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Comparative performance of capillary columns made with totally porous and core–shell particles coated with a polysaccharide-based chiral selector in nano-liquid chromatography and capillary electrochromatography

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ABSTRACT

In this study two types of silica particles, one fully porous and the other superficial porous (core–shell or fused-core) were modified with a polysaccharide-type chiral selector and evaluated for the separation of enantiomers in nano-liquid chromatography (nano-LC) and capillary electrochromatography (CEC). The major goal of this project was to critically evaluate the contribution of the "flow through particles" to enhancing peak efficiency in CEC compared to nano-LC. The better performance of fused-core silica particles compared with silica particles of comparable size but having through pores questions the previous assumption that "flow through particles" is the major contributor to enhancing peak efficiencies observed in CEC. In addition, based on the results of this study it is suggested that contrary to previous reports on core–shell particles behaving poorly in narrow bore columns, these materials are quite suitable for CEC, at least in capillary columns of 100 μm I.D.

1. Introduction

Capillary electrochromatography (CEC) is a separation technique governed by both chromatographic separation principles and electrokinetic migration of analytes [1,2]. If properly operated, CEC provides higher separation efficiency compared to chromatographic separation techniques under otherwise similar experimental conditions. This has been systematically proven in achiral [3–6] as well as chiral separations [7–13] using CEC. Faster intraparticle mass transfer due to flow through particles has been identified as one of the major contributions to the peak efficiency enhancement in CEC experiments compared with nano-liquid chromatographic (nano-LC) experiments performed on the same capillary column [7–13]. The fact that intraparticle mass transfer in CEC experiments is faster compared to nano-LC is unquestionable as it has been clearly illustrated experimentally by a lower C-term in the van Deemter equation [7,8], as well as based on the effects of silica pore size [9,10], ionic strength of the background electrolyte [10] and other factors [11,13] on peak efficiency in CEC. Core–shell silica particles which became commercially available recently have a nonporous core potentially prohibiting (or at least impeding) flow through particles. Thus, a comparative evaluation of the performance of totally porous and core–shell materials in nano-LC and CEC should provide experimental evidence of the contribution by intra-particle flow in enhancing peak performance in CEC compared to nano-LC.

In addition, the current opinion is that core–shell particles do not perform as well in smaller bore (2.1 mm I.D.) columns as in standard columns of 4.6 mm I.D. [14–17]. The advantages of chiral stationary phases (CSPs) based on core–shell silica in standard diameter HPLC columns have been recently demonstrated [18]. It seems interesting to evaluate the performance of core–shell particle based chiral capillary columns as such columns should prove even less efficient given their even lower column internal diameter to particle diameter ratio compared to 2.1 mm I.D. columns.

In order to answer the above questions, two polysaccharide-based CSPs were prepared by coating the same cellulose derivative on core–shell and totally porous silica particles. These materials were evaluated for the separation of enantiomers in nano-LC and in CEC.

2. Experimental

2.1. Chemicals and samples

All chemicals were of analytical reagent grade and used as received. Acetonitrile (MeCN), methanol (MeOH) and acetic acid

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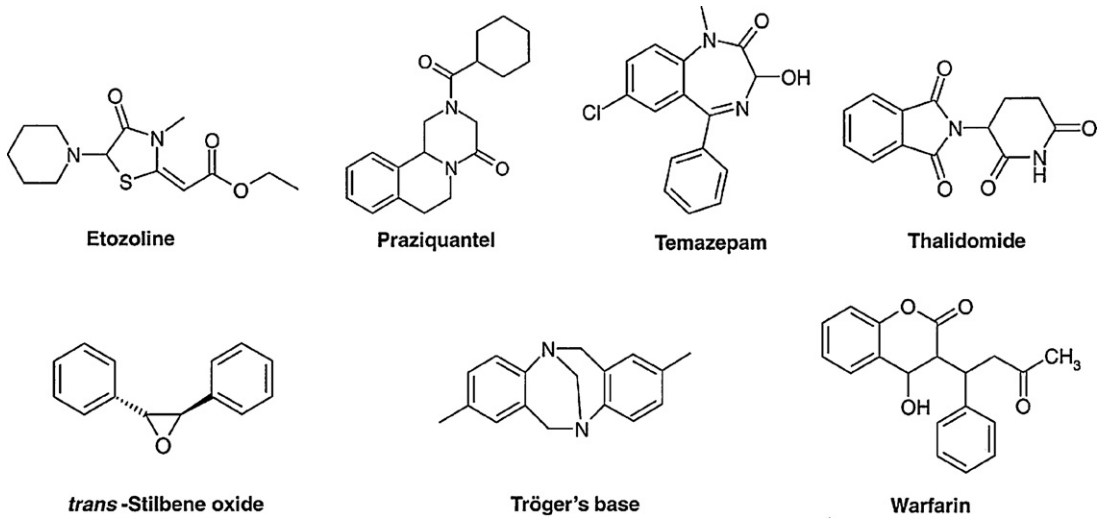

Fig. 1. Structure of chiral test compounds.

(AcOH) were supplied by Carlo Erba (Milan, Italy). Ammonium hydroxide solution (30%) was purchased from Riedel-de Haën (Seelze, Germany). Racemic praziquantel, temazepam, thalidomide, *trans*-stilbene oxide, Tröger's base and warfarin were purchased from Sigma Aldrich (St. Luis, MO, USA), while etozoline was kindly provided by the Institute of Pharmaceutical and Medicinal Chemistry, University of Münster (Germany). The chemical structures of the studied analytes are shown in Fig. 1. Thiourea, employed as a t_0 marker was from Sigma Aldrich.

A Milli-Q system (Millipore, Milford, MA, USA) was used for the preparation of deionized water employed in these experiments. Ammonium acetate solution was prepared by titrating appropriate volumes of AcOH with 1 M ammonia solution to the desired pH every week and stored at +4 °C. Mobile phases were prepared daily by mixing the suitable volumes of organic solvents and water or buffer solutions.

Stock standard solutions of analytes having a concentration of 1 mg/mL were prepared by dissolving the appropriate amount of each compound in MeOH (except for thalidomide, which was dissolved in MeCN). Working solutions were prepared by diluting the stock solutions with methanol: etozoline and temazepam were diluted to 0.1 mg/mL, flavanone to 0.05 mg/mL, while lopirazepam to 0.02 mg/mL. When not in use all solutions were stored at +4 °C.

2.2. Instrumentation

Accurate pH measurements of buffer solutions were performed with MicropH 2001 Meter (Crison, Barcellona, Spain). An ultrasonic bath model FS 100b Decon (Hove, UK) was used to sonicate solutions, slurry and capillary columns during the packing step. Fused-silica capillaries (75 μm I.D. × 375 μm O.D.) were purchased from Composite Metal Services (Hallow, Worcestershire, UK). A LC series 10 HPLC pump (Perkin Elmer, Palo Alto, CA, USA) was used for packing and for equilibrating the capillary columns with mobile phase. A Stereozoom 4 optical microscope (Cambridge instruments, Vienna, Austria) with illuminator was used to monitor the capillary packing process.

2.2.1. Capillary electrochromatography

CEC experiments were carried out with a ^{3D}CE capillary electrophoresis equipment (Agilent Technologies, Waldbronn, Germany) equipped with a UV–vis diode array detector (DAD). Detection was performed at 205 nm. Column temperature (25° C) was controlled by an air thermostating system. The ^{3D}CE Chemstation software (Rev. A.09.01, Agilent Technologies) was used for collecting and reprocessing data. Injection was done by applying 8 bar pressure at the inlet side of the capillary column for 0.3 min. The estimated injected volume of sample was about 60 nL followed by a plug of background electrolyte (8 bar × 0.1 min).

CEC runs were carried out using a background electrolyte containing a mixture of MeCN/H_2O (70:30, v/v) containing 5 mM ammonium acetate pH 4.5 (final concentration). Both ends of the capillary were pressurized at 8 bar. Optimum experimental conditions (highest enantiomeric resolution) were obtained at 20 kV. Van Deemter plots were constructed by applying different voltages in the range of 5–30 kV. At the end of the working day, the capillary ends were immersed in a mixture of MeOH/water (50:50, v/v).

2.2.2. Nano-liquid chromatography

Nano-LC experiments were carried out employing a laboratory assembled instrument including an HPLC Accela® pump from Thermo-Finnigan (San Jose, CA, USA), a modified injector valve from Enantiosep GmbH (Münster, Germany) and an on-column spectrophotometric detector Spectra 100 UV from Thermo Separation Products (St. Jose, CA, USA). The modified injector valve, equipped with a 50 μL loop, contained the mobile phase. The same valve was used for analyte injection by filling the loop with sample solution and switching the valve for 5–10 s (the injected volume was about 60 nL).

After sample injection, the injector was flushed with mobile phase (250 μL) and left filled with mobile phase. The mobile phase flow rate (mL/min) was reduced to nL/min range with a passive split system. For this purpose, a stainless steel T piece (Vici Valco, Houston, TX, USA) and PEEK fittings (50 cm × 130 μm I.D. from the pump and 70 cm × 50 μm I.D. for the waste position) were used. The splitter was connected to the injector by a stainless steel tube (5 cm × 500 μm I.D.). The mobile phase was contained only in the valve loop, while MeOH was the pumping solvent and was continuously recycled from waste. The mobile phase was changed times-to-times carefully considering the flow rate.

The capillary column was directly inserted into the modified injector in such a way that it was immersed practically dead-volume free either in the sample solution (during sample injection) or the mobile phase (during analysis).

The flow rate was estimated by connecting a 10 μL syringe (Hamilton, Reno, NV, USA) to the capillary column outlet through a Teflon tube (TF-350; LC-Packing, CA, USA) and by measuring the volume of mobile phase accumulated over 5 min. Nano-LC runs