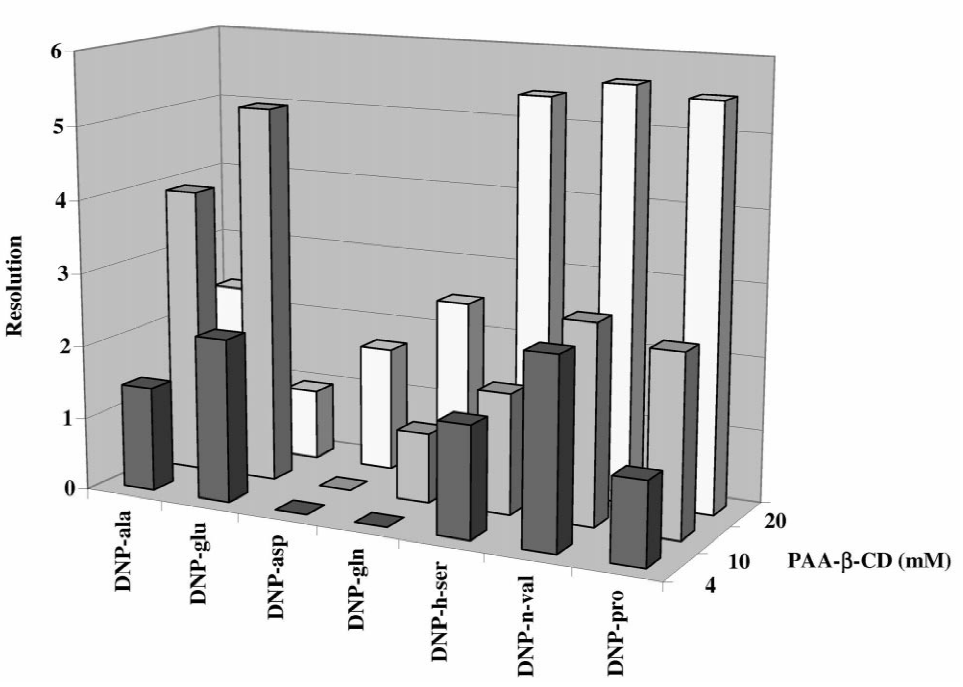

Fig. 7. Resolution of D,L-DNP amino acids as a function of CD polymer concentration in a polyacrylamide coated capillary. Separation conditions as in Fig. 4.

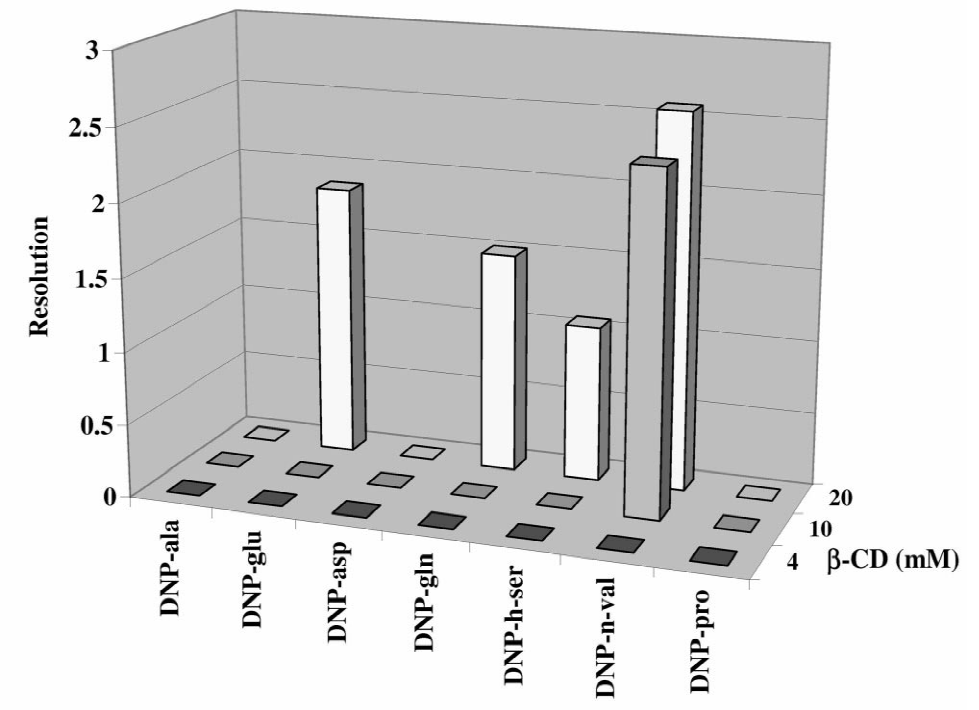

Fig. 8. Resolution of D,L-DNP amino acids as a function of native β-cyclodextrin concentration in a polyacrylamide coated capillary. Separation conditions as in Fig. 4.

the racemates were well resolved with the polymeric CD at 20 m*M* concentration. The observed selectivity enhancement may result from the presence of charges on the polymer that bears the CD molecules. CD attached to a charged polymer possesses a self-mobility opposite to that of the anlytes that may result in an increase in the separation factor independent of the selectivity of the host-guest complexation. A similar effect has been proposed to explain the extremely high enantioselectivity of other charged CD derivatives such as sulfobutyl ether of β-CD [10].

4. Conclusions

A new, water soluble CD containing polymer was obtained by a radical polymerization procedure. This polymer was used in capillary electrophoresis as a chiral selector for the separation of DNP-amino acid enantiomers. The polymer backbone, being positively charged at neutral or acidic pH, imparts to the cyclodextrin a cathodal electrophoretic mobility. The fact that the chiral selector has a mobility opposite to that of the analytes leads to a significant increase in enantioselective separation for all the test racemates. In addition, EOF reversal caused by dynamic adsorption of this polymer onto the silica surface can be efficiently used for the modification of separation parameters such as selectivity and efficiency of separation and migration time. Reversal of EOF is beneficial in enantioseparations of acidic compounds, significantly reducing analysis time and increasing peak efficiency.

Acknowledgements

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