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Novel β-cyclodextrin derivative functionalized polymethacrylate-based monolithic columns for enantioselective separation of ibuprofen and naproxen enantiomers in capillary electrochromatography

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ABSTRACT

A novel enantioselective polymethacrylate-based monolithic column for capillary electrochromatography was prepared by ring-opening reaction of epoxy groups from poly(glycidyl methacrylate-*co*-ethylene dimethacrylate) monolith with a novel β-cyclodextrin derivative bearing 4-dimethylamino-1,8-naphthalimide functionalities. Conditions for the ring-opening reaction with respect to different reaction parameters were thoroughly optimized to obtain high electroosmotic flow, separation efficiency and enantioselectivity for the analytes. The nonaqueous mobile phase composition regarding acetonitrile–methanol ratio and the concentration of electrolyte were examined to manipulate the hydrophobic inclusion and anion-exchange interaction between the analytes and chiral stationary phase. It was observed that in addition to β-cyclodextrin cavity, the electrostatic interaction exhibited pronounced influence on the enantioseparation of acidic analytes. Acidic enantiomers (ibuprofen and naproxen) could be separated with separation factor (α) values up to 1.08 and a maximum separation efficiency of 86 000 plates/m could be achieved.

1. Introduction

Monolithic column is a promising recent trend in capillary electrochromatography (CEC) and it represents an attracting alternative to packed-CEC due to their inherent advantages, such as fritless design, easy of preparation with adjustable porosity and pore diameter and fast diffusional mass transfer [1]. In the past decade, monolithic columns have been proved to be effective media for enantioselective separations in CEC and have gained considerable attentions [2,3]. The categories of materials that have been adapted for enantioselective monolithic columns include polyacrylamides [4–6], polymethacrylates [7–13], silica-based monoliths [14–18] and particle-fixed materials [19,20]. Among them, polymethacrylate monolithic columns exhibit some potential advantages and represent a promising direction for CEC applications, as they are rigid and tolerant of extreme pH and they can be prepared via a single-step polymerization reaction and their porous properties can easily be tailored by tuning the composition of the porogenic solvent and monomers in the starting polymerization solution [21]. Lämmerhofer and co-workers [7–9] have prepared polymethacrylate-based enantioselective monolithic columns by *in situ* copolymerization of different chiral monomers carrying pendant chiral ligands with other comonomers in the presence of porogenic solvent and the enantioselective monolithic columns demonstrated exceptional enantiomeric separation capacities towards the test solutes. However, most of the chiral monomers are not readily available and the pore structure optimization steps for every new chiral monomer to be introduced are tedious and time-consuming. Hence, a more generic post-modification strategy has been developed, which allows a subsequent immobilization of diverse chromatographic ligands onto preoptimized monolithic supports with reactive groups. In this case, chiral monomers can be eliminated and low consumption of bonded ligands is needed. This is especially valuable for the use of precious chiral selectors. Preinerstorfer et al. [10,11] have developed reactive thiol-modified monolithic columns, and chiral ligands as *O*-9-tert-butylcarbamoylquinine and (*S*)-*N*-(4-allyloxy-3,5-dichlorobenzoyl)-2-amino-3,3-dimethylbutanephosphonic acid were attached to the monolith through a radical addition reaction. Recently, Messina et al. [12,13] also demonstrated the feasibility of post-modification approach by immmobilization of ergot alkaloid-based chiral selector (+)-1-(4-aminobutyl)-(5R,8S,10R)-terguride onto a poly(glycidyl methacrylate-*co*-ethylene dimethacrylate) monolith.

Native and derivatized β-cyclodextrins (CDs) are doughnut-shaped cyclic oligosaccharides with a relatively hydrophobic cavity,

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and represent the most prominent and widely used chiral selectors. They have found many applications in enantioseparation by HPLC [22,23], capillary electrophoresis [24,25], GC [26]etc. To date, extensive studies focusing on β-CDs as the chiral selector mainly dealt with polyacrylamides, silica-based and particle-fixed monoliths [27,28], and their use for polymethacrylate monolithic columns has not reported yet. In this contribution, a novel β-cyclodextrin derivative with 4-dimethylamino-1,8-naphthalimide functionalities (DMAN-β-CD) was synthesized and served as the chiral selector of the reactive poly(glycidyl methacrylate-*co*-ethylene dimethacrylate) monolithic columns for enantioselective separation of racemic naproxen and ibuprofen.

2. Experiment

2.1. Instruments

Analysis was carried out on a Beckman P/ACE MDQ instrument (Beckman Coulter, Fullerton, CA, USA) equipped with a photo diode array detector (190–600 nm), automatic injector, a fluid cooled column cartridge (15–50 °C) and 32 Karat software. Fused-silica capillaries (100 μm I.D. × 375 μm O.D.) were purchased from Yongnian Photoconductive Fiber Factory (Hebei, China) and modified as in Section 2.3. An oven was purchased from Sopu instrument (Shanghai, China). NMR spectra were recorded on a Varian Mercury-VX300 spectrometer (Varian, Palo Alto, CA, USA).

2.2. Reagents and chemicals

All the chemicals in the experiment were of analytical grade. Double distilled water was used. Naproxen and ibuprofen enantiomers were purchased from Sigma (St. Louis, MO, USA). γ-Methacryloxypropyltrimethoxysilane (γ-MAPS) was obtained from the Chemical Plant of Wuhan University (Wuhan, China). 1-Propanol, 1,4-butanediol and azobisisobutyronitrile (AIBN) were purchased from Sinopharm Chemical Reagent Plant (Shanghai, China). Ethylene dimethacrylate (EDMA) and glycidyl methacrylate (GMA) from Acros Organics (Morris Plains, NJ, USA) were distilled under vacuum before use. DMAN-β-CD was designed and synthesized by Fu's group [29]. Mobile phases comprised of triethylamine (TEA) and acetic acid (HAc) at different molar ratios in acetonitrile–methanol mixture with different proportions. All the analytes were dissolved in methanol and diluted to the desired concentration. Mobile phases and the analytes were passed through 0.22 μm filter before use.

2.3. Preparation of poly(GMA-co-EDMA) monolith and immobilization of DMAN-β-CD

Column pretreatment and the preparation of the poly(GMA-*co*-EDMA) monoliths were according to our previously described procedure [29]. Briefly, after consecutive washes with 1.0 M NaOH, 0.1 M HCl and H_2O, the capillary was dried under nitrogen for 2 h. A solution of 50% (v/v) of γ-MAPS in methanol was charged into the capillary with a syringe. Then the capillary was sealed and placed in the oven at 50 °C for 12 h to vinylize the inner wall of the capillary. Finally, the capillary was rinsed with methanol and acetone and dried. 30% (v/v) GMA, 10% (v/v) EDMA, AIBN (1%, w/w, with respect to the monomers), 35% (v/v) 1-propanol, 20% (v/v) 1,4-butanediol and 5% (v/v) H_2O were mixed together. After sonicated for 10 min, the solution was degassed by purging with nitrogen for 10 min and injected into the vinylized capillary to a length of 30 cm, and then both ends of the capillary were sealed with rubbers. The capillary was submerged into a water bath at 60 °C for 16 h, followed by continuous rinses with ACN and methanol under the applied pressure of 40 bar to remove the unreacted chemicals. The monolithic column was then filled with a DMF solution containing 12% (w/v) DMAN-β-CD. With both ends sealed, it was heated at 70 °C for 12 h and subsequently washed with methanol and water. A solution of 0.5 M HCl–methanol (80/20, v/v) was pumped through the column for 2 h to hydrolyze the residual epoxide groups to diols. Finally, the column was rinsed with water to remove the acid and further conditioned with mobile phase prior to use. A detection window was created right after the end of the monolith using a flame. The resultant monolithic column was designated as DMAN-β-CD-GMA-EDMA column.

2.4. Electrochromatographic experiment

The capillary was cut to a total length of 40.2 cm, having a monolithic bed of 30 cm and an open segment of 10.2 cm, respectively. Samples were injected electrokinetically at −5 kV for 3 s. The separations were performed at an applied voltage of −20 kV at 20 °C. The detection wavelength was set at 214 nm for the analytes. An equal pressure of 50 psi was applied at both ends of the capillary. Acetone was used as the electroosmotic flow (EOF) marker. The mobility of EOF was calculated by the equation $\mu_{EOF} = L_t L_{eff}/Vt_m$, where L_t is the total length of the capillary (cm), L_{eff} is the effective length of the capillary (cm), V is the separation voltage (V), and t_m is the migration time of the neutral marker. The resolution (R_s) were calculated by the equation $R_s = 2(t_2 - t_1)/(w_1 + w_2)$, and the separation factor (α) was calculated by $\alpha = (t_2 - t_0)/(t_1 - t_0)$. Where t_1, t_2 and t_0 are the retention time for the 1st, 2nd eluted enantiomers and the EOF marker, respectively, and w_1 and w_2 are the peak widths of the racemates.

3. Results and discussion

3.1. Fabrication and optimization of DMAN-β-CD-GMA-EDMA column

For the present study, a highly crosslinked poly(GMA-*co*-EDMA) monolith was prepared according to our previously described method [29]. This monolith was then selected as the substrate for the immobilization of DMAN-β-CD as the chiral selector by means of ring opening reaction of the epoxy groups originating from glycidyl methacrylate. The structure of DMAN-β-CD and ibuprofen and naproxen isomers and the chemical reaction scheme are illustrated in Fig. 1. The chiral selector involves β-CD cavity as well as aromatic and tertiary amine groups, and the β-CD moieties can afford inclusion complexation towards enantiomers, while the positively charged aromatic and tertiary amine groups can generate anodic EOF and simultaneously provide a supportive secondary interaction (electrostatic interaction) with acidic enantiomers under acidic conditions, which may influence the enantioselectivity. The aforementioned characteristics are especially beneficial to the enantioseparation of acidic drug enantiomers, since their separation in traditional silica-based chiral stationary phases (CSPs) is not straightforward [30].

As sufficient amount of DMAN-β-CD loading is required to maintain high EOF and enantioselectivity, major variables of immobilization conditions, such as reaction temperature, reaction time and DMAN-β-CD concentration were examined, respectively. Naproxen and ibuprofen enantiomers were used as test solutes in this study. The influence of reaction temperature from 40 to 80 °C on the mobilities of EOF and the first elute of ibuprofen and naproxen enantiomers were investigated. As expected, using mobile phase of ACN–MeOH (70:30, v/v) mixture, containing 350 mM HAc and 5 mM TEA, an increase in anodic EOF mobility from −0.54 to -0.67×10^{-4} cm^2 V^{-1} s^{-1} was accompanied by an increase of reaction temperature from 40 to 70 °C, while the mobility for the first

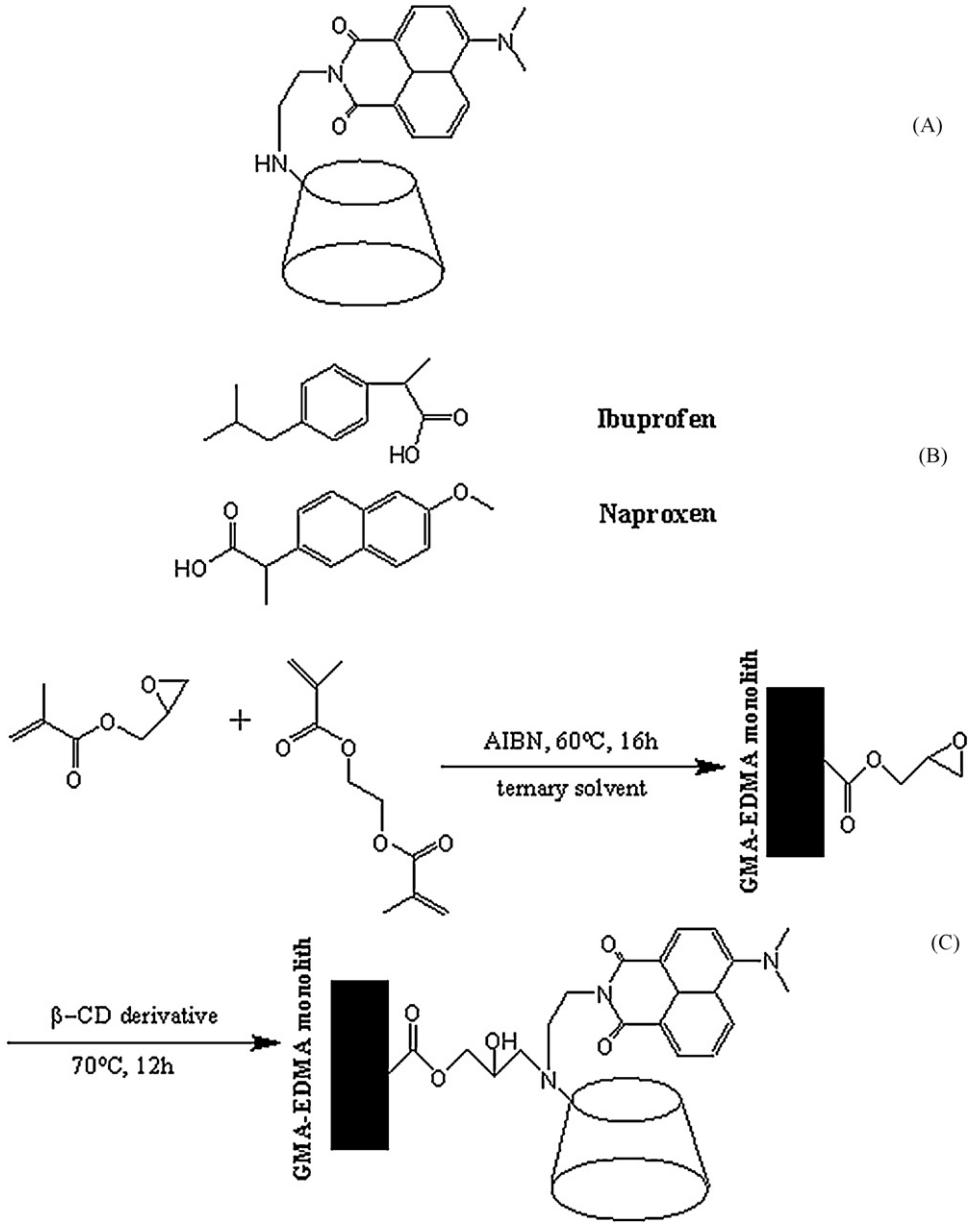

Fig. 1. The structures of DMAN-β-CD (A) and ibuprofen and naproxen (B) and reaction scheme for the synthesis of poly(GMA-*co*-EDMA) monolith and post-modification with DMAN-β-CD (C).

elute of ibuprofen and naproxen enantiomers over this temperature range decreased from -1.03×10^{-4} to -0.96×10^{-4} cm^2 V^{-1} s^{-1} and from -1.16×10^{-4} to -1.07×10^{-4} cm^2 V^{-1} s^{-1}, respectively. Since the chiral selector provides the driving force for EOF as well as retention sites towards the analytes, this variations should be resulted from an increase in it's loading, as the reaction temperature increased from 40 to 70 °C. However, at higher reaction temperature (>70 °C), on the other hand, the mobility of EOF decreased and the

Table 1
Effect of reaction time on CEC enantioseparation of ibuprofen and naproxen.[a].

Reaction time (h)	μ_0[b]	Ibuprofen			Naproxen		
		μ_1[b]	N[c]	R_s	μ_2[b]	N	R_s
4	−0.55	−1.06	7.78	1.43	−1.18	8.23	1.53
6	−0.61	−1.01	7.92	1.77	−1.12	8.35	1.82
8	−0.65	−0.98	7.86	2.04	−1.09	8.42	2.08
12	−0.67	−0.96	7.98	2.26	−1.07	8.59	2.34
14	−0.68	−0.95	7.83	2.26	−1.07	8.40	2.35

[a] Mobile phase: ACN–MeOH(70:30, v/v) mixture, containing 350 mM HAc and 5 mM TEA; reaction conditions: 20% (w/v) DMAN-β-CD was dissolved in DMF and the reaction temperature was set at 70 °C.

[b] μ_0, μ_1 and μ_2 were the mobilities of EOF marker (10^{-4} cm^2 V^{-1} s^{-1}) and the first elute of ibuprofen and naproxen enantiomers, respectively.

[c] N is the average theoretical plates count in 10 000 plates/m.

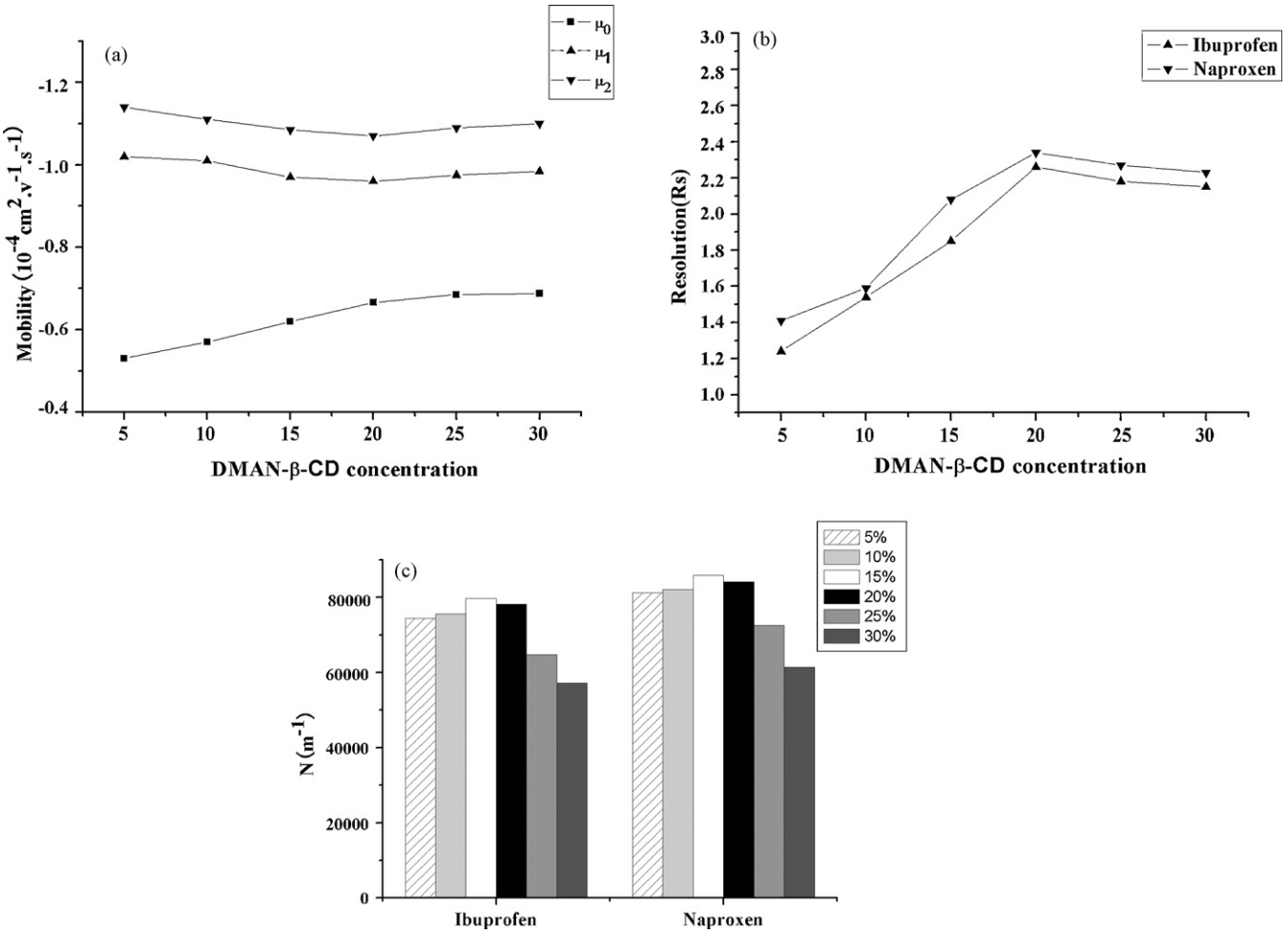

Fig. 2. Influence of DMAN-β-CD concentration used on (a) the mobilities of the EOF (■) and the first eluted enantiomers of ibuprofen (▲) and naproxen (▼), (b) the resolution (R_s), and (c) the separation efficiency (N). Notation on (c), DMAN-β-CD concentration varied from 5 to 30% (w/v); 5% (slash bars), 10% (light gray bars), 15 (white bars), 20% (black bars), 25% (gray bars) and 30% (dark gray bars). Mobile phase: ACN–MeOH (70:30, v/v) mixture, containing 350 mM HAc and 5 mM TEA; Reaction conditions: DMAN-β-CD was dissolved in DMF with concentration from 5 to 30% (w/v), reaction was proceeded for 12 h at 70 °C.

mobilities of the analytes were slightly higher than those for reaction temperature of 70 °C, which indicated that the ring opening reaction was set off at higher temperature. As a result, 70 °C reaction temperature was selected for subsequent study. Table 1 summarizes the dependence of reaction time on mobilities of the EOF and the first elute, column efficiency and resolution of the two enantiomers. It could be concluded that prolonged reaction time brought more DMAN-β-CD to the monolithic matrix and thus increased EOF mobility and decreased mobilities for the analytes were obtained. Since more DMAN-β-CD yielded an increase in the available sites for enantioselective interactions, hence resolution for both the two enantiomers were enhanced. Nevertheless, as any further increase in reaction time from 12 h led to the loss of efficiency, reaction time of 12 h represented the optimal compromise in terms of separation efficiency and resolution. The DMAN-β-CD concentration over the range of 5–30% (w/v) on the mobilities of EOF and the analytes, resolution and column efficiency were investigated, as shown in Fig. 2. Steady variations of mobility of EOF were recorded using DMAN-β-CD concentration from 5 to 20%, and the values almost reached a plateau and varied a little as the concentration was raised to 25% (Fig. 2a). It suggested that the chiral selector immobilized to the monolithic support almost no longer increased with any further increase of DMAN-β-CD concentration. In addition, the amount of DMAN-β-CD immobilized to the monolithic support would result in a difference in electrochromatographic properties of the column, such as resolution and separation efficiency. It could be seen from Fig. 2b, the resolution for both enantiomers improved dramatically as the DMAN-β-CD concentration increased from 5 to 20% and any further increase of DMAN-β-CD concentration resulted in moderate loss of resolution. Consequently, the optimal resolution was obtained at DMAN-β-CD concentration of 20%. Since the non-stereo interaction stemming from the monolithic support was minimized with the increase of DMAN-β-CD concentration, which led to an increase in R_s values, we could infer that the loss in resolution at DMAN-β-CD concentration higher than 20% could be attributed to the presence of more densely immobilized naphthalimide moieties, which restricted the access of the analytes to the stereoselective centre of β-CD derivative. Fig. 2c shows that the separation efficiency differed not substantially at DMAN-β-CD concentration from 5 to 20% (ranging 74 400–78 200 and 81 200–84 100 m^{-1}, respectively, for the first elute of the two enantiomers), nevertheless it decreased dramatically at higher DMAN-β-CD concentrations. It seemed that the concentration of 20% DMAN-β-CD was more acceptable considering both the resolution and separation efficiency. Taking into account all the effects, 20% DMAN-β-CD subjected to a reaction of 12 h at 70 °C was proved to be the optimal immobilization condition. As mentioned by Peters [31], enantioselectivity in polymethacrylate monolithic column was hampered by non-specific interactions between the racemates and the hydrophobic backbone of poly(GMA-*co*-EDMA) mono-

lith, we increased the hydrophilic of DMAN-β-CD-GMA-EDMA column by hydrolyzing the unreacted 2,3-epoxypropyl groups to diols. However, only an increase of 8% in resolution of ibuprofen enantiomers was observed for the hydrolyzed column (R_S = 2.26) compared with that for unhydrolyzed one (R_S = 2.10), while the value is 11% for naproxen enantiomers. It seemed that most of the epoxy groups have reacted with DMAN-β-CD during the ring opening reaction.

3.2. Effect of mobile phase on enantioseparations

In general, β-CD derivative-based chiral selectors were widely used for CEC in either aqueous or nonaqueous mobile phases [27,32]. Compared with aqueous conditions, nonaqueous CEC (NA-CEC) turned out to be advantageous with respect to low current and hence reducing the risk of excessive Joule heating. Volatile organic mobile phase is also well suited for on-line coupling with electrospray ionization mass spectrometry (ESI-MS). In addition, nonenantioselective interactions, such as hydrophobic interactions, are weakened in nonaqueous conditions. Thus, the enantioselectivity is often higher than that under the aqueous mobile phase, especially for lipophilic polymethacrylate-based monolithic CSPs [17,32,33]. Accordingly, from a practical point of view, NA elution condition was employed throughout the present study, which consisted of acetonitrile and methanol mixture as the mobile phase and HAc as well as TEA as the electrolytes.

3.2.1. Influence of solvent composition

The interior of the cavity of DMAN-β-CD and the poly(GMA-*co*-EDMA) monolith are relatively hydrophobic, which can afford hydrophobic interactions towards the analytes. The former is of necessity to provide inclusion with the analytes for enantiodiscrimination, while the latter is regarded as non-specific interaction that has deleterious effects. Variation in solvent composition is proved to be an effective means to regulate these interactions and thus the enantioseparations. As a consequence, the effect of acetonitrile–methanol ratio at a constant electrolyte concentration (350 mM HAc and 5 mM TEA) on electroosmotic characteristics and electrochromatographic behaviour of both ibuprofen and naproxen enantiomers was investigated. As illustrated in Fig. 3a, the mobility of EOF decreased from -0.78×10^{-4} to -0.48×10^{-4} cm^2 V^{-1} s^{-1} with the increase of methanol concentration, and this can be readily explained by the variation in dielectric constant to viscosity ratio and by the effect of mobile phase polarity on the ζ-potential. The increase of methanol concentration over the range studied had a positive effect on the retention, which implied that the hydrophobic interaction between the CSPs and the analytes played an important role in the retention process. Since both DMAN-β-

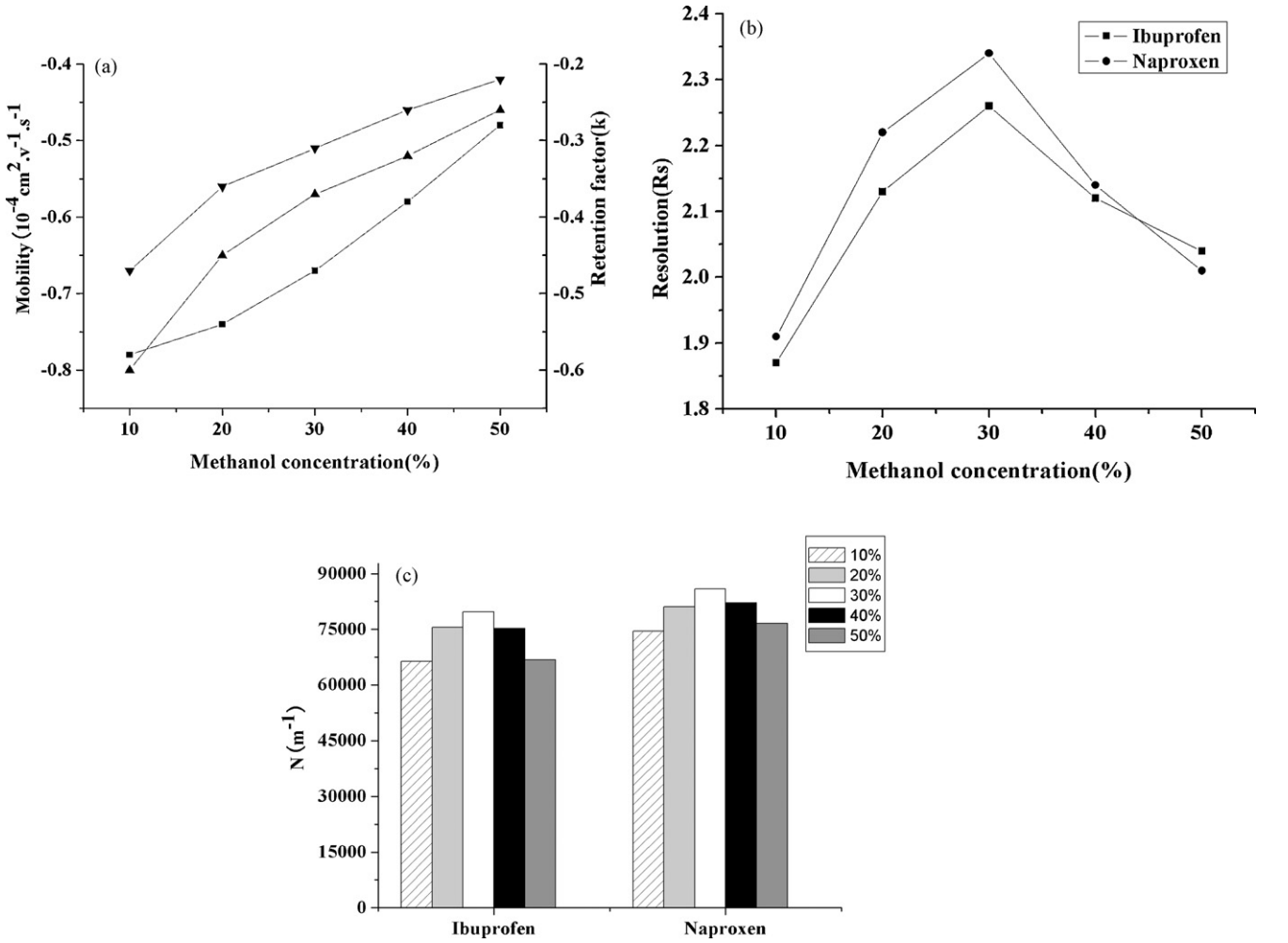

Fig. 3. Effect of acetonitrile–methanol ratio in the mobile phase on (a) the EOF mobility (■) and retention factor for the first eluted enantiomers of ibuprofen(▼) and naproxen(▲), (b) the resolution (R_s), and (c) the separation efficiency (N). Notation on (c), methanol concentration varied from 10 to 50% (v/v), 10% (slash bars), 20% (light gray bars), 30% (white bars), 40% (black bars) and 50% (dark gray bars). Experimental conditions: various acetonitrile–methanol mixture containing 350 mM HAc and 5 mM TEA in the mobile phase.

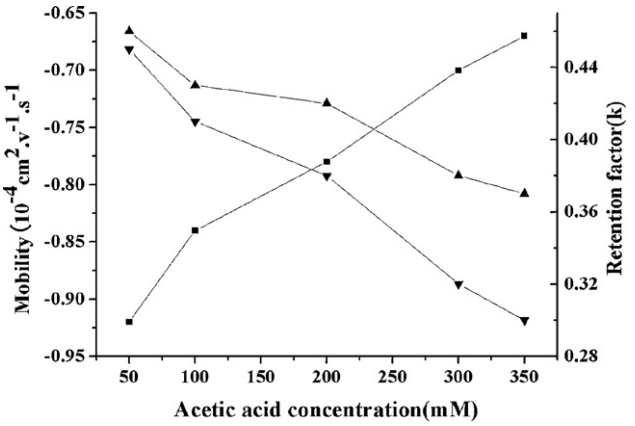

Fig. 4. Influence of HAc concentration in the mobile phase (at a constant HAc-TEA ratio of 70:1) on (a) the mobilities of the EOF (■) and the retention factor for first eluted enantiomers of ibuprofen (▲) and naproxen (▼). Experimental conditions: ACN–MeOH (70:30, v/v) mixture containing various of HAc concentrations.

CD and the poly(GMA-*co*-EDMA) monolith can afford hydrophobic interaction and compete with each other, which might influence the recognition process, and thus the effect of methanol concentration on the resolution was investigated as shown in Fig. 3b. The resolution increased with the increase of methanol concentration from 10 to 30%, in contrast, the dependence is *vice versa* as methanol concentration further increased from 30 to 50%. The result indicated that more methanol favored the hydrophobic interaction between DMAN-β-CD and the analytes, and thus eventually enhanced the enantioseparation for the analytes at methanol concentration from 10 to 30%. However, as the methanol concentration is above 30%, the increased hydrophobic interaction between the poly(GMA-*co*-EDMA) monolith and the analytes eventually prevailed over that between DMAN-β-CD and the analytes and this non-specific interaction was detrimental to the enantioseparation. As a result, the resolution tended to decrease and the decrease of corresponding column efficiency over this methanol concentration range might be regarded as a clue to the predominance of the non-specific interaction (Fig. 3c). With regards to optimal efficiency and resolution, conditions with an ACN–MeOH ratio of 70:30 (v/v) were considered to be useful to continue further studies.

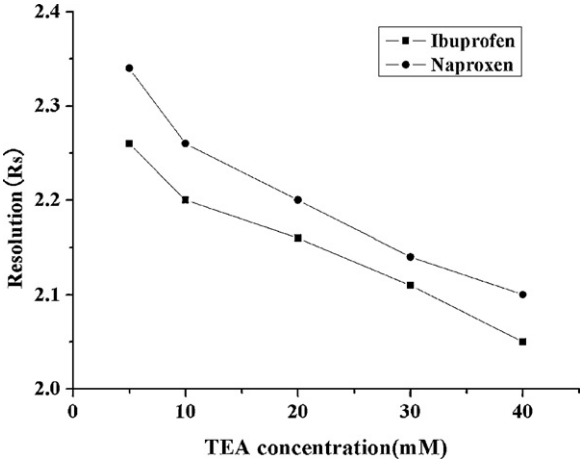

Fig. 5. Effect of TEA concentration in the mobile phase (at a constant HAc concentration of 350 mM) on the resolution (R_s) for ibuprofen and naproxen enantiomers. Experimental conditions: ACN–MeOH (70:30, v/v) mixture, containing 350 mM HAc and various amounts of TEA.

3.2.2. Influence of electrolyte concentration

Since surface exposed aromatic and tertiary amine moieties of DMAN-β-CD are positively charged and can serve as a weak anion exchanger under acidic conditions, to comply with the weak exchange mechanism as well as provide a substantial anodic EOF, a sufficient amount of HAc is required, which can act as counter-ion to control the retention of the analytes. Co-ion as TEA was also considered. Ibuprofen and naproxen have pK_a values of 4.2 and 4.9, respectively (Merck Index). Consequently, they should be protonated and negatively charged under acidic conditions. Therefore, their separation process is associated with a combination of intertwined equilibria, namely inclusion between DMAN-β-CD and enantiomers, electrostatic interaction between the anion exchanger and counter-ion, as well as the interaction between co-ion and solute ion, which all may mutually affect one another through competitive interference and eventually affected the enantiodiscrimination process. The last two interactions can be regulated by the variation of apparent pH in the mobile phase; however, changes in the counter-ion concentration appeared to be more effective and convenient. As already discussed, excessive HAc is required to certify the CSPs is positively charged and higher electrolyte concentration can be used under NA-CEC conditions, the HAc concentration ranging from 50 to 400 mM (at a constant HAc-TEA ratio of 70:1) on the EOF mobility, retention factor of the first elute of the enantiomers, resolution and separation efficiency was studied. Although as expected, the EOF substantially reduced from 0.92×10^{-4} to 0.67×10^{-4} $cm^2\ V^{-1}\ s^{-1}$ with the increase of HAc concentration, due to the decrease of double layer thickness and ζ-potential (Fig. 4), the lower capacity of the chiral anion exchanger at higher counter-ion concentrations led to lower retention factors for both of enantiomers (Fig. 4) and an improvement in separation efficiency was also achieved (figure not shown). These results confirmed that the anion-exchange mechanism is in operation. However, as the HAc concentration was raised to 400 mM, the separation was hampered by excessive Joule heating, which resulted in either loss of current or a decrease in reproducibility. It is worth to note that the resolution for both enantiomers remained almost constant with the variation of HAc concentrations. Generally, enantioselectivity is strictly related to the association and/or dissociation at different rates between the chiral selector and the enantiomers, mainly driven by inclusion of β-CD cavity towards to analytes in this study. The constant R_s values within the HAc concentration range can be attributed to the similar inclusion behaviours of β-CD cavity. As a result, in order to obtain fast elution without loss in separation efficiency and resolution, 350 mM HAc concentration was needed. TEA was used as

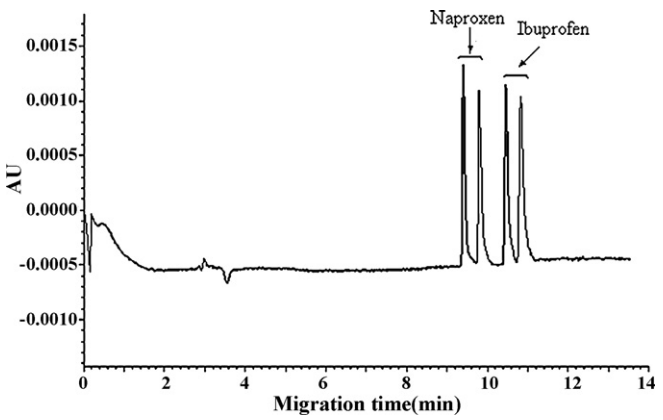

Fig. 6. Simultaneous enantioseparation of ibuprofen and naproxen enantiomers in a single run on DMAN-β-CD-GMA-EDMA column. Experimental conditions: mobile phase, ACN–MeOH(70:30, v/v), containing 350 mM HAc and 5 mM TEA.

Table 2
Within- and between-column repeatability of migration time and separation efficiency for DMAN-β-CD-GMA-EDMA column using ibuprofen and naproxen enantiomers as test solutes.[a].

Analyte	Migration time (min)	RSD_t (%)	N[b]	RSD_N (%)
First eluted naproxen enantiomers	9.40	0.72[c] (4.12)[d]	8.59	1.05[b] (5.87)[c]
Last eluted naproxen enantiomers	9.82	0.78 (4.08)	8.02	1.04 (5.92)
First eluted ibuprofen enantiomers	10.53	0.82 (4.34)	7.98	1.08 (6.06)
Last eluted ibuprofen enantiomers	10.80	0.88 (4.56)	7.76	1.10 (6.28)

[a] Experimental conditions are the same as in Fig. 8.
[b] N is the average theoretical plates count in 10 000 plates/m.
[c] Within-column runs: number of measurements = 5.
[d] Column-to-column runs: number of columns = 4.

the co-ion in our study, tended to compete with anion exchanger for the interaction with the enantiomers and hence affected the enantiodiscrimination. Since the pH is not defined in nonaqueous mobile phase, the variation of the base-to-acid ratio can be used instead, which is equivalent to the change of the apparent pH. As a result, the ionization of the chiral selectors and the analytes would vary with different base-to-acid ratios and resultantly affected the enantiodiscrimination. We studied the influence of the concentration of TEA ranging from 5 to 25 mM on the R_S for the enantiomers of ibuprofen and naproxen, while the HAc concentration was kept constant at 350 mM. As it is illustrated in Fig. 5, the resolution decreased with increasing in the TEA concentration measured, and 5 mM represented the optimum. Two reason might be responsible for this phenomenon: On one hand, the electrostatic interaction between the chiral selector and the analytes kept varying with as the TEA concentration increased, which led to the variation in resolution; on the other hand, since electrostatic competition exists between the positive charged chiral selector and TEA towards the analytes, the decrease in resolution can readily be explained by the increased competition effect with chiral selector.

3.2.3. Simultaneous separation of ibuprofen and naproxen enantiomers

Under the optimized experimental conditions described in the preceding section, simultaneous enantioseparation of ibuprofen and naproxen enantiomers on the β-CD derivative modified monolithic column could be achieved and the representative electrochromatogram is shown in Fig. 6. Both of the enantiomers can be baseline enantio-resolved a single run only within 12 min. The column efficiencies ranging from 75 000 to 86 000 plates/m for the two enantiomers can be achieved. The resolutions were 2.26 and 2.34 for ibuprofen and naproxen racemates, respectively, which corresponding to separation factors (α) of 1.05 and 1.08, respectively.

3.3. Repeatability and stability of the column

Repeatability and stability are two important factors for the evaluation of monolithic columns. The within- and between-column repeatabilities of the DMAN-β-CD modified monolithic column for the two enantiomers were examined in terms of migration time and separation efficiency. The results were summarized in Table 2. The RSDs of the migration time and separation efficiency for the test solutes were not more than 0.88% and 1.10% for five consecutive within-column runs, which suggested satisfactory within-column repeatability. For different batches of polymerization mixture ($n = 4$), column-to-column repeatability is acceptable here, with RSDs of migration time and separation efficiency in the range of 4.12–4.56% and 5.87–6.28%, respectively. No significant change in performance of the column was observed during a test of period of three hundred consecutive runs (with RSDs of the migration time here all within 1.62%), which indicated that DMAN-β-CD modified monolithic columns had a good stability and long lifetime.

4. Conclusions

A new β-cyclodextrin derivative has been successfully employed for the preparation of enantioselective poly(GMA-*co*-EDMA) monolithic column for capillary electrochromatography. After optimization of post-modification steps, sufficient β-cyclodextrin derivative was immobilized onto the monolithic support to afford substantial EOF and good enantioseparation performance. Two acidic enantiomers were successfully resolved on this β-cyclodextrin derivative modified monolithic column with good enantioselectivities and columns efficiency. The mobile phase composition with regards to acetonitrile–methanol ratio and electrolyte concentration had a significant influence on the retention and enantioseparation of the enantiomers. Reasonable within- and between-column repeatabilities and excellent column stability offer a promising prospect of the utility of this novel monolithic column to enantiomeric analysis of acidic pharmaceuticals in CEC.

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