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Short communication

# Enantioseparation of basic chiral compounds on a clindamycin phosphate-silica/zirconia hybrid monolith by capillary electrochromatography

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ABSTRACT

An organic–inorganic silica/zirconia hybrid monolithic capillary column was prepared by sol–gel process in a fused-silica capillary by using triethoxysilylpropylcarbamate (TEOSPC) derivative of clindamycin phosphate (CLIP) as a chiral selector. A sol solution consisting of $6 \times 10^{-3}$ M of polyethylene glycol, 1 M of water, 2 M of acetic acid and 0.04/0.96 ratio of CLIP-TEOSPC/Zr-Bu resulted in homogeneous monolith having well defined through-pores and tightly anchored to the capillary wall. The column was employed for capillary electrochromatographic enantioseparation of eight basic chiral drugs in mobile phases consisting of acetonitrile, methanol and ammonium acetate (AA, as the electrolyte). Effects of the compositions of solvents and electrolyte in the mobile phase, applied voltage and capillary temperature on chiral separation were investigated. The highest resolution values were obtained with mobile phases consisting of 40/60 MeOH/ACN and 100 mM AA (for citalopram, Tröger's base, indapamide, metoprolol, cetirizine and atropine) and 35/65 MeOH/ACN and 100 mM AA (for sertraline and propranolol) using −10 kV applied voltage at 25 °C.



## 1. Introduction

Chiral selector (CS)-coated/modified zirconia monoliths (ZMs) have been successfully employed for enantioseparation in recent years [1–6]. Clindamycin phosphate (CLIP)-modified ZM resulted in successful enantioseparation of several acidic and basic drugs using CLIP-saturated polar organic mobile phases (MPs) consisting of methanol (MeOH), acetonitrile (ACN) and ammonium acetate (AA) [5]. Despite attaining successful enantioseparation of a range of analytes, certain limitation were observed while using CS-modified monoliths, i.e., limited choices of MPs in view of the solubility of coated CSs in a number of solvents, and requirement to use CS-saturated MPs to compensate potential leaching of CS from the coated phase [1–6].

To address these limitations, the present work was designed to prepare and evaluate CS-incorporated silica/zirconia hybrid monoliths (SZHMs). CLIP-incorporated ZHMs (CLIP-SZHMs) were synthesized by sol–gel method. CLIP was chosen as the CS as it has shown excellent enantiodiscrimination abilities in capillary electrophoresis (CE), micellar electrokinetic chromatography (MEKC) and capillary electrochromatography (CEC) in recent years [5,7,8]. The preliminary results for CEC enantioseparation of eight basic drugs and a comparison of performance and robustness of CLIP-incorporated SZHM with CLIP-coated ZM have been presented.

## 2. Experimental

### 2.1. Chemicals and reagents

Fused silica capillaries (50 μm I.D., 365 μm O.D.) were from Polymicro Technologies (Phoenix, AZ, USA). CLIP, 3-triethoxysilylpropyl isocyanate, zirconium n-butoxide (Zr-Bu) in n-butanol, acetic acid, AA and polyethylene glycol (PEG) (MW = 10,000 g/mol) were purchased from Aldrich (Milwaukee, WI, USA). HPLC-grade ACN and MeOH were from J.T. Baker (Phillipsburg, NJ, USA). Water was purified with an Elgastat UHQ water purification system (Bucks, UK). The pure standards of chiral compounds were purchased from Aldrich (Milwaukee, WI, USA) (Fig. S1 in supplementary information, SI).

### 2.2. Instrumentation

Agilent HP $^{3D}$CE system (Palo Alto, CA, USA) equipped with a diode-array UV detector, a ±30 kV power supply, an external nitrogen pressure and, the ChemStation software was used. A

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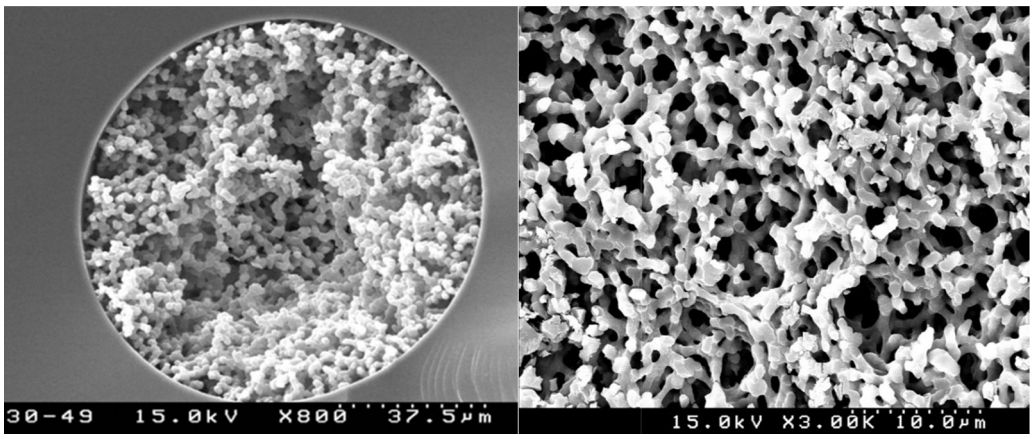


**Fig. 1.** SEM images of CLIP-SZHM at different magnifications.

scanning electron microscope (Hitachi, FE-SEM S-4100, Japan) was used to examine the morphology of the ZMs.

### 2.3. Preparation of silica/zirconia hybrid monolith

The CLIP-SZHMs were prepared by sol–gel technique. A hydrolysis solution was prepared by dissolving 0.06 g of PEG in a solution consisting of 18 μL of water, 114 μL of acetic acid and 430 μL of n-butanol with ultrasonication. After PEG was dissolved completely, 0.03 g of 3-triethoxysilylpropylcarbamate derivative of CLIP (CLIP-TEOSPC; synthetic procedure and characterization data have been provided in SI) was added to the hydrolysis solution and ultrasonication was applied for 10 min which was followed by 5-min degassing. After addition of 440 μL of zirconium butoxide, the resulting mixture was injected into the pretreated [2] fused silica capillary up to a certain length by syringe. The filled capillary was sealed with septa and allowed to react at 25 °C in an incubator for 5 h. Then, the capillary was heated at 150 °C for 6 h in a GC oven. The capillary was cooled to room temperature and characterized by SEM.

### 2.4. CEC conditions

The MPs were prepared by dissolving appropriate amount of AA (as an electrolyte) in MeOH/ACN mixtures. The MeOH/ACN mixtures were chosen for their high EOF and satisfactory solubility for analytes and electrolyte [9]. The chiral compounds were dissolved in the MP at a concentration of 0.1 mg/mL except MET which was dissolved at the same concentration in MeOH. The MP and sample solutions were filtered with 0.45-μm nylon membrane filter and degassed before use. The column was conditioned with the MP using −10 kV applied voltage and 10 bar pressure (for both inlet and outlet vials) at 25 °C for at least 2 h prior to CEC run.

## 3. Results and discussion

### 3.1. Preparation of zirconia hybrid monolith

During optimization several factors were taken into consideration, which include tight anchoring of monolithic material onto the capillary wall, ease of injection of a sol solution into capillary so that monolith synthesis will be repeatable, and presence of well-defined through-pores in the monolith so that potential blocking problem can be avoided. Although literature reveals that monoliths with smaller domain size usually exhibit a smaller plate height [10] a compromise was required where monoliths with larger domains were preferred to address the aforementioned issues.

As a point of initiation to optimize sol–gel composition for SZHM, different concentrations of CLIP-TEOSPC were added in the sol solution of an optimized composition found in the preparation of the native ZM [2]. The molar ratio of CLIP-TEOSPC/Zr-Bu may affect the morphology and porosity properties of the final monolithic material in addition to its analytical performance. Therefore, monoliths were synthesized with different molar ratios of CLIP-TEOSPC/Zr-Bu in the sol solution consisting of $5 \times 10^{-3}$ M PEG, 2 M acetic acid and 1 M water. None of the compositions resulted in fully filled capillary and thus different concentrations of PEG were employed. PEG forms strong hydrogen bonds with hydroxyl groups bonded directly to zirconium atoms of growing zirconia oligomer. The effect of PEG was investigated at different concentrations with fixed amounts of acetic acid (2 M), water (1 M) and CLIP-TEOSPC/Zr-Bu ratio (0.04/0.96). The particle and through-pore sizes were found to increase with increasing PEG concentration (Fig. S2A, SI). While fully filled capillaries were obtained with 6.0 and $7.0 \times 10^{-3}$ M PEG concentrations, on account of too-dense monolith in the latter case the former concentration was chosen for further optimization.

Subsequently, monoliths were synthesized with different molar ratios of CLIP-TEOSPC/Zr-Bu keeping other compositions constant, i.e., $6.0 \times 10^{-3}$ M PEG, 2 M acetic acid and 1 M water. At 0.04/0.96 and 0.06/0.94 molar ratios of CLIP-TEOSPC/Zr-Bu, fully filled capillaries having monolithic structures were obtained (Fig. S2B, SI). However, due to small pore sizes the capillaries were observed to be easily blocked in the latter case. Thus effect of acetic acid concentration was studied in sol solutions composed of 0.04/0.96 molar ratio of CLIP-TEOSPC/Zr-Bu, $6 \times 10^{-3}$ M PEG and 1 M water. Acetic acid concentration influences gelation process as the catalyst in hydrolysis and condensation reactions. Monolithic structures having well-defined through-pores and tightly anchored onto the capillary wall were obtained at 1.5 and 2.0 M (Fig. S2C, SI). However, in the case of 1.5 M concentration too-fast gelation resulted in difficulty in injection of the solution into the capillary and hence less reproducible monolithic structure. At higher concentration (2.5 M) of acetic acid, particle aggregation was observed. Further, the effect of concentration of water was investigated ranging from 0.5 to 1.5 M in sol solutions composed of 0.04/0.96 molar ratio of CLIP-TEOSPC/Zr-Bu, PEG ($6 \times 10^{-3}$ M) and water (1 M) (Fig. S2D, SI). At a lower concentration (0.5 M), monolith was not attached to the capillary wall, and at a higher concentration (1.5 M) monolith formation was not observed. As 1.0 M water addition resulted in monolith having well defined through pores and tightly anchored with capillary wall, it was considered the optimized concentration.

The optimal composition of the sol solution was therefore found to be $6 \times 10^{-3}$ M of PEG, 1 M of water, 2 M of acetic acid and 0.04/0.96 ratio of CLIP-TEOSPC/Zr-Bu. The SEM images