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Sulfated and sulfonated polysaccharide as chiral stationary phases for capillary electrochromatography and capillary electrochromatography–mass spectrometry

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Abstract

The applications of polysaccharide phenyl carbamate derivatives as chiral stationary phases (CSPs) for capillary electrochromatography (CEC) are often hindered by longer retention times, especially using a normal-phase (NP) eluent due to very low electroosmotic flow (EOF). Therefore, in this study, we propose an approach for the aforementioned problems by introducing two new types of negatively charged sulfate and sulfonated groups for polysaccharide CSPs. These CSPs were utilized to pack CEC columns for enantioseparation with a NP eluent. Compared to conventional cellulose tris(3,5-dimethylphenyl carbamate) or CDMPC CSPs, the sulfated CDMPC CSP (sulfur content 4.25%, w/w) shortened the analysis time up to 50% but with a significant loss of enantiomeric resolution (~60%). On the other hand, the sulfonated CDMPC CSP (sulfur content 1.76%, w/w) not only provided fast throughput but also maintained excellent resolving power. In addition, its synthesis is much more straightforward than the sulfated one. Furthermore, we studied several stationary phase parameters (CSP loading and silica gel pore size) and mobile phase parameters (including type of mobile phase and its composition) to evaluate the throughput and enantioselectivity. Using the optimized conditions, a chiral pool containing 66 analytes was screened to evaluate the enantioselectivity under three different mobile phase modes (i.e., NP, polar organic phase (POP) and reversed-phase (RP) eluents). Among these mobile phase modes, the RP mode showed the highest success rate, whereas some degree of complementary enantioselectivity was observed with NP and POP. Finally, the feasibility of applying this CSP for CEC–MS enantioseparation using internal tapered column was evaluated with NP, POP and RP eluents. In particular, the NP-CEC–MS provided significantly enhanced sensitivity when methanol was replaced with isopropanol in the sheath liquid. Using aminoglutethimide as model chiral analyte, all three modes of CEC–MS demonstrated excellent durability as well as excellent reproducibility of retention time and enantioselectivity.

Keywords

Sulfonated polysaccharide; Chiral stationary phase loading; Pore size; Normal-phase CEC–MS; Reversed-phase CEC–MS; Polar organic phase CEC–MS

1. Introduction

Capillary electrochromatography (CEC), a hybrid of high-performance liquid chromatography (HPLC) and capillary electrophoresis (CE), is one of the latest developments in separation technologies, which has attracted growing interest in recent years. Concerning enantiomeric separations, CEC has demonstrated great potential as an alternative technique because of its excellent separation efficiency and enantioselectivity in comparison to HPLC. High efficiency is obtained in CEC mainly because of the flat flow profile of electroosmotic flow (EOF), which significantly reduces the band-broadening by eddy diffusion [1–3]. Furthermore, compared to conventional open-tubular CE, the use of bonded chiral stationary phases (CSPs) in the CEC column are beneficial for achieving excellent enantiomeric selectivity and sample loading capacity. Other positive aspects of CEC include low cost of operation, low consumption of stationary phase and sample, as well as convenient for coupling to mass spectrometric (MS) detection.

Several types of HPLC CSPs have been applied for CEC, which include proteins [4], cyclodextrins [5], macrocyclic antibiotics [6,7], imprinted polymers [8], chiral acrylamides [9], polysaccharide derivatives [10], anion exchangers [11] and brush-type CSP [12,13]. Polysaccharide CSPs are often considered as one type of the most successful HPLC CSPs due to their broad enantioselectivity, outstanding efficiency and stability [14,15]. Typically, these CSPs are prepared by coating or covalent-bonding polysaccharide phenylcarbamates (e.g., cellulose tris(3,5-dimethylphenyl carbamate) (CDMPC)) and phenyl esters on the surface of macroporous silica particles [16]. The intermolecular interactions, such as hydrogen-bonding and π – π interactions are predominant for chiral recognition with these CSPs. Hence, normal-phase (NP) eluent is often adopted since hydrogen-bonding, and π – π interactions could be maximized under this condition.

Like the other HPLC CSPs, the feasibility of polysaccharide derivatives as CEC CSPs also has been investigated [10,17–32]. Using a reversed-phase (RP) or polar organic phase (POP) condition, these CSPs demonstrated excellent resolving power for various classes of analytes. However, a major limitation of these polysaccharide CSPs for CEC is very long analysis time, particularly with the use of NP eluents. As mentioned earlier, the EOF generated by the applied electric field is the major driving force for CEC. However, the use of a NP eluent (e.g., a mixture containing hexane and ethanol) in CEC provides poor conductivity and very low EOF. In addition, neutral polysaccharide coating on the silica particles also hinders the EOF primarily by blocking the accessibility of ionizable groups (e.g., amino groups on aminopropyl silica or silanols on bare silica) to the counterions in mobile phase.

To enhance the EOF by polysaccharide CSPs, several approaches have been reported. For example, Girod et al. [10,25] suggested to decrease the loading of CSP from 20% (w/w) to 2–5% (w/w). However, the enantioselectivity was compromised with low CSP loading. Very recently, Zou [30,32] introduced positively charged polysaccharide CSPs. These CSPs were synthesized by covalent bonding of cellulose phenylcarbamate derivatives on diethylenetriamino-propylated or methacryloyl diethylene-triaminopropylated silica. To increase the throughput, a column with only 8cm pack bed was used with short-end injection. Because the secondary aminogroups on the silica gel surface acquire positive charge with acidic mobile phase, anodic EOF was obtained using these CSPs [31]. However, these

positively charged polysaccharide CSPs often require multistep synthesis (typically 6–7 steps), which could be very time consuming. Tobler et al. [33], Zarbl et al. [34] and Hebenstreit et al. [35] reported EOF enhancement for brush-type CSPs by introducing small-molecular weight chiral selector with sulfonate or phosphate groups.

In this study, we explore the use of a new type of negatively charged polysaccharide CSPs to enhance the EOF profile in CEC and CEC coupled to mass spectrometry. These CSPs are prepared by coating negatively charged cellulose 3,5-dimethyl phenyl carbamate derivatives (e.g., sulfonated or sulfated CDMPC) on silica gel. Similar to aforementioned neutral polysaccharide CSPs, the accessibility of silanol groups on these CSPs is also limited by the polysaccharide coating (Fig. 1). However, the strong cation-exchange groups (e.g., sulfated or sulfonated groups) covalently bonded to the polysaccharide CSP, are highly accessible to the mobile phase counter ions (Fig. 1). Thus, the magnitude of EOF is dominated by the abundance of these negatively charge sulfonated or sulfated groups on the CSP surface instead of silanol groups on the silica gel. Furthermore, the use of these CSPs could be very advantageous because high CSP loading is generally needed for polysaccharide CSPs to achieve better enantioselectivity. In particular, the chromatographic data in this work with sulfonated CDMPC clearly show that the loading amount has no significant impact on the magnitude of EOF.

There are three major objectives included in this research project. First, a series of two negatively charged CDMPC CSPs, namely sulfated and sulfonated CDMPC CSPs were synthesized. The electrochromatographic behaviors, throughput and enantioselectivity of these two CSPs were compared with non-charged CDMPC CSPs using a NP eluent. Second, after investigation of several mobile phase and stationary phase parameters, a total of 66 analytes were screened to evaluate the throughput and enantioselectivity of the sulfonated CDMPC as CSP under NP, POP and RP modes of CEC. Finally, the feasibility of applying this CSP for a rugged enantiomeric analysis was evaluated in all three aforementioned modes of CEC coupled to MS. To the best of our knowledge, this is the first time that the feasibility of a NP-CEC–MS is demonstrated for enantiomeric analysis.

2. Experimental

2.1. Reagents and chemicals

The Kromosil 5 μm 100Å and 300Å bare silica gel particles were kindly donated by Dr. P.K. Dutta of Akzo Nobel (Dobbs Ferry, NY, USA). The Nucleosil 5 μm 1000Å and 4000Å bare silica gel particles were obtained from Macherey-Nagel (Easton, PA, USA). The HPLC grade organic solvents, acetonitrile (ACN), hexane, tetrahydrofuran (THF), methanol (MeOH), ethanol (EtOH), and isopropanol (IPA) were purchased from Fisher (Springfield, NJ, USA). Ammonium formate (NH_4COOH), acetic acid (HOAc), diethylamine (DEA), cyclohexylamine (HA), microcrystalline cellulose, 3,5-dimethylphenyl isocyanate, trityl chloride, anhydrous pyridine, anhydrous N,N-dimethylformamide (DMF), hydrogen chloride–1.25 M in methanol ($\text{HCl}\text{--}\text{MeOH}$), sulfur trioxide–pyridine complex ($\text{SO}_3\text{--Py}$), sulfur trioxide–N,N-dimethylformamide complex ($\text{SO}_3\text{--DMF}$) and most of the chiral analytes (as racemic mixture, Fig. 2) were purchased from Sigma–Aldrich (Milwaukee, WI, USA). All analytes were dissolved in EtOH at a concentration of 1 mg/mL and stored at -20°C before their use. Water used in this study was purified by a Barnstead Nanopure II Water System (Dubuque, IA, USA).

2.2. Synthesis of 6-sulfated cellulose tris(3,5-dimethylphenyl carbamate) (6- $\text{SO}_4\text{--CDMPC}$)

The 6- $\text{SO}_4\text{--CDMPC}$ was prepared through reacting cellulose bis(3,5-dimethylphenyl carbamate) (6-OH-CDMPC) with $\text{SO}_3\text{--DMF}$ (Fig. 3). Briefly, 4 g of cellulose (~25 mmol) was dried under vacuum at 60°C for 12 h. The resulting dried cellulose was first reacted with 10

g trityl chloride (~36 mmol) in 60 mL of pyridine to form trityl ether with the hydroxy group at the 6-position of the glucose unit (step a, Fig. 3). About 12 mL of 3,5-dimethylphenyl isocyanate (~85 mmol) was added to convert the free hydroxyl groups (including 2- and 3-positions) to phenyl carbamate (step b, Fig. 3). Next, the resulting cellulose derivative was stirred in 500 mL of 1% HCl-methanol solution for 1 day to hydrolyze trityl ester and activate the 6-position hydroxyl group resulting in 6-OH-CDMPC (step c, Fig. 3). Next, nearly 2 g of synthesized 6-OH-CDMPC was dissolved in 40 mL anhydrous DMF and the sulfated reaction was conducted in the presence of 0.6 g $\text{SO}_3 \cdot \text{DMF}$ at 100 °C for 48 h (step d, Fig. 3). The reaction mixture was cooled to room temperature and slowly poured into a beaker containing ~3 L of methanol. The obtained white precipitate was filtered and dried under vacuum at 60 °C for 12 h. The product (6- SO_4 -CDMPC, yield ~40%) and starting material (6-OH-CDMPC) were characterized by elemental analysis (Fig. 4), ^{13}C NMR, and IR. The IR spectrum of the product displayed 3319 (NH), 1717 (C=O), 1615 and 765cm^{-1} (C_6H_6) peaks. The NMR spectra were obtained using a Varian Unity +300MHz spectrometer using $[\text{D}_6]\text{pyridine}$ (pyridine- d_6) as the solvent.

6-OH-CDMPC— ^{13}C NMR (pyridine- d_6 solvent; 75MHz): δ (ppm) = 19.6, 21.2, 53.6, 60.5, 63.6, 69.2, 73.8, 78.8, 92.9, 103.1, 106.0, 117.0, 117.4, 118.5, 123.0, 123.3, 123.6, 125.1, 128.5, 129.3, 130.6, 135.0, 135.3, 135.6, 138.4, 139.2, 141.1, 149.4, 149.8, 150.2, 152.1, 152.2, 153.2, 154.9 and 156.8.

6- SO_4 -CDMPC— ^{13}C NMR (pyridine- d_6 solvent; 75MHz): δ (ppm) = 21.2, 63.6, 73.8, 78.8, 94.7, 103.0, 117.4, 123.0, 123.3, 123.6, 125.0, 135.0, 135.3, 135.6, 138.4, 139.2, 149.4, 149.9, 150.1, 153.2 and 154.9.

2.3. Synthesis of sulfonated cellulose tris(3,5-dimethylphenyl carbamate) (CDMPC- SO_3)

The CDMPC- SO_3 was prepared through sulfonation of CDMPC with $\text{SO}_3 \cdot \text{Py}$ (Fig. 4). First, the CDMPC was synthesized by reacting cellulose with 3,5-dimethylphenyl isocyanate according to the procedure reported by Okamoto et al. [16]. Around 4 g of cellulose (~25 mmol) was dried under vacuum at 60 °C for 12 h. Then, 60 mL anhydrous pyridine was added into a round-bottom flask containing the dried cellulose. This mixture was allowed to stir and heat at 100 °C for 4 h. After slowly adding 12 mL 3,5-dimethylphenyl isocyanate (~85 mmol) into the flask, the mixture was stirred at 100 °C for 48 h. Next, the reaction mixture was cooled to room temperature and slowly poured into a beaker containing ~3 L of methanol followed by stirring for 1–2 h. The obtained white precipitate was filtered and dried under vacuum at 60 °C for 12 h. The yield of the product (CDMPC) was 95%. Elemental analysis showed that the product consisted of C at 64.72%, H at 6.19% and N at 6.78. The IR spectrum displayed 3318cm^{-1} (NH), 1718cm^{-1} (CO), 1615cm^{-1} and 764cm^{-1} (C_6H_6) peaks.

Next, 3 g of dried CDMPC was dissolved in 50 mL anhydrous pyridine. After the CDMPC was completely dissolved, $\text{SO}_3 \cdot \text{Py}$ was added into the resulting solution and the mixture was stirred at 100 °C for 48 h. The reaction mixture was cooled to room temperature and ~70% pyridine was removed by rotoevaporation. The concentrated mixture was slowly poured into a beaker containing ~3L of methanol and stirred for 1–2 h. The obtained white precipitate was filtered and recrystallized with THF followed by drying under vacuum at 60 °C for 12 h. The product (CDMPC- SO_3 , yield 70%) was characterized by elemental analysis and ^{13}C NMR. The NMR chemical shifts of the CDMPC- SO_3 are essentially the same as that of 6- SO_4 -CDMPC (see Supplementary material).

2.4. Preparation of cellulose phenyl carbamate CSPs

The cellulose phenyl carbamate CSPs were prepared through coating bare silica gel particles as described by Chankvetadze [36]. Typically, 0.1 g of synthesized cellulose phenyl carbamate

derivative was dissolved in ~10 mL of THF. The resulting THF solution was added to 0.5 g of bare silica gel followed by evaporation under vacuum. After all the solvent was removed, the CSP was dried under vacuum at 60 °C for 12 h.

2.5. CEC column fabrication

The fused silica capillaries (O.D. 375 μm , I.D. 100 μm), obtained from Polymicro Technologies (Phoenix, AZ, USA) were used to fabricate the columns for CEC-UV and CEC-electrospray ionization (ESI)-MS. The packing procedure was similar to the one reported earlier [37], except that packing solvent and slurry solvent were replaced with hexane/IPA at 90:10 (v/v) instead of ACN. After flushing the column bed with water for 2 h, the inlet and outlet frits were fabricated with a home-made frit burner. A typical CEC-UV column consists of 20 cm packed and 13.5 cm unpacked segment (including 4.5 cm from the inlet side and 9 cm from the outlet side) while a typical CEC-MS column consists of 20 cm packed and 40 cm unpacked segment.

2.6. CEC-UV and CEC-ESI-MS instrumentation

The CEC-UV experiments were carried out with an Agilent capillary electrophoresis system (Palo Alto, CA, USA) interfaced to a diode-array detector. The CEC-ESI-MS experiments were carried out with the same CE instrument interfaced to a single quadrupole mass spectrometer, Agilent 1100 series MSD. An Agilent 1100 series HPLC pump equipped with a 1:100 splitter was used to deliver the sheath liquid. The Agilent Chemstation and CE-MS add-on software (V 10.02) were used for instrument control and calculation of chiral resolution (R_s), selectivity (α), and separation efficiency (N). The N value is reported as the average value of the two enantiomers in this study.

2.7. CEC-UV and CEC-ESI-MS conditions

All of the mobile phases and sheath liquids were degassed for 30 min before use. Before CEC run, the column was preconditioned with the desired mobile phase using a HPLC pump (Lab Alliance, State College, PA, USA) at ~2000 psi for 2 h followed by further conditioning on the CEC instrument for another 2 h. Each injection was performed through high flushing, i.e., applying 6 bars and 6 kV for 6 s. For CEC-UV, separation voltage was set at 10–30 kV (depending on the CEC mode), employing a voltage ramp of 3kV/s. During the separation, an external pressure of 11 bar was applied to inlet buffer vial to suppress bubble formation. For CEC-ESI-MS, separation voltage was set at 15–30 kV (depending on the CEC mode) along with the same voltage ramp and external pressure at inlet as CEC-UV. The following conditions were used unless otherwise stated: sheath liquid, MeOH/H₂O (90:10, v/v) containing 50 mM NH₄OAc, flow rate, 5.0 $\mu\text{L}/\text{min}$; capillary voltage, +3000 V; fragmentor voltage, 80 V; drying gas flow rate, 5L/min; drying gas temperature, 130 °C; nebulizer pressure, 4 psi. The selective ion-monitoring (SIM) mode was set at corresponding polarity to monitor the desired protonated ($[\text{M}+\text{H}]^+$) or deprotonated ($[\text{M}-\text{H}]^-$) molecular ions for each investigated analyte.

3. Results and discussion

3.1. Preparation and characterization of negatively charged CSPs

In this study, two different schemes were developed for synthesizing sulfated and sulfonated cellulose derivatives. For the synthesis of 6-SO₄-CDMPC, an intermediate, 6-OH-CDMPC was first prepared with the procedure developed by Chankvetadze [36]. Next, through reaction with SO₃-DMF, the intermediate was converted regioselectively to 6-position sulfated product, which contained 4.25% sulfur (Fig. 3). By comparing the ¹³C NMR spectra of 6-OH-CDMPC with 6-SO₄-CDMPC in Fig. 3, it was noted that ¹³C NMR signal of 6-position carbon in the anhydro glucose unit shifted from 60.8 ppm to 63.7 ppm, which supported the conversion of

6-OH to the sulfated product [38]. Besides the 6-SO₄-CDMPC, we also prepared another type of negatively charged polysaccharide CSP, CDMPC-SO₃. As compared to the former, the synthesis procedure for the latter (sulfur content 1.76%) is much simplified, which only involves two steps (Fig. 4).

3.2. Comparison of negatively charged CSPs

The electrochromatographic behaviors of 6-SO₄-CDMPC and CDMPC-SO₃ were investigated with a normal-phase eluent. For comparison purpose, CDMPC and 6-OH-CDMPC coated on bare silica gel at 20% (w/w) were also studied. The enantiomers of Troger's base (TB) and 2,2,2-trifluoro-1-(9-anthryl)ethanol (TFAE) were utilized to evaluate these CSPs. As displayed in Fig. 5a–h, the retention time of both analytes showed a significant decrease in the order of: CDMPC ≈ 6-OH-CDMPC > CDMPC-SO₃ > 6-SO₄-CDMPC. When comparing the EOF of all of these four CSPs (Fig. 5 inset), an opposite order, i.e., 6-SO₄-CDMPC > CDMPC-SO₃ > CDMPC ≈ 6-OH-CDMPC was observed. Therefore, the analysis times for both of the test analytes were shortened by ~50% when using 6-SO₄-CDMPC (Fig. 5c and g). Because 6-SO₄-CDMPC contains a very high content of sulfur (4.25%, w/w), the highest charge density of sulfate groups led to the highest magnitude of EOF. On the other hand, CDMPC-SO₃ consists of moderate amount of sulfur (1.76%, w/w), resulting in the second fastest EOF. These data clearly suggest that a strong correlation exists between the magnitude of EOF and the content of ionizable groups on the CSP.

Several trends are noted when comparing the chiral resolution (R_s) and enantioselectivity (α) values of the four polysaccharide derivatives. For both of TB and TFAE, 6-OH-CDMPC provided lower R_s and α values (Fig. 5b and f, respectively) than CDMPC (Fig. 5a and e) mainly because of the non-enantioselective hydrogen-bonding interaction between 6-position hydroxyl groups on the 6-OH-CDMPC and the chiral analytes. The 6-SO₄-CDMPC led to the lowest R_s and α values for both analytes among all four CSPs (Fig. 5c and g). In particular, TB was less than baseline resolved ($R_s = 1.3$) with this CSP. This remarkable loss on chiral resolving power of 6-SO₄-CDMPC could be related to the existence of abundant charged sites (sulfate group) as well as hydrogen-bonding sites, which strongly decrease the chiral recognition. In addition, these sulfate groups, which are directly attached to cellulose backbone might cause unfavorable disorientation of the highly ordered polysaccharide structure reducing the enantioselectivity [14]. On the other hand, CDMPC-SO₃ showed a ~35% reduced retention time compared to CDMPC (Fig. 5d and h). Note that for TB, this CSP provided similar R_s and α values as CDMPC. Regarding TFAE, the R_s value on CDMPC-SO₃ was 50% lower than CDMPC. However, the enantiomers were still more than baseline resolved.

According to the chromatographic results discussed above, two factors could contribute to faster separation without compromising the resolution using CDMPC-SO₃: (1) lower content of ionizable groups resulting in less non-enantioselective interactions and (2) the sulfonated groups are attached on phenyl rings instead of cellulose backbones, resulting in less disturbance of high-ordered structure of cellulose. Overall, note that the CDMPC-SO₃ CSP provided a reasonable compromise between chiral R_s and analysis time. Furthermore, the synthesis of this CSP (Fig. 4) is much simpler and involves only two steps compared to the four steps required for the synthesis of 6-SO₄-CDMPC (Fig. 3). Therefore, the CDMPC-SO₃ CSP was selected as the optimum CSP for further characterization.

3.3. Ion-exchange retention for CDMPC-SO₃

Due to the existence of sulfonate groups on CDMPC-SO₃, this CSP is likely to demonstrate a certain degree of cation-exchange behaviors. To confirm the aforementioned hypothesis, the enantioseparation and retention trends of pindolol were studied with an acidic methanol as mobile phase, in which NH₄COOH concentration was varied from 2mM to 8mM. As the ionic

strength increased, the increase of dead time (t_0) indicated that the magnitude of the EOF is gradually decreased due to the reduced double-layer thickness (Fig. 6). Despite a decrease in EOF, a significant reduced retention of the positively charged pindolol clearly suggests the presence of cation-exchange effect. While the CDMPC-SO₃ CSP showed fairly high enantioselectivity for (±)-pindolol, this pair of enantiomers was barely baseline resolved because severe peak tailing led to poor efficiency. Although it is not clear at this time, the possible reasons for peak splitting observed in Fig. 6 is most likely related to either a mismatch between the %composition of methanol in the running buffer and injected electrolyte or a baseline turbulence caused by t_0 .

3.4. Optimizing the %loading and pore size

Before evaluation of the enantioselectivity for a broad range of the analytes, we first investigated the loading effects of CDMPC-SO₃ CSP, silica gel particle size and pore size. The polysaccharide selector is usually coated or covalently bonded on the silica gel. Previous studies [25,29] have shown that higher loading of polysaccharide CSP on silica gel could improve the enantiomeric resolutions but result in prolong analysis time due to the poor EOF. In this study, CDMPC-SO₃ was coated on silica gel at 10% (w/w) and 20% (w/w) and the performance was compared using methanol as mobile phase. In contrast to the results obtained on CDMPC CSP by Girod et al. [25] and Chankvetadze et al. [29], Table 1 shows that the magnitude of EOF velocity under these two loadings were essentially the same ($8.3 \times 10^{-5} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$ and $8.8 \times 10^{-5} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$) for loading at 10% (w/w) and 20% (w/w), respectively. This different trend of EOF and chiral selector loading observed in this study is probably related to a different mechanism for the EOF generation on the CDMPC-SO₃ CSP as compared to CDMPC CSP. In particular, with the use of this CSP, the EOF generation is mainly relied on negatively charged sulfonate groups on the coating surface instead of silanol groups on the silica gel surface. Although the contribution of EOF from silanol group could be masked at higher CSP loading, there was no net change in the magnitude of EOF. As a result, no significant change in the analysis times for TB and aminoglutethimide (AGT) was observed at 1000 Å silica with two different CSP loadings (Table 1). However, better resolutions were achieved at high chiral selector loading. For example, with CSP loading at 20% (w/w), the R_s values for both analytes were significantly improved due to much better selectivity as compared to CSP loading at 10% (w/w). Previous studies [14,25,29] have shown that higher loading of polysaccharide selector on silica gel could improve the enantiomeric resolutions but result in prolong analysis time due to the poor EOF.

The HPLC studies have shown that the use of wide-pore silica is one of the key factors to improve the polysaccharide CSP loading. Due to the macromolecular size of polysaccharide chiral selector, wide-pore silica particles showed much more success than the small-pore size particles mainly due to better pore accessibility with the former, which in turn improves the CSP loading capacity [14,24]. Girod et al. [24] noticed in their CEC study using CDMPC-coated CSP that the silica gel particles with larger pore size provided faster EOF and higher efficiency due to enhanced perfusion. In this study, the CDMPC-SO₃ was coated onto 5 μm silica gel particles with pore size of 300 Å, 1000 Å and 4000 Å. However, as listed in Table 1 under a fixed CSP loading of 20% (w/w), a slight decrease in EOF and longer retention times for the enantiomers of TB and AGT were observed when the silica pore size is increased. Hence, the lowest R_s and α values were achieved with 4000 Å silica although it provided slightly better efficiency among the three silica of variable pore size.

3.5. Evaluation of CDMPC-SO₃ under various mobile phase modes

It has been well recognized that the type of mobile phase and its composition have strong impact on the enantioselective interactions between the CSP and the analyte. Thus, followed by the optimization of the stationary phase, the effects of mobile phase type and composition

were explored with the enantioseparations of several selected analytes, including TB, AGT, flavanone, phenylthiohydantoin (PTH) leucine, and benzoin. Three mobile phase modes, the NP, POP and RP modes [14,39], were investigated on the CDMPC-SO₃ as discussed below.

Chen et al. [31] found that the hexane content in mobile phase had strong impact on the magnitude of EOF, analyte retention as well as selectivity and resolution using polysaccharide CSP under NP-CEC conditions. In this study, we compared the performances of two NP eluents containing hexane/EtOH at 20:80 and 40:60 (v/v), 8mM NH₄COOH using PTH-leucine and benzoin as model analytes. A slight decrease in the EOF mobility (from $2.2 \times 10^{-5} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$ to $2.0 \times 10^{-5} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$) was noticed when the hexane content increased from 20% (condition A, Table 2) to 40% (condition B, Table 2, v/v), which is in agreement with the observation of Chen et al. [31]. As a result, longer retention of benzoin and PTH-leucine were also observed. Furthermore, the reduction of EtOH (as a polar solvent and a hydrogen-bonding donor) content in the mobile phase only slightly increased the retention factors and enantioselectivity, which led to the improvements in the chiral resolution (Table 2, last column). It was also found that when hexane content in the mobile phase was more than 50% (v/v), a much slower EOF was obtained (data not shown). Therefore, for the NP-CEC studies, a mobile phase containing hexane:EtOH at 40:60 (v/v) was adopted.

For the POP mode, several solvents such as MeOH, EtOH and ACN have been utilized as mobile phases for polysaccharide CSPs [10,27]. Girod et al. [10] found that the use of EtOH could improve both separation efficiency and selectivity but at the expense of prolonged retention time. Later, Chankvetadze et al. [27] noticed a certain degree of complementarity on the enantioseparations for a few chiral analytes with MeOH or ACN as mobile phase. Because 100% ACN caused a slight swelling of the CDMPC-SO₃, which in turn led to column clogging, our efforts were mainly focused on the comparison of 99:1 MeOH/HOAc (condition C, Table 2) and 99:1 EtOH/HOAc (condition D, Table 2) using AGT and flavanone as test analytes. With the replacement of MeOH with EtOH in the mobile phase, a significant reduction on the magnitude of EOF was observed (from $8.7 \times 10^{-5} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$ to $2.2 \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$). As a result, the retentions of both analytes were almost doubled even though the applied voltage was increased from 10 kV to 20 kV (condition D, Table 2). In addition, note that the increase in capacity factor is most likely due to higher viscosity of ethanol (Table 2). Thus, better enantiomeric resolutions of both analytes were achieved with the use of EtOH than MeOH (Table 2).

In the RP modes, we compared two mobile phases containing 40% (v/v) ACN (condition F, Table 2) and 70% (v/v) ACN (condition E, Table 2), respectively. A low pH mobile phase was used because it provided good peak shape and a smooth baseline. The mobile phase containing 70% (v/v) ACN provided slightly faster EOF ($1.4 \times 10^{-4} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$) than the 40% (v/v) ACN ($1.2 \times 10^{-4} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$) due to lower viscosity but much higher eluting strength. Baseline resolutions of model compounds, such as (±)-TB were easily achieved with the use of 70% (v/v) ACN. However, for the other model compounds like PTH-Leu and flavanone, no sign of enantiomeric resolution were obtained due to poor retention and chiral selectivity (Table 2). When reducing the ACN content (v/v) in the mobile phase to 40% (condition F, Table 2), significant improvements on the retention factor, efficiency and chiral selectivity were observed for both PTH-Leu and flavanone, which in turn enhanced the enantiomeric resolutions. Note that the analysis time for flavanone was 3–5 times longer with 40% (v/v) ACN than with 70% (v/v) ACN (Table 2), and TB cannot be eluted even within 2 h. As a result, both of the 40% and 70% (v/v) ACN could be utilized for the RP-CEC studies. Therefore, 40% (v/v) ACN should be applied for the poorly retained compounds, whereas 70% (v/v) ACN could be used to elute the highly retained analytes.

Similar to aforementioned NP condition, the CDMPC-SO₃ resulted in significantly shorter analysis time than CDMPC under POP and RP conditions (Fig. 7 and Fig. 8, respectively). As mentioned earlier, on CDMPC-SO₃, the sulfonate groups are in direct contact with the mobile phase counter ions, which results in stronger EOF. The overall *N* of enantiomers seems to be higher using CDMPC-SO₃ than CDMPC in the RP, whereas the *N* values seem to be comparable in the POP mode of CEC. The *R_s* and α values of AGT and warfarin in POP (Fig. 7) are comparable to those obtained in the RP (Fig. 8) using either CDMPC or CDMPC-SO₃ as CSP. However, it is interesting to note for TB, POP-CEC (Fig. 7) provided at least twofolds higher *R_s* and *N* values than RP mode of CEC. Furthermore, the enantiomers of TFAE also showed much higher *R_s* in POP-CEC using CDMPC. On the other hand, baseline *R_s* was still obtained in POP or RP modes of CEC using CDMPC-SO₃ as CSP with a significant decrease in analysis time.

3.6. Chiral screening with CDMPC-SO₃ CSP

To understand the enantioselectivity and resolving power of the CDMPC-SO₃ CSP, a total of 66 chiral compounds listed in Table 3 were selected for chiral screening. The analytes were first classified into neutral, acidic and basic compounds, and then discussed in group based on their structural similarity. The classification is based on the charged state of the analyte under the pH conditions of the mobile phase used in CEC. Various mobile phases under NP, POP and RP modes, which were optimized in the previous section, were used to evaluate the chromatographic parameters, such as capacity factor *k'*₁, *N*, α and *R_s*. In the following discussion, the analytes were classified based on their structural similarity and acid/base characteristics, and the enantioselectivity as well as the resolution values were investigated.

3.6.1. Neutral analytes—Benzoin and its derivatives (analytes 4–8, Table 3) were best resolved under RP, in which almost all of them were baseline separated, except hydrobenzoin. With NP eluent, three out of five benzoin derivatives showed resolution. In contrast, under POP condition, only benzoin and benzoin methyl ether can be resolved. The lower *R_s* can be related to the poor retention (or low *k'*₁) under NP and POP, which limited the enantioselective interactions. In addition, note that steric interaction seemed to be important in chiral recognition under NP condition. For example, comparing NP separation of benzoin, benzoin methyl ether and benzoin ethyl ether, the α values decreased from 1.599 to 1.000 as the size of substitution group linked to the chiral center increased from hydroxyl to ethoxy group.

For the three phenyl alcohols (analytes 10–12, Table 3), no resolution was observed with NP and POP eluents due to poor retention. However, partial resolutions for α -cyclopropylbenzyl alcohol and 1-phenylpropanol were obtained with RP eluent. Furthermore, hydrogen bonding seems to play a crucial role in the chiral recognition. For example, *trans*-2-phenylcyclohexanol (analyte 11, Table 3) with hydrogen-bond donor hydroxyl group cannot be enantioresolved under any of the three mobile phase modes. In contrast, its analog, 2-phenylcyclohexanone (analyte 9, Table 3) with hydrogen-bonding acceptor, ketone group can be easily resolved under all three modes.

All three benzodiazepines (analytes 14–16, Table 3) showed excellent α and *R_s* with the RP eluent and were much more than baseline separated. Interestingly, all three analytes can be also resolved with POP but with lower retention and *R_s*. However, NP showed *R_s* only for oxazepam. No clear trend can be seen for the enantioseparations of this group of structurally similar analytes.

Among the four barbiturates studied (analytes 17–20, Table 3), only mephobarbital showed enantioresolution under three modes of CEC with RP modes providing the highest *R_s* value. Comparing the structures of all four barbitals (Fig. 4), mephobarbital is the only barbital, which

contains an aromatic ring next to the chiral center. Clearly, the possible π - π interaction is important for chiral recognition for this type of analyte.

For three phenylthiohydantoins (analytes 21–23, Table 3), no resolution was observed with NP and POP eluents because of very poor retention. Using RP eluent, baseline resolution for 5-(4-hydroxyphenyl)-5-phenylhydantoin and partial resolution for 5-(4-methylphenyl)-5-phenylhydantoin were obtained, whereas 5-methyl-5-phenyl-hydantoin was not even partially resolved. Note that the first two hydantoins (analytes 22 and 23) have two aromatic rings linked to the chiral center while 5-methyl-5-phenyl-hydantoin (analyte 21) only has one aromatic ring. Therefore, a combination of π - π interaction and steric interaction could be used to explain why some phenylthiohydantoins were better resolved than the others.

The PTH-amino acids (analytes 24–38) were best resolved under NP, in which 13 out of 15 of PTH-amino acids (including cationic PTH-arginine) showed enantioselectivity. The POP and RP eluents can resolve five and eight PTH-amino acids, respectively. Note under all three modes, both PTH-alanine and PTH-serine remained unresolved, which might be related to the size and substitution group in their side chains. For the former, a small side chain, methyl group could be the key; while for the latter, the non-enantioselective hydroxyl group in the side chain could actually undermine the chiral recognition.

Different degree of chiral R_s was achieved for several neutral compounds, including TFAE, flavanone, and glutethimide (analytes 1–3, Table 3) under three mobile phase modes. Besides the chiral compounds containing an asymmetrically substituted carbon atom, we also investigated the enantioseparations for chiral compounds lacking a stereogenic carbon atom. For example, the atropisomers, including 1,1'-bi-2-naphthol (analyte 41, Table 3) and 1,1'-binaphthyl-2,2'-diamine (BNA, analyte 61, Table 3) were separated under both NP and RP modes. However, no R_s was achieved for chiral chelate, Co(acac)₃ (analyte 40, Table 3) in any of the three modes of CEC.

3.6.2. Acidic analytes—For the acidic analytes, our efforts were mainly focused on warfarin and its analogs, non-steroidal anti-inflammatory drugs (NSAIDs) and N-dinitrobenzoyl (DNB) leucine. Besides these aforementioned compounds, initially our study also included dansyl amino acids, β -phenoxy propionic acids, and 1,1'-binaphthyl-2,2'-diyl-hydrogen-phosphate. However, the latter three compounds were not eluted even after adding 0.2% (v/v) trifluoroacetic acid in the mobile phase.

Warfarin and its analogs (analytes 42–48, Table 3) were best resolved under NP, in which almost all of them were baseline separated, except 10-hydroxywarfarin, which was less than baseline resolved ($R_s = 1.2$). With RP eluent, slightly lower R_s values were obtained, but still four out of seven warfarin and its analogs were baseline resolved. In contrast, under POP condition, only warfarin was baseline separated, whereas no R_s was observed for 10-hydroxywarfarin. The relatively lower enantioselectivity of 10-hydroxywarfarin could be associated with the substitution of the hydroxyl group, which is located next to the chiral center and could disturb the hydrogen bonding between the analyte and chiral selector. Furthermore, comparing warfarin with coumachlor, the former demonstrated a lower retention but higher α values. These results indicated the chlorine substitution enhances the retention but does not improve the enantioselective recognition.

Unlike warfarin and its analogs, the CDMPC-SO₃ provided poorer R_s for the NSAIDs (analytes 49–56, Table 3). Among the three modes, the RP provided the best resolving power, in which four out of eight NSAIDs showed some degree of enantioselectivity. Indoprofen is the only NSAID, which was baseline resolved in RP and partially resolved under NP and POP. These results indicate that for this group of analytes, a bulky substitution group at the *para* position

could be important for chiral recognition. The enantiomers of DNB-leucine (analyte 57, Table 3) can only be resolved under NP conditions, whereas no R_s was obtained under RP and no peak was observed even after 200min for POP eluent.

3.6.3. Basic analytes—Also, the CDMPC-SO₃ CSP showed good chiral selectivity for several basic analytes, including TB, AGT, BNA, PTH-arginine (analytes 58, 59, 61 and 62, Table 3) and β -blockers (analytes 60, 63–67, Table 3). Significantly higher resolution was observed for TB and AGT under NP and POP than the RP mode. The AGT (analyte 59) demonstrated longer retention and better α than its neutral analog, glutethimide (analyte 3). This result confirmed the importance of amino group and positive charge for the retention and chiral recognition.

Unlike the other basic analytes, (β -blockers demonstrated poor retention and enantiomeric resolutions using the mobile phases acidified with HOAc. Because these analytes were positively charged under this condition, they eluted even faster than EOF, which dramatically undermined the chiral recognition. Although pindolol (analyte 60, Table 3) was still baseline resolved under NP and POP, and partially separated under RP, severe peak tailing and lower separation efficiency were observed. By replacing HOAc content in the mobile phase with DEA or HA, the retention and peak shape of these β -blockers were remarkably improved. Among the three modes, the NP provided the best resolving power, in which baseline resolution for pindolol and oxprenolol, and partial resolution for propranolol and carteolol were obtained. Note that pindolol is the only β -blocker showing resolution under all three modes. In particular, under the RP condition, the R_s value as high as 9.2 was achieved. However, no R_s was observed for talinolol.

Generally, using CDMPC-SO₃ CSPs with NP and POP eluents, the analytes showed much lower retention ($k'_1 < 1$) than RP eluent. Comparison of three modes of mobile phase (Fig. 9) reveals that the RP eluent provided the highest success rate for resolution (65%), by which ~40% of the analytes provided very good-to-excellent resolution ($R_s > 1.5$). The NP eluent led to the second best success rate at ~60%, by which 20% of the analytes had $R_s > 1.5$. Finally, the POP eluent only provided 40% success rate and only 10% of the analytes were baseline resolved. Overall, among all 53 compounds showing various degree of resolution in any mode, 21 analytes were commonly resolved under all three mobile phase modes.

3.7. Applications of CDMPC-SO₃ CSP for CEC-MS

In previous sections, we have shown that CDMPC-SO₃ CSP is an excellent CSP for a broad range of chiral analytes under various mobile phase conditions in chiral CEC-UV. Therefore, in this section, we report our investigation of the feasibility of applying this CSP for NP, POP and RP CEC-MS. Furthermore, the ruggedness of CEC-MS was studied using internal tapered columns packed with CDMPC-SO₃ CSP.

First, the CEC-MS enantioseparations using CDMPC-SO₃ CSP were conducted with a NP eluent. Because no report on NP CEC-MS has been published before, initial sheath liquid composition was selected based on our previous CEC-MS studies under POP [40]. However, significantly high noise levels were observed when a sheath liquid containing 90% (v/v) MeOH was utilized (Fig. 10a). When this sheath liquid was replaced with 90% (v/v) EtOH or IPA, the noise level was significantly suppressed, which in turn improved the detection sensitivity. For example, the signal-to-noise ratio (S/N) of TB was enhanced more than 10-folds with the 90% (v/v) IPA (Fig. 10b). This difference in observed noise level could be caused by the longer chain alcohol (e.g., IPA), which has better miscibility with hexane than MeOH. Besides TB and warfarin (Fig. 11a and b), other analytes, such as AGT, BNA, coumachlor, mephobarbital and DNB-leucine were also resolved under NP CEC-MS condition (Table 4) with excellent efficiency (Table 4).

The use of CDMPC-SO₃ CSP in CEC-MS enantioseparations (Fig. 11c–f) under POP and RP conditions (conditions B–E, Table 4) was also explored. Unlike the aforementioned NP CEC-MS, a sheath liquid based on 90% (v/v) MeOH showed good compatibility under these two modes. Using POP conditions, TB, warfarin (Fig. 11c and d), AGT, coumachlor, pindolol and mephobarbital could be resolved with MeOH or EtOH as mobile phase (Table 4). The replacement of MeOH with EtOH in the mobile phase slightly improved the resolution for TB, but the analysis time almost doubled (condition C in Table 4 vs. Fig. 11c). Under RP condition, a mobile phase containing 70% (v/v) ACN provided the shortest analysis time along with high efficiency for TB and warfarin (Fig. 11e and f), coumachlor as well as several other acidic, basic and neutral enantiomers, which can be ionized in the gas phase (Table 4). The resolution for poorly retained compounds could be significantly improved by lowering ACN content in mobile phase from 70% to 40%. For example, mephobarbital, 5-(4-hydroxy-phenyl)-5-phenylhydantoin, lorazepam, oxazepam, along with six PTH-amino acids were at least partially resolved under this condition (Table 4).

To understand the effects on detection sensitivity by using different CEC-MS modes, the S/N ratios of TB and warfarin in positive or negative ion modes of ESI were plotted in Fig. 11g. Overall, the highest sensitivity is obtained under RP mode for both of the analytes. The NP mode led to the lowest sensitivity because of aforementioned ESI-MS signal suppression by hexane in the mobile phase.

Finally, the CEC-MS reproducibility studies were conducted on internal tapered columns packed with CDMPC-SO₃ CSP. The AGT was utilized as test solute for 20–30 injections each day for 3 consecutive days. The data showed excellent precision for retention time (columns 3 and 4, Table 5) and α (column 6, Table 5) during the test. Under all three CEC-MS modes, the intra-day RSD for retention time of each enantiomer was within 0.3–2.1% and the inter-day RSD was 1.3–2.2%. On the other hand, the α values showed minimum variation with intra-day RSD of only 0.04–0.2% and inter-day RSD 0.1–0.2% (Table 5). Although the values of average efficiency and resolution showed relatively higher fluctuations (inter-day RSD 11–17% and 4.8–10%, respectively), clearly the column maintained excellent N and R_s throughout the investigation.

4. Concluding remarks

For the first time, two negatively charged polysaccharide CSPs, namely 6-SO₄-CDMPC and CDMPC-SO₃ were prepared and evaluated for CEC applications. With different content of cation-exchange groups bonded on the two CSPs, faster EOF enhances the throughput of CEC chiral analysis using a NP eluent. However, CDMPC-SO₃ CSP maintained the enantiomeric resolving power of CDMPC better than 6-SO₄-CDMPC. The electrochromatographic behavior of a positively charged analyte, pindolol under mobile phases with different ionic strength clearly indicated that the cationic-exchange mechanism is operative on CDMPC-SO₃. In addition, CSP loading and silica particle pore size had strong impact on the throughput and enantioselectivity. For example, the increase of the CDMPC-SO₃ CSP loading favorably improved the enantioselectivity while the magnitude of EOF kept unchanged. This independence of EOF with CSP loading confirmed our hypothesis that the sulfonate groups instead of silanols on the silica gel are the major source for EOF generation. Furthermore, the use of a large pore size (1000 Å) silica gel demonstrated good compromise between the throughput and enantioselectivity.

Using the chiral screening approach, the enantioselectivity of the CDMPC-SO₃ CSP was evaluated with NP, POP and RP modes of CEC. This CSP demonstrated excellent resolving power for a broad range of analytes, such as benzoin derivatives, phenyl alcohol, benzodiazepines, barbiturates, PTH amino acids, warfarin analogs, NSAIDs, and β -blockers.

Among the three mobile phase modes investigated, the RP mode showed the highest success rates. For example, up to 65% analytes displayed good R_s and ~40% were baseline resolved in RP.

The feasibility of applying the developed CSP for chiral CEC–MS was investigated under various mobile phase conditions. The type of alcohol in sheath liquid is critical for the detection sensitivity in NP CEC–MS. Lower noise level and better detection sensitivity were achieved by replacing MeOH in sheath liquid with IPA because the latter provides good miscibility with hexane. Furthermore, several basic, acidic and neutral analytes were resolved under the CEC–MS condition with excellent N and R_s . In addition, internal tapered columns packed with CDMPC-SO₃ demonstrated excellent durability (60–90 injections for 3 days) and precision of retention time (intra-day RSD 0.3–2.1%, inter-day RSD 1.3–2.2%) and α (intra-day RSD 0.04–0.2%, inter-day RSD 0.1–0.2%). The ruggedness of CEC–MS was explored by switching the mobile phase mode from NP to RP or *vice versa* using EtOH as replacement solvent to flush the column for 3 h. Very reproducible enantioselectivity was obtained when changing from NP to RP or *vice versa*.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Appendix

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi: 10.1016/j.chroma.2008.11.082.

References

- Bartle, KD.; Cikalo, MG.; Robson, MM. Capillary Electrochromatography. Vol. 1st ed.. Bartle, KD.; Myers, ZP., editors. Cambridge, UK: Royal Society of Chemistry; 2001. p. 1
- Dittmann MM, Wienand K, Bek F, Rozing GP. LC-GC 1995;13:803.
- Dittmann MM, Rozing GP. J. Chromatogr. A 1996;744:63.
- Lloyd DK, Li S, Ryan P. J. Chromatogr. A 1995;694:285.
- Lelievre F, Yan C, Zare RN, Gareil P. J. Chromatogr. A 1996;723:145.
- Karlsson C, Karlsson L, Armstrong DW, Owens PK. Anal. Chem 2000;72:4394. [PubMed: 11008775]
- Dermaux A, Lynen F, Sandra P, High Resolut J. Chromatogr 1998;21:575.
- Owens PK, Karlsson L, Lutz ESM, Andersson LI. Trends Anal. Chem 1999;18:146.
- Maruska A, Ericson C, Vegvari A, Hjerten S. J. Chromatogr. A 1999;837:25.
- Girod M, Chankvetadze B, Blaschke G. J. Chromatogr. A 2000;887:439. [PubMed: 10961332]
- Lammerhofer M, Tobler E, Lindner W. J. Chromatogr. A 2000;887:421. [PubMed: 10961331]
- Wolf C, Spence PL, Pirkle WH, Cavender DM, Derrico EM. Electrophoresis 2000;21:917. [PubMed: 10768777]
- Wolf C, Spence PL, Pirkle WH, Derrico EM, Cavender DM, Rozing GP. J. Chromatogr. A 1997;782:175.

14. Okamoto Y, Yashima E. *Angew. Chem., Int. Ed* 1998;37:1021.
15. Thayer A. *Chem. Eng. News* 2005;83:49.
16. Okamoto Y, Kawashima M, Hatada K. *J. Am. Chem. Soc* 1984;106:5357.
17. Francotte E, Jung M. *Chromatographia* 1996;42:521.
18. Otsuka K, Mikami C, Terabe S. *J. Chromatogr. A* 2000;887:457. [PubMed: 10961333]
19. Kawamura K, Otsuka K, Terabe S. *J. Chromatogr. A* 2001;924:251. [PubMed: 11521871]
20. Mayer S, Briand X, Francotte E. *J. Chromatogr. A* 2000;875:331. [PubMed: 10839153]
21. Mangelings D, Hardies N, Maftouh M, Suteu C, Massart DL, Vander Heyden Y. *Electrophoresis* 2003;24:2567. [PubMed: 12900869]
22. Mangelings D, Maftouh M, Massart DL, Vander Heyden Y. *Electrophoresis* 2004;25:2808. [PubMed: 15352013]
23. Mangelings D, Tanret I, Matthijs N, Maftouh M, Massart DL, Vander Heyden Y. *Electrophoresis* 2005;26:818. [PubMed: 15714567]
24. Girod M, Chankvetadze B, Blaschke G. *Electrophoresis* 2001;22:1282. [PubMed: 11379949]
25. Girod M, Chankvetadze B, Okamoto Y, Blaschke G. *J. Sep. Sci* 2001;24:27.
26. Chankvetadze B, Kartoza I, Breitzkreutz J, Girod M, Knobloch M, Okamoto Y, Blaschke G. *J. Sep. Sci* 2001;24:251.
27. Chankvetadze B, Kartoza I, Breitzkreutz J, Okamoto Y, Blaschke G. *Electrophoresis* 2001;22:3327. [PubMed: 11589297]
28. Chankvetadze B, Kartoza I, Yamamoto C, Okamoto Y, Blaschke G. *J. Pharm. Biomed. Anal* 2003;30:1897. [PubMed: 12485732]
29. Chankvetadze L, Kartoza I, Yamamoto C, Chankvetadze B, Blaschke G, Okamoto Y. *Electrophoresis* 2002;23:486. [PubMed: 11870751]
30. Chen X, Jin W, Qin F, Liu Y, Zou H, Guo B. *Electrophoresis* 2003;24:2559. [PubMed: 12900868]
31. Chen X, Zou H, Ye M, Zhang Z. *Electrophoresis* 2002;23:1246. [PubMed: 12007123]
32. Chen X, Qin F, Liu Y, Liang K, Zou H. *Electrophoresis* 2004;25:2817. [PubMed: 15352014]
33. Tobler E, Lammerhofer M, Wuggenig F, Hammerschmidt F, Lindner W. *Electrophoresis* 2002;23:462. [PubMed: 11870749]
34. Zarbl E, Lammerhofer M, Woschek A, Hammerschmidt F, Parenti C, Cannazza G, Lindner W. *J. Sep. Sci* 2002;25:1269.
35. Hebenstreit D, Bicker W, Lammerhofer M, Lindner W. *Electrophoresis* 2004;25:277. [PubMed: 14743480]
36. Chankvetadze B. *Methods in Molecular Biology* (Totowa, NJ, United States). Gerald Gübitz, MG.; Schmid, editors. Totowa, NJ: Humana Press; 2004. p. 387
37. Zheng J, Shamsi SA. *J. Chromatogr. A* 2003;1005:177. [PubMed: 12924792]
38. Richter A, Klemm D. *Cellulose* 2003;10:133.
39. Tachibana K, Ohnishi A. *J. Chromatogr. A* 2001;906:127. [PubMed: 11215884]
40. Zheng, J.; Shamsi Shahab, A. Chapter 14. In: Subramanian, G., editor. *Chiral Separation Techniques*. Weinheim: Wiley-VCH; 2007. p. 480

Fig. 1. Schematic of negatively charged CDMPC CSP containing covalent-bonded cation-exchange groups, which are high accessible to the mobile phase counter ions. The cation-exchange groups (e.g., sulfate) are denoted as “ X^- ”.

Neutral analytes

Neutral analytes (II)

Acidic analytes

warfarin and its analogs

warfarin: $R_4', R_6, R_7, R_8, R_{10} = H$
 coumachlor: $R_4' = Cl, R_6, R_7, R_8, R_{10} = H$
 4'-hydroxy warfarin: $R_4' = OH, R_6, R_7, R_8, R_{10} = H$
 6-hydroxy warfarin: $R_6 = OH, R_4', R_7, R_8, R_{10} = H$
 7-hydroxy warfarin: $R_7 = OH, R_4', R_6, R_8, R_{10} = H$
 8-hydroxy warfarin: $R_8 = OH, R_4', R_6, R_7, R_{10} = H$
 10-hydroxy warfarin: $R_{10} = OH, R_4', R_6, R_7, R_8 = H$

DNB-leucine

Basic analytes

Fig. 2.

Structures of chiral analytes investigated in this study (classified as neutral, acidic and basic compounds). The chiral center is denoted as “*”.

Fig. 3. Synthetic scheme and ¹³C NMR spectra for 6-sulfated cellulose tris(3,5-dimethylphenyl carbamate) (6-SO₄-CDMPC).

Elemental Analysis for 4 CSPs

Element	Elemental Analysis Found (%)			
	CDMPC	6-OH CDMPC	6-Sulfated CDMPC	CDMPC-SO ₃
C	64.78	64.26	57.06	61.27
H	6.24	6.24	5.40	5.89
N	6.82	6.56	6.61	6.79
S	0	0	4.25	1.76

Fig. 4. Synthetic scheme for sulfonated cellulose tris(3,5-dimethylphenyl carbamate) (CDMPC-SO₃). The bottom part shows the elemental analysis (C, H, N and S) data for CDMPC, 6-OH-CDMPC, 6-SO₄-CDMPC and CDMPC-SO₃.

Fig. 5. Comparison of NP CEC enantioseparations of (±)-TB (a–d) and (±)-TFAE (e–h) using four different cellulose 3,5-dimethylphenyl carbamate CSPs. Mobile phase: Hex/EtOH/HOAc 40:60:1 (v/v/v) containing 8 mM NH_4COOH , 30kV. For other conditions see Section 2.

Fig. 6. Comparison of CEC enantioseparations of ($\pm$)-pindolol with different NH_4COOH content in POP eluents using CDMPC- SO_3 CSP. Mobile phase: MeOH/HOAc 99:1 (v/v) containing (a) 2 mM; (b) 4 mM; (c) 8 mM NH_4COOH , 10 kV. For other conditions see Section 2.

Fig. 7. Comparison of POP CEC enantioseparations of four chiral compounds using CDMPC (a–d) and CDMPC-SO₃ (e–h). Mobile phase: EtOH/HOAc 99:1 (v/v) containing 8 mM NH₄COOH, 20kV. For other conditions see Section 2.

Fig. 8. Comparison of RP CEC enantioseparations of four chiral compounds using CDMPC (a–d) and CDMPC-SO₃ (e–h). Mobile phase: ACN/H₂O 70:30 (v/v) containing 5 mM NH₄COOH at pH 3.5, 15kV. For other conditions see Section 2.

Fig. 9.

Bar plot showing the enantiomeric resolution values from the chiral screening with NP, POP and RP eluents using CDMPC-SO₃ CSP. The R_s values were classified as poor ($R_s = 0$), fair-to-good ($0 < R_s < 1.5$) and good-to-excellent ($R_s > 1.5$). For conditions of all three modes see Table 3.

Fig. 10.

Plots (a) and (b) showing the effects of different alcohols as sheath liquids on the noise level (calculated by six times standard deviation) and signal-to-noise (S/N) for NP CEC-MS enantioseparation of ($\pm$)-TB. Mobile phase: Hex/EtOH/HOAc 40:60:1 (v/v/v) containing 8 mM NH_4COOH , 30kV. For other conditions see Section 2.

Fig. 11. Electropherograms of CEC-MS enantioseparations and S/N plot of ($\pm$)-TB and ($\pm$)-warfarin with different modes of mobile phase using CDMPC-SO₃ CSP. (a and b) NP; (c and d) POP; (e and f) RP; (g) plot of S/N for ($\pm$)-TB and ($\pm$)-warfarin. For the conditions see Table 4 or Section 2.

Table 1

Polar organic CEC enantioseparations of Troger's base and aminogluthethimide using sulfonated CDMPC CSP coated on different pore size of silica gel particles.

Pore size (Å)	CSP loading (% w/w)	EOF (cm ² V ⁻¹ s ⁻¹)	Analytes	t _{R2} (min)	N	a	R _s
300	20	1.0 × 10 ⁻⁴	TB	20.6	12,000	1.612	4.0
			AGT	17.2	12,800	1.406	2.2
1000	10	8.3 × 10 ⁻⁵	TB	22.1	12,500	1.401	3.1
			AGT	19.4	21,000	1.230	1.9
	20	8.8 × 10 ⁻⁵	TB	22.7	15,800	1.735	5.3
			AGT	19.3	14,000	1.417	2.5
4000	20	8.7 × 10 ⁻⁵	TB	25.0	15,000	1.299	3.0
			AGT	19.5	19,000	1.324	2.0

Condition: mobile phase MeOH/HOAc 99:1 (v/v) containing 8mM NH₄COOH, 10kV.

Table 2
CEC enantioseparations using sulfonated CDMPC CSP with different modes of mobile phases.

Condition	EOF (cm ² V ⁻¹ s ⁻¹)	Analytes	t _{R2} (min)	k' ₁	k' ₂	N	α	R _s
Normal phase								
A	2.2 × 10 ⁻⁵	Benzoin	23.0	0.13	0.16	25,000	1.216	1.0
A		PTH-leucine	22.2	0.13	0.15	15,000	1.144	0.5
B	2.0 × 10 ⁻⁵	Benzoin	25.6	0.16	0.21	19,500	1.362	1.7
B		PTH-leucine	25.7	0.18	0.22	15,900	1.211	1.0
Polar organic phase								
C	8.7 × 10 ⁻⁵	AGT	19.3	0.25	0.36	14,000	1.417	2.5
C		Flavanone	15.16	0.09	–	6,100	1.000	0
D	2.2 × 10 ⁻⁵	AGT	36.3	0.39	0.56	15,000	1.431	3.5
D		Flavanone	30.4	0.27	0.3	14,600	1.097	0.6
Reversed phase								
E	1.4 × 10 ⁻⁴	TB	10.1	0.60	0.69	17,500	1.145	1.7
E		PTH-Leu	6.66	0.12	0.12	4,000	1.000	0
E		Flavanone	8.55	0.44	0.44	8,700	1.000	0
F	1.2 × 10 ⁻⁴	PTH-Leu	22.6	2.03	2.40	7,200	1.181	2.1
F		Flavanone	40.6	4.55	4.72	13,000	1.037	0.8

Conditions: (A) mobile phase hexane/EtOH/HOAc 20:80:1 (v/v/v) containing 8 mM NH₄COOH, 30 kV; (B) mobile phase hexane/EtOH/HOAc 40:60:1 (v/v/v) containing 8 mM NH₄COOH, 30kV; (C) mobile phase MeOH/HOAc 99:1 (v/v) containing 8 mM NH₄COOH, 10kV; (D) mobile phase EtOH/HOAc 99:1 (v/v) containing 8mM NH₄COOH, 20kV; (E) mobile phase 70% (v/v) ACN containing 5mM NH₄COOH at pH 3.5, 15 kV; (F) mobile phase 40% (v/v) ACN containing 5mM NH₄COOH at pH 3.5, 15kV.

Table 3

Comparison of CEC enantioseparations with normal phase, polar organic phase and reversed-phase eluents using sulfonated CDMPC CSP.

Analyte	Normal phase ^a			Polar organic phase ^b			Reversed phase ^c			
	<i>k'</i> ₁	<i>a</i>	<i>R</i> _s	<i>k'</i> ₁	<i>a</i>	<i>R</i> _s	Mobile phase	<i>k'</i> ₁	<i>a</i>	<i>R</i> _s
Neutral analytes										
1	0.09	2.568	4.1	0.08	1.710	1.7	E	0.37	1.301	1.6
2	0.21	1.226	1.4	0.34	1.082	0.6	F	4.55	1.037	0.8
3	0.22	1.180	0.8	0.25	1.151	0.9	F	2.04	1.248	5.1
4	0.09	1.599	1.7	0.19	1.136	0.6	F	1.49	1.164	3.3
5	0.09	1.525	1.4	0.25	1.000	0.0	F	1.35	1.166	3.3
6	0.10	1.000	0.0	0.14	1.000	0.0	F	8.37	1.138	2.1
7	0.14	1.210	0.9	0.17	1.100	0.5	F	1.83	1.174	3.8
8	0.13	1.000	0.0	0.19	1.000	0.0	F	1.27	1.046	0.8
9	0.17	1.192	1.0	0.18	1.231	1.2	F	1.85	1.088	2.1
10	0.00	1.000	0.0	0.04	1.000	0.0	F	0.57	1.083	1.2
11	0.00	1.000	0.0	0.05	1.000	0.0	F	1.19	1.000	0.0
12	0.00	1.000	0.0	0.02	1.000	0.0	F	0.54	1.073	1.1
13	0.09	1.193	0.5	0.06	1.000	0.0	F	0.12	1.167	0.6
14	0.35	1.000	0.0	0.05	5.003	3.3	F	1.01	1.405	5.2
15	0.31	1.737	2.9	0.20	1.647	1.1	F	1.00	1.482	5.3
16	0.57	1.000	0.0	0.43	1.260	1.1	F	1.69	1.343	4.9
17	0.32	1.000	0.0	0.22	1.000	0.0	F	0.83	1.000	0.0
18	0.22	1.216	1.4	0.14	1.148	1.0	F	0.96	1.163	2.2
19	0.05	1.000	0.0	0.03	1.000	0.0	F	0.45	1.000	0.0
20	0.05	1.000	0.0	0.03	1.000	0.0	F	0.71	1.000	0.0
21	0.11	1.000	0.0	0.08	1.000	0.0	F	0.16	1.000	0.0
22	0.11	1.000	0.0	0.04	1.000	0.0	F	0.29	1.264	1.6
23	0.12	1.000	0.0	0.08	1.000	0.0	F	1.58	1.063	0.9
24	0.54	1.333	3.1	0.27	1.356	1.1	F	0.06	1.000	0.0
25	0.19	1.147	0.6	0.17	1.000	0.0	F	1.74	1.109	1.7
26	0.31	1.157	1.2	0.23	1.216	1.0	F	2.15	1.000	0.0

Analyte	Normal phase ^a			Polar organic phase ^b			Reversed phase ^c					
	<i>k'</i> ₁	<i>a</i>	<i>R</i> _s	<i>k'</i> ₁	<i>a</i>	<i>R</i> _s	Mobile phase	<i>k'</i> ₁	<i>a</i>	<i>R</i> _s		
27	PTH-serinine	0.26	1.000	0.0	0.18	1.000	0.0	F	0.09	1.000	0.0	
28	PTH-alanine	0.23	1.000	0.0	0.18	1.000	0.0	F	0.38	1.000	0.0	
29	PTH- α -amino-butyric acid	0.22	1.093	0.5	0.21	1.000	0.0	F	0.84	1.000	0.0	
30		PTH-leucine	0.20	1.194	1.0	0.18	1.000	0.0	F	2.03	1.181	2.1
31		PTH-methionine	0.33	1.092	0.6	0.17	1.000	0.0	F	1.86	1.105	1.5
32	PTH- α -amino-caprylic acid	0.18	1.138	0.5	0.18	1.000	0.0	F	0.20	1.000	0.0	
33		PTH-norvaline	0.22	1.144	0.6	0.19	1.000	0.0	F	1.09	1.123	1.3
34		PTH-proline	0.49	1.116	1.2	0.31	1.093	0.5	F	1.56	1.000	0.0
35	PTH-tryptophan	0.51	1.097	0.8	0.30	1.099	0.5	F	3.45	1.074	1.1	
36	PTH-tyrosine	0.27	1.055	0.4	0.18	1.000	0.0	F	0.68	1.000	0.0	
37	PTH-valine	0.22	1.176	1.0	0.17	1.156	0.5	F	1.06	1.071	1.0	
38	PTH-norleucine	0.16	1.165	0.7	0.16	1.000	0.0	F	1.23	1.085	1.3	
39	Thalidomide	0.04	1.000	0.0	0.08	1.000	0.0	F	0.66	1.000	0.0	
40	Co(acac) ₃	0.15	1.000	0.0	0.06	1.000	0.0	F	0.26	1.000	0.0	
41	1,1'-Bi-2-naphthol	0.20	1.185	1.0	0.11	1.000	0.0	F	2.99	1.145	2.7	
Acidic analytes												
42	Warfarin	0.17	1.794	4.0	0.23	1.408	2.1	E	0.22	1.365	2.4	
43	Coumachlor	0.30	1.198	1.5	0.45	1.119	0.9	E	0.54	1.204	2.2	
44	4'-Hydroxywarfarin	0.11	1.706	2.2	0.15	1.290	0.6	F	0.14	1.285	1.4	
45	6-Hydroxywarfarin	0.11	1.704	2.3	0.12	1.313	0.6	F	0.11	1.328	1.2	
46	7-Hydroxywarfarin	0.07	2.915	3.6	0.07	1.795	1.1	F	0.22	1.426	2.9	
47	8-Hydroxywarfarin	0.14	1.688	2.7	0.11	1.416	1.0	F	0.15	1.319	1.5	
48	10-Hydroxywarfarin	0.15	1.286	1.2	0.13	1.000	0.0	F	0.14	1.127	0.6	
49	Carprofen	0.17	1.116	0.5	0.14	1.000	0.0	F	3.46	1.026	0.5	
50	Feneprofen	0.29	1.000	0.0	0.11	1.000	0.0	F	1.94	1.000	0.0	
51	Flurbiprofen	0.29	1.000	0.0	0.17	1.000	0.0	F	2.29	1.000	0.0	
52	Ibuprofen	0.19	1.000	0.0	0.09	1.000	0.0	F	1.91	1.025	0.5	
53	Ketoprofen	0.31	1.000	0.0	0.19	1.000	0.0	F	0.94	1.000	0.0	
54	Naproxen	0.38	1.000	0.0	0.19	1.000	0.0	F	1.42	1.000	0.0	
55	Indoprofen	0.34	1.095	0.7	0.28	1.082	0.6	F	1.12	1.091	1.5	

Analyte	Normal phase ^a			Polar organic phase ^b			Reversed phase ^c				
	<i>k'</i> ₁	<i>a</i>	<i>R</i> _s	<i>k'</i> ₁	<i>a</i>	<i>R</i> _s	Mobile phase	<i>k'</i> ₁	<i>a</i>	<i>R</i> _s	
56	Suprofen	0.32	1.000	0.0	0.25	1.000	0.0	F	1.09	1.037	0.6
57	DNB-leucine	1.15	1.142	1.8	No peak			F	2.10	1.000	0.0
Basic analytes											
58	Troger's base	0.17	1.578	2.8	0.31	1.759	4.8	E	0.60	1.145	1.7
59	Aminoglutethimide	0.45	1.546	4.3	0.43	1.403	3.5	E	0.14	1.563	2.5
60	Pindolol	n/a	1.330	3.6	n/a	1.230	1.7	E	1.30	1.623	1.0
61	1,1'-Binaphthyl-2,2'-diamine	0.60	1.063	0.6	0.52	1.000	0.0	F	10.18	1.136	1.2
62	PTH-arginine	0.21	1.093	0.5	0.17	1.000	0.0	F	0.65	1.266	2.4
Basic analytes											
63	Carteolol	0.07	1.707	1.0	0.08	1.000	0.0	G	0.07	1.000	0.0
64	Oxprenolol	0.02	3.704	1.5	0.04	1.000	0.0	G	0.62	1.000	0.0
60	Pindolol	0.09	3.051	3.7	0.05	1.605	0.8	G	5.27	1.397	9.2
65	Propranolol	0.13	1.366	1.0	0.11	1.071	0.3	G	2.33	1.000	0.0
66	Talinolol	0.08	1.000	0.0	0.06	1.000	0.0	G	6.07	1.030	0.8

^a Conditions same as condition B in Table 2.

^b Conditions same as condition D in Table 2.

^c Mobile phases E and F same as conditions E and F in Table 2, respectively.

^d Mobile phase hexane/EtOH/DEA 40:60:1 (v/v/v) containing 8mM NH₄COOH, 30kV.

^e Mobile phase EtOH/DEA 99:1 (v/v) containing 8mM NH₄COOH, 20kV.

^f Mobile phase G 40% (v/v) ACN containing 5 mM NH₄COOH at pH 10 (adjusted with cyclohexylamine), 15kV.

Table 4
CEC-MS in three different modes using a capillary packed with 5 μ m 1000 Å sulfonated CDMPC CSP.

Mode	Condition	Analytes	SIM	t_{R2} (min)	N	α^a	R_s
Normal phase	A	Aminogluthimide	POS 245	47.1	22,000	1.066	2.4
	A	Coumachlor	NEG 307	70.3	18,000	1.018	0.6
	A	Mephobarbital	NEG 245	59.8	25,000	1.015	0.6
	A	Pindolol	POS 251	63.0	2,000	1.327	3.1
	A	DNB-leucine	NEG 296	97.8	16,000	1.030	1.1
	A	1,1'-Binaphthyl-2,2'-diamine	POS 285	65.5	20,000	1.020	0.6
	B	Aminogluthimide	POS 245	39.5	39,000	1.043	2.2
Polar organic phase	B	Coumachlor	NEG 307	41.7	53,000	1.020	1.2
	B	Pindolol	POS 251	22.9	1,000	1.120	0.8
	C	Troger's base	POS 251	68.4	52,000	1.058	3.1
	C	Mephobarbital	NEG 245	62.8	36,000	1.010	0.6
	D	1,1'-Binaphthyl-2,2'-diamine	POS 285	24.5	28,000	1.024	1.0
	D	Aminogluthimide	POS 245	20.9	56,000	1.045	2.6
	D	Coumachlor	NEG 307	23.3	28,000	1.031	1.3
Reversed phase	E	1,1'-Bi-2-naphthol	NEG 285	70.6	24,000	1.082	3.0
	E	Indoprofen	NEG 280	33.2	34,000	1.025	1.1
	E	Mephobarbital	NEG 245	35.4	53,000	1.042	2.3
	E	Lorazepam	POS 320	40.0	5,000	1.129	2.0
	E	Oxazepam	POS 287	34.2	16,000	1.128	3.1
	E	PTH-isoleucine	NEG 247	41.5	42,000	1.041	2.1
	E	PTH-leucine	NEG 247	44.7	25,000	1.070	2.7
	E	PTH-norleucine	NEG 247	45.3	29,000	1.024	1.0
	E	PTH-norvaline	NEG 233	36.4	37,000	1.029	1.4
	E	PTH-tryptophan	NEG 320	49.4	17,000	1.028	0.9
	E	PTH-valine	NEG 233	32.0	26,000	1.020	0.8
	E	5-(4-Hydroxyphenyl)-5-phenylhydantoin	NEG 267	24.5	32,000	1.027	1.2

Conditions: (A) mobile phase hexane/EtOH/HOAc 40:60:1 (v/v/v) containing 8 mM NH_4COOH , 30 kV, sheath liquid 90% IPA (v/v) containing 5mM NH_4OAc ; (B) mobile phase MeOH/HOAc 99:1 (v/v) containing 8 mM NH_4COOH , 10 kV, sheath liquid 90% MeOH (v/v) containing 5mM NH_4OAc ; (C) mobile phase EtOH/HOAc 99:1 (v/v) containing 8 mM NH_4COOH , 20 kV; (D) mobile phase

70% (v/v) ACN containing 5mM NH₄COOH at pH 3.5, 15kV; (E) mobile phase 40% (v/v) ACN containing 5mM NH₄COOH at pH 3.5, 15kV, sheath liquid 90% MeOH (v/v) containing 5mM NH₄OAc.

^a Selectivity is calculated by the ratio of R_2/R_1 .

Table 5

Intra-day and inter-day reproducibility of retention times, separation efficiency, selectivity, and resolution for AGT in three different CEC-MS modes using a capillary packed with 5 μm 1000 Å sulfonated CDMPC CSP for 3 consecutive days.

Day	Run	Average retention time (min) (RSD, %)		Average efficiency (RSD, %)	Average selectivity	Average resolution
		S-AGT	R-AGT			
Normal-phase mode ^a						
1	20	44.2 (2.0)	47.1 (2.1)	22,000 (17)	1.066 (0.2)	2.4 (9)
2	20	43.6 (1.6)	46.4 (1.6)	24,000 (21)	1.066 (0.2)	2.5 (13)
3	20	43.4 (0.3)	46.3 (0.4)	24,000 (10)	1.067 (0.1)	2.5 (6)
Overall	60	43.7 (1.7)	46.6 (1.7)	24,000 (17)	1.066 (0.2)	2.5 (10)
Polar organic phase mode ^b						
1	20	37.9 (1.8)	39.5 (1.8)	39,000 (8)	1.043 (0.09)	2.1 (3.9)
2	20	39.1 (1.0)	40.7 (1.0)	41,000 (11)	1.043 (0.04)	2.1 (4.1)
3	20	38.7 (2.9)	40.3 (2.5)	38,000 (13)	1.044 (0.05)	2.1 (6.0)
Overall	60	38.5 (2.2)	40.2 (2.0)	39,000 (11)	1.043 (0.14)	2.1 (4.8)
Reversed-phase mode ^c						
1	30	20.0 (0.7)	20.9 (0.7)	56,000 (7)	1.045 (0.1)	2.6 (4)
2	30	20.0 (0.3)	20.9 (0.3)	62,000 (8)	1.044 (0.1)	2.7 (4)
3	30	19.5 (0.4)	20.3 (0.4)	73,000 (5)	1.045 (0.1)	2.8 (3)
Overall	90	19.8 (1.3)	20.7 (1.5)	63,000 (15)	1.044 (0.1)	2.7 (6)

^a Conditions same as condition A in Table 4.

^b Conditions same as condition B in Table 4.

^c Conditions same as condition D in Table 4.