Very Fast Enantioseparation in High-performance Liquid Chromatography Using Cellulose Tris(3,5-dimethylphenylcarbamate) Coated on Monolithic Silica Support

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The combination of the high chiral recognition ability of cellulose tris(3,5-dimethylphenylcarbamate) (CDMPC) with the favorable dynamic properties of a silica-based monolithic chromatographic support allowed the baseline enantioseparation of 2,2,2-trifluoro-1-(9-anthryl)ethanol with the retention times of 7.2 and 18.5 s for the first and second eluted enantiomers, respectively. The separation was accomplished in 30 s which may be the shortest time in which baseline HPLC enantioseparations have been reported.

Fast chromatographic methods are becoming more prevalent in the last few years due to the rapid developments in combinatorial chemistry, genomics, and proteomics where a large number of analytes are generated. The analytical run time is dependent upon the retention characteristics of an analyte, the mobile phase flow rate, column length and dynamic characteristics of the separation bed. Several attempts have been made in the past in order to shorten the analysis time for HPLC enantioseparations.[1–3] Two basic limitations associated with conventional particulate silica packing materials used in HPLC, when attempting to shorten the analysis time by increasing the linear flow-rate of a mobile phase are; a) increasing pressure drop, and b) increasing the peak broadening due to resistance to mass transfer. In addition, the enantiomer recognition ability of a chiral selector used must be high in order to allow sufficient enantioseparation in a short time. Polysaccharide derivatives meet the last requirement as illustrated in numerous studies during the past 20 years.[4–6] One of the highest enantioseparation factors ever achieved in chiral HPLC was recently reported using these materials.[6]

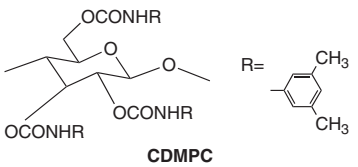

Figure 1. Structure of cellulose tris(3,5-dimethylphenylcarbamate).

On the other hand, recent studies have indicated that monolithic silica materials successfully meet the other two requirements mentioned above for shortening the analysis time. In particular, the pressure drop through a monolithic silica bed is significantly lower and the plate height dependence on the linear flow rate of the mobile phase is much flatter compared to the columns packed with particulate silica materials.[7–9] The advantages of the aforementioned favorable characteristics of silica-based monolithic materials for analytical,[7–9] as well as for preparative-scale separations[10] have been already noticed. However, the potential advantages of silica-based monolithic columns for enantiomeric separations (after their chiral modification) have not yet been explored. This paper reports a preliminary attempt in order to combine the high enantiomer resolving ability of polysaccharide-type chiral stationary phases, in particular that of cellulose tris(3,5-dimethylphenylcarbamate) (CDMPC, Figure 1) with the favorable dynamic properties of monolithic silica materials.

The fabrication of commercially available monolithic silica columns involves its cladding in a poly(ether ether ketone) (PEEK) envelope by treatment at higher temperatures at which the polysaccharide phenylcarbamates are thermally unstable. Owing to this reason, the chiral modification of a commercially available Chromolith SpeedRod column, RP-18e (50 × 4.6 mm)

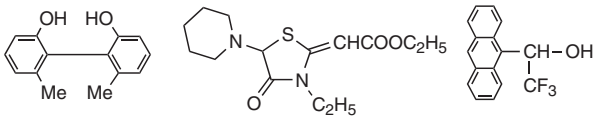

Figure 2. Structures of racemates.

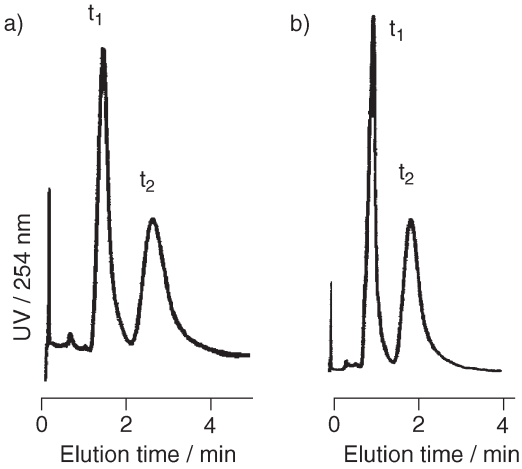

Figure 3. Enantioseparation of 2,2′-dihydroxy-6,6′-dimethylbiphenyl (a) and piprozolin (b) using in situ coated 12% (w/w) CDMPC on Chromolith SpeedRod as chiral stationary phase. Column: 4.6 × 50 mm; mobile phase: hexane/2-propanol 90/10 (v/v); flow rate: 2 mL/min.

(Charge No. B270755) from Merck KGaA (Darmstadt, Germany) was performed in situ by filing the column with a 50 mg/mL solution of CDMPC in acetone followed by slow evaporation of the solvent at a room temperature. This procedure was repeated two times. The coating of CDMPC was ca. 12% based on the weight increase of the column.

The enantioseparations were performed using hexane/2-propanol in the ratio 90/10 (v/v) as the mobile phase in the flow rate range of 1–20 mL/min. The low backpressure of the monolithic silica column even after coating with a significant amount of the polysaccharide derivative (in fact, no increase in the backpressure was observed after the coating) in combination with the high chiral recognition ability of CDMPC enabled the enantioseparations of the enantiomers of an axially chiral biphenyl derivative and chiral drug, piprozolin (for chemical structures, see Figure 2), in a rather short time (Figure 3). Moreover, in the case of 2,2,2-trifluoro-1-(9-anthryl)ethanol, it was possible to increase the linear flow rate to 20 mL/min and achieve a baseline separation of enantiomers with the analysis time below 30 s (the elution times were 7.2 and 18.5 s for the first and the second eluted enantiomers, respectively) (Figure 4). The enantioseparation on a seconds scale opens new possibilities for the application of chiral HPLC for the monitoring of fast chemical processes involving enantiomer conversion. In principle, further shortening of the analysis time seems feasible from the side of the chromatographic process itself but this may involve some serious technical requirements on the pumps, detectors and integrators (recorders) involved in the separation and its documentation.

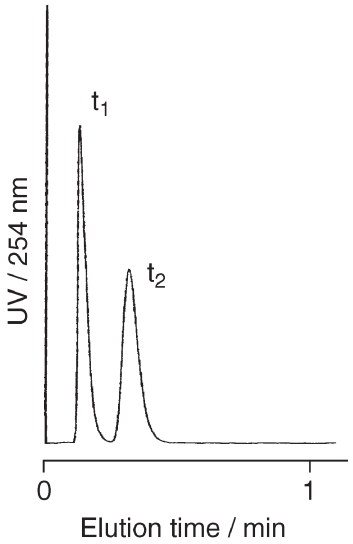

Figure 4. Enantioseparation of 2,2,2-trifluoro-1-(9-anthryl)ethanol using CDMPC in situ coated 12% (w/w) on Chromolith SpeedRod as chiral stationary phase. Column: 4.6 × 50 mm; mobile phase: hexane/2-propanol 90/10 (v/v); flow rate: 20 mL/min.

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