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# Stationary phase based on cellulose dodecanoate physically immobilized on silica particles for high-performance liquid chromatography

Andressa T. Vieira, Rosana M.N. Assunção, Anizio M. Faria\*

Instituto de Ciências Exatas e Naturais do Pontal, Universidade Federal de Uberlândia, 38304-402 Ituiutaba, MG, Brazil

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## ABSTRACT

The chemical agent free preparation of a stationary phase using a natural macromolecule was the focus of this paper. Thermal immobilization of cellulose dodecanoate on silica particles was used for the preparation of a stationary phase without the use of chemical reagents. Cellulose modification was performed to produce a hydrophobic macromolecule with solubility in common organic solvents. The new stationary phase was characterized morphologically and physico-chemically, presenting as spherical particles immobilized with a thin cellulose dodecanoate layer. The degree of substitution of cellulose dodecanoate was 1.7, which resulted in a separation mechanism in reversed phase mode, but with lower hydrophobicity and higher steric selectivity, which are properties from cellulose. These characteristics resulted in a stationary phase with intrinsic selectivity that was able to separate mixtures of polar drugs, homologs of an anionic surfactant and omeprazole isomers, which are not well resolved in typical C<sub>18</sub> phases. Considering that cellulose is a natural polymer and the preparation method of stationary phase involves only physical processes of silica modification, the final material presents as a stationary phase with specific retention properties coming from both dodecanoate and cellulose.

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## 1. Introduction

Stationary phases for reversed-phase liquid chromatography (RP-LC) are traditionally obtained from synthetic processes involving high-purity, expensive chemical reagents and requiring strict control of the experimental conditions to produce more reproducible materials. Furthermore, the separation selectivity provided by such agents did not yet resolve some classes of compounds, especially those more polar ones or having structural similarities [1–10].

One possibility of making the stationary phase preparation in a more simple and accessible process would be the use of molecules obtained from natural sources using a method of preparation that does not require expensive and unsustainable chemical agents. Cellulose presents itself as an advantageous candidate for this purpose. Among the many advantages of cellulose, its biodegradability, hydrophilicity, chirality, wide availability and ease of chemical

modification can be highlighted [11–13]. However, cellulose is characterized as high molar mass fibers, with a high degree of crystallinity and insoluble in most common organic solvents. These limitations hinder its use as a separation medium for liquid chromatography with adequate performance and selectivity. Despite this cellulose characteristics, Lehnert et al. [14] prepared a stationary phase based on unmodified cellulose to efficiently separate mixtures of vitamins or sugars in hydrophilic interaction liquid chromatography (HILIC) concept. However, due to the ease of chemical modification, it's possible to use cellulose as a stationary phase in other HPLC modes by their derivatization to a substance that is soluble in some organic solvent, since in the form of solution its attachment to the chromatographic support can be favored [15–20]. In literature [15–34] the most use of cellulose derivative as HPLC stationary phase has focused on the enantioseparation, our idea is to use the cellulosic derivative as a differentiated selectivity material that provides adequate separation of mixtures with poor resolutions as those constituted by polar compounds or by compounds with similar physical and chemical properties.

The attachment of cellulose derivative on silica gel as HPLC stationary phase can be performed by some methods, such as photo irradiation [33]; linkage with diisocyanates [34]; click chemistry

\* Corresponding author at: Instituto de Ciências Exatas e Naturais do Pontal, Universidade Federal de Uberlândia, Rua Vinte, 1600, Bairro Tupã, Ituiutaba 38304-402, Brazil.

E-mail address: [anizio@ufu.br](mailto:anizio@ufu.br) (A.M. Faria).

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[35]; linkage at the reactive terminal [36,37]; or others. All these methods use some chemical agent to immobilize cellulose derivative on silica. In this work, cellulose dodecanoate (cellulose laurate) was evaluated as a reversed stationary phase for HPLC using a physical preparation method without the use of chemical reagents to coating silica particles. The method of preparation of cellulose dodecanoate phases was based on the thermal immobilization of polymers on inorganic oxides which results in an efficient coating of support particles, independently of the binding strength of support active sites with the polymer [38–41]. Cellulose dodecanoate was the focus of this work since studies indicate that a carbon chain between 12–14 atoms can be provided intermediate selectivity to  $C_{18}$  and  $C_8$  phases, resulting in adequate separations in the same chromatographic run for compounds with different polarity [42,43]. In addition, the use of a cellulose derivative with a longer carbon chain, increasing the hydrophobicity of the material, makes possible the evaluation of cellulose contribution (polarity and chirality) in the selectivity of separation. The thermal immobilization of cellulose dodecanoate on silica particles was optimized and the retention properties of the obtained material were evaluated. The potentiality of use as a reversed phase in the separation of mixtures with low resolution in commercial  $C_{18}$  chemically bonded phases was evaluated.

## 2. Experimental

### 2.1. Chemicals

Kromasil silica (5  $\mu\text{m}$ , 100 Å) was obtained from Akzo Nobel (Bohus, Sweden). HPLC-grade methanol (MeOH), chloroform and acetonitrile (ACN) were obtained from JT Baker (Xalostoc, Mexico). Microcrystalline cellulose analytical-grade was obtained from Synth (Diadema, Brazil). Dodecanoyl chloride (~99%), *N,N*-dimethylacetamide (DMAc, 99.9%) and lithium chloride (99%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Analytical grade solvents methanol, ethanol, acetone and toluene was acquired from Qhemis (São Paulo, Brazil). All compounds from test mixtures were analytical standard (>99% purity) and purchased from Sigma-Aldrich. Solvents were filtered through a 0.22  $\mu\text{m}$  PTFE membrane (Millipore, Bedford, MA, USA).

### 2.2. Synthesis of cellulose dodecanoate

Cellulose dodecanoate was synthesized in two steps. In the first step, 2.00 g of microcrystalline cellulose were added in 16 ml of pyridine under constant stirring. This mixture was placed in an ice water bath. 16 ml of dodecanoyl chloride dissolved in 16 ml of dimethylacetamide were added dropwise under stirring for 15 min. The mixture was stored for 48 h at 8 °C. The product ester was precipitated into 500 ml ice water, filtered and then was washed with ethanol and distilled water, then again washed with acetone and distilled water, then the produced ester was dried at room temperature for 24 h. After that, the product was placed in an oven at 50 °C until completely dried [44].

In the second step, 2.00 g of previously dried material were placed in 67 ml of DMAc under reflux and stirred for 2 h. Then, 4.800 g of lithium chloride were added under reflux and stirred for 1 h. Heating was then stopped, leaving the mixture under stirring and ambient temperature overnight. Then, 16 ml of dodecanoyl chloride dissolved in 16 ml of DMAc were added dropwise under stirring. The mixture was stored for 48 h at 8 °C. Solid material was filtered and then was washed with methanol and distilled water, then again washed with acetone and distilled water, then the produced ester was dried at 50 °C overnight. The dried material

obtained was cellulose dodecanoate used as the polymeric coating of chromatographic support.

### 2.2.1. Degree of substitution in cellulose dodecanoate

Degree of substitution (DS) in cellulose dodecanoate was determined by titration according to the procedure adapted from Chauvelon et al. (1999) [45], through a saponification reaction of 0.1 g of cellulose dodecanoate, dispersed in 5.0 ml of ethanol, with 5.0 ml of NaOH (0.25 mol/l) for 24 h. After this period, 10.00 ml HCl (0.25 mol/l) was added to the system, which was left to stand for 30 min. Next, the mixture was titrated using a standard 0.25 mol/l NaOH solution, using phenolphthalein as indicator. This procedure was repeated in triplicate.

### 2.3. Preparation of cellulose dodecanoate thermally immobilized on silica particles

Spherical silica particles were dried in air at 140 °C for 12 h and added to a 2.7% (w/v) solution of cellulose dodecanoate (DoDC) in toluene, obtaining a specific mass of 0.5 g DoDC/g silica. The mixture was slowly stirred for 3 h at room temperature, sonicating eventually for 10 min. After that, the solution was placed in a fume hood to allow the complete solvent evaporation, without stirring, at room temperature. Dried material was placed in an oven to induce thermal immobilization that was performed at 120 °C for 8 h.

After immobilization, the non-immobilized DoDC fraction was removed from the material by sequential washing with toluene, MeOH, and MeOH:H<sub>2</sub>O (1:1) and discarding of supernatant after centrifugation at 2500 rpm for 4 min. Si(DoDC) stationary phase was dried at 60 °C for 1 h prior to packing the columns.

Stainless steel type 316 columns (50 mm  $\times$  4 mm i.d.) were slurry packed using 10% slurries (w/v) of Si(DoDC) phases in chloroform, applying a constant packing pressure of 60 MPa with methanol as propulsion solvent, using a Restek packing in a box kit (Bellefonte, PA, USA). Columns were conditioned for ~1 h with mobile phase at 0.4 ml/min prior to the chromatographic evaluations.

### 2.4. Physicochemical and morphological characterizations

Small portions of Si(DoDC) were submitted for morphological and physicochemical characterizations. The carbon content of Si(DoDC) phases was obtained by elemental analyses, using a Model CHN-2400 Perkin-Elmer Analyzer (Shelton, CT, USA). Specific surface area, pore volume and pore diameter of Si(DoDC) phases were obtained with an equipment ASAP 2010 Analyzer (Micromeritics, Norcross, GA). Approximately 10 mg of Si(DoDC) samples were outgassed at 0.004 atm and 120 °C for 24 h prior to analysis. A morphological evaluation of Si(DoDC) was completed with the images of its particles. Samples were sputter-coated with gold and then examined with a Tescan model Vega3 scanning electron microscope (Brno, Czech Republic) at 20 kV.

The thermal stability of Si(DoDC) phase was studied using samples of ~5 mg, heating from 25 °C to 600 °C at 10 °C/min in an inert atmosphere, using a Shimadzu instrument DTG-60H model (Tokyo, Japan). Samples of DoDC and Si(DoDC) were measured by infrared spectroscopic using a JASCO FTIR-4100 spectrometer. Samples were prepared using potassium bromide (KBr) pellets in a sample:KBr ratio of 1:100. The infrared spectral range was evaluated from 400 to 4000  $\text{cm}^{-1}$  using a resolution of 4  $\text{cm}^{-1}$  and 32 scans/min.

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