SHORT COMMUNICATION

Chiral separation using cyclodextrins as mobile phase additives in open-tubular liquid chromatography with a pseudophase coating

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The chiral separation of various analytes (dichlorprop, mecoprop, ibuprofen, and ketoprofen) was demonstrated with different cyclodextrins as mobile phase additives in open-tubular liquid chromatography using a stationary pseudophase semipermanent coating. The stable coating was prepared by a successive multiple ionic layer approach using poly(diallyldimethylammonium chloride), polystyrene sulfonate, and didodecyldimethyl ammonium bromide. Increasing concentrations (0–0.2 mM) of various native and derivatized cyclodextrins in 25 mM sodium tetraborate (pH 9.2) were investigated. Chiral separation was achieved for the four test analytes using 0.05–0.1 mM β-cyclodextrin (resolution between 1.11 and 1.34), γ-cyclodextrin (resolution between 0.78 and 1.27), carboxymethyl-β-cyclodextrin (resolution between 1.64 and 2.59), and 2-hydroxypropyl-β-cyclodextrin (resolution between 0.71 and 1.76) with the highest resolutions obtained with 0.1 mM carboxymethyl-β-cyclodextrin. %RSD values were <10%. This is the first demonstration of chiral open-tubular liquid chromatography using achiral chromatographic coatings and cyclodextrins as mobile phase additives.

KEYWORDS
chiral separation, cyclodextrins, open-tubular liquid chromatography, semipermanent coating, stationary pseudophase coating

1 | INTRODUCTION

The importance of chiral analysis in analytical chemistry is underscored by the number of research articles dedicated to chirality [1–10]. The growth of chiral analysis research was driven by the fact that enantiomers or chiral molecules—a pair of molecules whose mirror images are nonsuperimposable—have different biological activities and potencies. The need to differentiate between two enantiomers to assess enantiomeric purity of commercial and new chemicals gave rise to the development of various chiral analytical techniques based on CE [1, 5], GC [11], and LC [2, 6, 7].

Article Related Abbreviations: CD, cyclodextrin; CM-β-CD, carboxymethyl β-CD; CSP, chiral stationary phase; DDAB, didodecyldimethylammonium bromide; HP-β-CD, 2-hydroxypropyl-β-CD; OT-LC, open-tubular LC; PDDA, poly(diallyldimethylammonium chloride); PSS, polystyrene sulfonate; SMIL, successive multiple ionic layer

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Two strategies are typically employed in chiral LC. The most common strategy is where the chiral selector is either coated or immobilized onto a solid stationary phase. This is called the chiral stationary phase (CSP) [12]. There are various commercial CSPs available in the market based on different selectors such as antibiotics, alkaloids, amylose, cellulose, crown ethers, small molecules, and cyclodextrins (CDs) [6, 7, 13]. However, chiral LC CSPs are expensive and have typically limited lifetimes. Another strategy is by using chiral mobile phase additives such as antibiotics, ligand exchangers, and CDs [14, 15]. This method is implemented in an achiral stationary phase such as C-18 [15, 16]. This approach is a cheaper and more flexible alternative to CSPs.

Open-tubular LC (OT-LC) uses a chromatographic coating on the walls of narrow inner diameter columns [17–22]. OT-LC separations may offer advantages such as low sample and consumable requirements, zero backflow, and no column clogging. We have been exploring surfactant aggregates as pseudophase coatings in OT-LC [23–25]. In particular, the double-chained surfactant didodecylmethyl ammonium bromide (DDAB) was shown as a semipermanent stationary pseudophase in OT-LC [25]. Unlike the use of long-chain ionic surfactants (e.g., cetyltrimethylammonium bromide), no surfactant (i.e., DDAB) was added in the mobile phase [26].

CDs are oligocyclic molecules that consist of 5–10 glucose residues linked together by an α(1→4) glycosidic linkage [27]. The glucose residues in CDs can be unmodified (i.e., native) or modified with various moieties such as hydroxyalkyl, methyl, or carboxymethyl groups (i.e., derivatized). CDs have a toroidal, truncated cone structure with a hydrophilic surface and a hydrophobic interior. CDs can form inclusion complexes with various small molecules. When the molecules involved are chiral, the process is enantioselective. This ability has been exploited for the chiral separation of analytes in various LC methods [28–34]. CDs have been successfully used as CSP coatings in OT-capillary electrochromatography [35] but not in OT-LC. Moreover, based on our Scopus search using keywords “open-tubular liquid chromatography,” “cyclodextrin,” and “mobile phase additive,” CDs have not been used as chiral mobile phase additives in OT-LC.

In this paper, we explored the use of CDs as chiral mobile phase additives in OT-LC for the first time. OT-LC was performed using DDAB as chromatographic coating inside fused-silica capillaries for the separation of model analytes (dichlorprop, mecoprop, ibuprofen, and ketoprofen). We studied the stability of coatings prepared from (1) single flushing of capillary with coating solutions containing DDAB, β-CD/DDAB, and β-CD/DDAB-NaCl, and (2) successive multiple ionic layer (SMIL) [36] approach using poly(diallyldimethylammonium chloride) (PDDA), polystyrene sulfonate (PSS), and DDAB. The most stable semipermanent pseudophase coating with SMIL approach was then used to study chiral OT-LC of the model analytes using different CDs (native (α-, β-, γ-CD) and derivatized (carboxymethyl-β-CD [CM-β-CD], 2-hydroxypropyl-β-CD [HP-β-CD], heptakis(2,3,6-tri-O-methyl)-β-CD, and quaternary ammonium β-CD)) in the mobile phase.

2 | MATERIALS AND METHODS

2.1 | Chemicals and general equipment

Chemicals (CDs, DDAB, HPLC-grade ACN and methanol, phosphoric acid, polyelectrolytes [PDDA and PSS], sodium tetraborate decahydrate, sodium hydroxide, and test model analytes) were from Sigma-Aldrich (New South Wales, Australia) or Fluka Analytical (St. Louis, MO, USA). Purified water with a resistivity of 18.2 MΩ·cm was obtained from a Milli-Q system (Millipore, Bedford, MA, USA). Stock solutions of 100 mM sodium tetraborate (pH 9.2), 5 mM DDAB, and CDs were prepared in purified water. Also 1% (w/v) polyelectrolyte solutions were prepared in 25 mM sodium tetraborate (pH 9.2). The coating solutions of 0.5 mM DDAB, 0.5 mM DDAB with 0.5 mM β-CD, and 0.5 mM DDAB with 0.5 mM β-CD and 50 mM NaCl were prepared in 25 mM sodium tetraborate (pH 9.2). All mobile phases were prepared by mixing appropriate volumes of sodium tetraborate and CD stock solutions with purified water. All solutions were sonicated and then filtered with 0.45 μm syringe filter prior to use.

Analyte stock solutions (2 mg/mL) were prepared in methanol or in Milli-Q water. These stock solutions were stored at 4–8 °C when not in use. Samples for injection (50 μg/mL each) were prepared by mixing appropriate volumes of analyte stock solution with the 25 mM sodium tetraborate (pH 9.2). For analytes prepared in methanol, the solvent was evaporated before reconstitution with 25 mM sodium tetraborate (pH 9.2).

2.2 | Open-tubular LC instrumentation and procedure

OT-LC was conducted using a commercial CE system (Agilent, Santa Clara, USA) equipped with a 50 μm id × 375 μm od fused-silica capillary. The total capillary length was 30 cm (21.5 cm from the inlet to the UV detector set at 200 nm). The capillary temperature was kept at 25°C. Sample solution was introduced at the inlet end of the capillary via pressure at 25 mbar for 3 sec (~2 mm plug).
Pressure separation was performed by applying 50 mbar (maximum permissible pressure by the instrument to acquire data) from the inlet. Void time was ~2.7 min.

### 2.3 Preparation of coated capillaries

New capillaries were conditioned with 0.1 M NaOH (~1 bar for 10 min) followed by purified water (~1 bar for 5 min) prior to use. For coatings prepared by single flushing with coating solution, the capillary was conditioned prior to each run as follows: purified water (3 capillary volumes), 0.1 M H₃PO₄ (6 capillary volumes), purified water (3 capillary volumes), 1:1 (v/v) ACN/methanol mixture (6 capillary volumes), purified water (3 capillary volumes), 0.1 M NaOH (6 capillary volumes), purified water (3 capillary volumes), coating solution (12 capillary volumes), 25 mM sodium tetraborate (pH 9.2) (3 capillary volumes), and mobile phase (10 capillary volumes). One capillary volume is ~1 bar for 0.2 min.

For SMIL-coated capillary, 1% (w/v) PDDA and 1% (w/v) PSS were flushed alternately into the capillaries at ~1 bar for 5 min, with a water rinse at ~1 bar for 2 min in between. The PDDA-PSS-coated capillaries were conditioned prior to each run as follows: 25 mM sodium tetraborate (pH 9.2) (3 capillary volumes), 0.5 mM DDAB in 25 mM sodium tetraborate (pH 9.2) (12 capillary volumes), 25 mM sodium tetraborate (pH 9.2) (3 capillary volumes), and mobile phase (10 capillary volumes). New capillaries were prepared each day as recommended in our previous study [25].

### 3 RESULTS AND DISCUSSION

#### 3.1 Stability of didodecyl(dimethylammonium) bromide coatings prepared by single flush with various coating solutions and successive multiple ionic layer approach

DDAB and CDs are known to form inclusion complexes [37]. Therefore, it is likely that CDs in the mobile phase will affect the stability of DDAB admicelles at the inner capillary wall surface/liquid interface. We therefore investigated the stability of single flush DDAB coatings prepared from different coating solutions in OT-LC using a mobile phase that contains a CD. The coating solutions were (1) 0.5 mM DDAB, (2) 0.5 mM DDAB with 0.5 mM β-CD, and (3) 0.5 mM DDAB with 0.5 mM β-CD and 50 mM NaCl in 25 mM sodium tetraborate (pH 9.2). The mobile phase was 0.1 mM β-CD in 25 mM sodium tetraborate (pH 9.2). The capillary was conditioned with the mobile phase in between runs. The separation of model analytes was not repeatable between the first two runs, suggesting a change in the aggregation of DDAB at the interface during separation. Indeed, when a higher concentration of CD (0.4 mM) in the mobile phase was used, a cathodic electroosmotic flow was observed after one OT-LC run. This suggested a predominantly negatively charged inner capillary wall surface due to the removal of DDAB aggregates from the interface.

An SMIL approach is known to provide stable coatings for CE separations [38, 39]. Here, we used SMIL approach by successive coating with PDDA, PSS, and finally, DDAB. The mobile phase was also 0.1 mM β-CD in 25 mM sodium tetraborate (pH 9.2), and the capillary was conditioned with the mobile phase in between runs. Similar separations of model analytes were obtained between the first two runs, suggesting that the DDAB admicelles were not affected by the CDs in the mobile phase during the OT-LC run. To further ensure repeatable results, we implemented a DDAB flush in between runs using the SMIL column.

#### 3.2 Cyclodextrins as chiral mobile phase additives in open-tubular LC

Chiral OT-LC of the model analytes using SMIL-coated capillaries was then investigated using mobile phases containing increasing concentrations (0–0.2 mM) of a native or derivatized CD. Among the native CDs, chiral separation was observed with β- and γ-CD only. Among the derivatized CDs, chiral separation was observed with HP-β-CD and CM-β-CD. Figures 1 (for native CDs β- and γ-CD) and 2 (for derivatized CDs HP-β-CD and CM-β-CD) show the plot between [CD] and retention factors (k) of dichlorprop (Figures 1A and 2A), mecoprop (Figures 1B and 2B), ibuprofen (Figures 1C and 2C), and ketoprofen (Figures 1D and 2D). k was calculated using the formula: $k = (t_R - t_0)/t_0$, where $t_0$ and $t_R$ are the void time and analyte retention time, which were obtained from the chromatograms, respectively. The experiments were done in triplicate with %RSD values of <10%. A general trend of decrease in k values of analytes with the increase of [CD] in the mobile phase was observed. More importantly, the change in k between two enantiomers indicated chiral separation. For the native CDs (see Figure 1; blue trace for β-CD and yellow trace for γ-CD), the change in k was observed for dichlorprop and ibuprofen at [β-CD] or [γ-CD] = 0.1 mM. The change in k was observed for mecoprop and ketoprofen at [β-CD] or [γ-CD] between 0.05 and 0.1 mM. For the derivatized CDs (see Figure 2; black trace for HP-β-CD and red trace for CM-β-CD), the change in k was observed for all analytes at [HP-β-CD] = 0.1 mM. The change in k was observed for dichlorprop, ibuprofen, and ketoprofen at [CM-β-CD] between 0.05 and 0.2 mM. The change in...
FIGURE 1 Effect of β- and γ-cyclodextrin (CD) on $k$ values of model analytes: dichlorprop (A), mecoprop (B), ibuprofen (C), and ketoprofen (D). Analyte concentration was 50 μg/mL. The successive multiple ionic layer (SMIL) coating was described in Section 2.3. Mobile phase was 0.1 mM of CD in 25 mM sodium tetraborate (pH 9.2). Legends for (A) are applicable to (B), (C), and (D). Other conditions are described in Section 2.2.

FIGURE 2 Effect of 2-hydroxypropyl-β-CD (HP-β-CD) and carboxymethyl-β-CD (CM-β-CD) on $k$ values of model analytes: dichlorprop (A), mecoprop (B), ibuprofen (C), and ketoprofen (D). Analyte concentration was 50 μg/mL. The successive multiple ionic layer (SMIL) coating was described in Section 2.3. Mobile phase was 0.1 mM of cyclodextrin (CD) in 25 mM sodium tetraborate (pH 9.2). Legends for (A) are applicable to (B), (C), and (D). Other conditions are described in Section 2.2.

$k$ was observed for mecoprop at [CM-β-CD] between 0.05 and 0.1 mM.

Table 1 summarizes the analyte resolution ($R_s$) values for the model analytes and the conditions described in Figures 1 and 2. Regardless of the effective CD found (β-CD, γ-CD, HP-β-CD, and CM-β-CD), optimum chiral separations were obtained at [CD] = 0.1 mM. Complete resolutions ($R_s \geq 1.2$) were obtained in nine out of the 29 conditions tested that showed chiral separation.

Figure 3 (top chromatograms) shows representative chiral OT-LC chromatograms of dichlorprop (A), mecoprop (B), ibuprofen (C), and ketoprofen (D) with a CD in the mobile phase. The bottom chromatograms in Figure 3A–D were obtained when no CD was added in the mobile phase. Among all CDs at [CD] = 0.1 mM, the best chiral separation for the model analytes was observed using CM-β-CD in the mobile phase (see Figure 3A–D and Table 1). The results clearly demonstrate the use of CDs as mobile phase additives in OT-LC using an SMIL coating with DDAB.

4 CONCLUDING REMARKS

The use of CDs as chiral mobile phase additives in OT-LC was described for the first time. OT-LC was with an SMIL-coated capillary with DDAB as the final coating that acted as the chromatographic pseudophase. Using β-CD, γ-CD, HP-β-CD, and CM-β-CD in the mobile phase with [CD] = 0.1 mM, chiral separation was observed for dichlorprop, mecoprop, ibuprofen, and ketoprofen, with CM-β-CD demonstrating the best chiral separation for all four analytes. The advantage of our method is that it is cheap and simple to prepare, and does not experience clogging from back pressure [25]. Also, the method uses
TABLE 1  Analyte resolutions with different cyclodextrins (CDs) in the mobile phase

| Analyte     | Rs [β-CD], mM | [γ-CD], mM | [HP-β-CD], mM | [CM-β-CD], mM |
|-------------|---------------|------------|---------------|---------------|
|             | 0.05 | 0.1 | 0.2 | 0.05 | 0.1 | 0.2 | 0.05 | 0.1 | 0.2 | 0.05 | 0.1 | 0.2 |
| Dichlorprop | 0    | 1.11 | 0 | 0 | 1.02 | 0 | 0 | 1.06 | 0 | 0.76 | 1.67 | 1.49 |
| Ibuprofen   | 0    | 1.34 | 0 | 0 | 1.24 | 0 | 0 | 1.76 | 0 | 0.72 | 2.55 | 1.46 |
| Ketoprofen  | 1.08 | 1.13 | 0 | 0.84 | 0.78 | 0 | 0.65 | 0.71 | 0 | 0.63 | 1.64 | 1.38 |
| Mecoprop    | 1.17 | 1.22 | 0 | 1.02 | 1.27 | 0 | 0.35 | 0.93 | 0 | 0.80 | 2.59 | 0 |

*Note: All determinations were done in triplicates with %RSD between 1 and 10%.

FIGURE 3  Representative chiral open-tubular LC (OT-LC) (top) and OT-LC (bottom) analyses of model analytes. Analyte concentration was 50 μg/mL. The successive multiple ionic layer (SMIL) coating was described in Section 2.3. Mobile phase was 0.1 mM (top) and 0 mM (bottom) carboxymethyl-β-CD (CM-β-CD) in 25 mM sodium tetraborate (pH 9.2). Other conditions are described in Section 2.2.

small amounts of reagents and produces small amounts of waste. However, the separation efficiencies obtained in our method is not comparable to that of more established methods.

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CONFLICT OF INTEREST
The authors have declared no conflict of interest.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available from the corresponding author upon reasonable request.

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