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Preparation and evaluation of immobilized 4-methylbenzoylcellulose stationary phases for enantioselective separations

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ABSTRACT

A photochemical method for immobilizing polysaccharide derivatives on silica gel has been developed and applied to 4-methylbenzoyl cellulose (PMBC). The photochemically immobilized materials have been used as chiral stationary phases (CSPs) for the chromatographic separation of the stereoisomers of chiral molecules. Through to the immobilization which makes the chromatographic material insoluble in almost all organic solvents, there no restriction regarding the kind of solvent used in the mobile phase. This feature permits to considerably extend the possibilities to improve the selectivity of the separations and or the solubility of the solute in the mobile phase.

The influence of various parameters such as immobilization process, cross-linker type and amount on the chromatographic properties and chiral recognition ability of the resulting CSPs has been investigated using a set of chiral molecules. The impact of the amount of coated polysaccharide material on chiral recognition ability was also examined.

1. Introduction

Numerous chiral stationary phases are currently available for the analytical and preparative chromatographic separation of enantiomers [1,2]. With these tools, it is now possible to resolve almost all racemic compounds by chromatography [3]. While the technique has become the method of choice for the analytical determination of the optical purity of chiral compounds, it is also routinely applied as a means to obtain optically pure substance in preparative amounts up to several tonnes [4,5]. Among all available chiral stationary phases, those derived from polysaccharides [6–11] have been the most used ones, due to the easy modulation of their chiral recognition properties and their relatively high loading capacity [5]. However, one major drawback of the first generation of polysaccharide-based phases was their moderate to high solubility in many organic solvents such as tetrahydrofurane, dioxane, toluene, chlorinated solvents, or ethyl acetate. This feature considerably reduced the choice of mobile phase, thus limiting the possibility of increasing selectivity, of varying retention time, and of improving the solubility of the racemate. In order to improve their properties with respect to this shortcoming, various approaches aiming to immobilize polysaccharide derivatives have been described. *Okamoto* and his group reported on first attempts to immobilize cellulose on silica gel through a dicarbamate linkage using diphenyl diisocyanate as a crosslinking agent in 1987 [12] but it appeared that the presence of the cross-linker negatively affects selectivity as the number of linkage increases. Improvement of this approach has permitted to prepare more stable and more efficient immobilized polysaccharide phases [13]. Other approaches have been developed, most of them includes the utilization of vinyl derivatives of polysaccharides. They have been reviewed a few years ago [11]. More recently another immobilization technique has been developed by Okamoto and his group and is based on the intermolecular polycondensation of polysaccharide derivatives and silica gel, both bearing triethoxysilyl groups [14,15].

We report here on the elaboration of a different immobilization concept for the preparation of insoluble polysaccharide stationary phases under very mild conditions, based on a photochemical approach. Preliminary and partial results arising from this concept have been described in an earlier patent [16].

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2. Experimental

2.1. Instrumentation

The high-performance liquid chromatography (HPLC) systems used in this study consisted of 1) a Shimadzu SPD-6AV pump, a variable wavelength Shimadzu LC-6A UV–vis detector in series with a Perkin-Elmer (Model 241) polarimeter and a Reodyne injector fitted with a 20-microliter sample loop; the apparatus was connected to a PC and the data were managed with the GINA-NT (Bional AG, Dietikon, Switzerland) chromatographic software; 2) a Shimadzu LC-10AD pump, a variable wavelength Shimadzu SPD-10A UV–vis detector in series with a Jasco OR-990 chiral detector and a Reodyne injector fitted with a 20-microliter sample loop; the apparatus was connected to a PC loaded with the Shimadzu chromatographic software (version 1.62).

2.2. Synthesis of the stationary phases

2.2.1. Low molecular weight cellulose

150 g of microcrystalline cellulose (Avicel, Merck) are suspended in a mixture of 1.2 l toluene, 150 ml acetic acid, and 3 ml trifluoromethylsulfonic acid. 450 ml of acetic acid anhydride is added dropwise to this suspension. This mixture is then stirred at room temperature for 15 h. The resulting cellulose triacetate suspension is filtered, washed tree times with methanol, and dried under vacuum at 80 °C for 6 h.

Cellulose triacetate obtained as above are suspended in 1.3 l isopropanol. 320 ml of hydrazine monohydrate is added dropwise to this suspension. The mixture is stirred at 60 °C for 60 h. The suspension is filtered, washed twice with isopropanol and once with methylene chloride, and dried under vacuum at 80 °C for 6 h. Yield: 142 g low molecular weight cellulose. The degradation degree of the cellulose was characterized by a sharp decrease in viscosity of its para-methylbenzoate derivative from 0.75dl/g to 0.43dl/g, measured in chloroform at 25 °C.

2.2.2. Preparation of (2,5-dihydro-3,4-dimethyl-2,5-dioxo-pyrrol-1-yl)-acetyl chloride (cross-linking spacer A)

183.2 g of (2,5-dihydro-3,4-dimethyl-2,5-dioxo-pyrrol-1-yl)-acetic acid [17] are suspended in 360 ml toluene. The solution is heated under reflux for 16 h using a water separator. During that period, approximately 40 ml of toluene/water are distilled off azeotropically. The solution is then cooled to 70 °C and 76.3 ml of thyonyl chloride are added dropwise in the course of 90 min. As soon the evolution of gas has ceased (approx. 2 h), the temperature is increased to 90 °C for 2 h and then to 110 °C for 30 min. After cooling, the solution is concentrated. The liquid residue is distilled and the fraction boiling at 182–184 °C is collected. Yiel: 172.5 g (85.5%). Elemental analysis; Calc.: C 46.6; H 4.00; N 5.95; O 23.81; Cl 17.58. Found: C 49.42; H 4.21; N 6.71; O 22.93; Cl 16.83. ^{1}H NMR (CDCl3): 2.00 (s, CH3), 4.63 (s, CH2)

2.2.3. Preparation of 4-(2,5-dihydro-3,4-dimethyl-2,5-dioxo-pyrrol-1-yl)-benzoic acid chloride (cross-linking spacer B)

48 g (0.35 mol) of 4-aminobenzoic acid are dissolved in sodium hydroxide solution (14 g of NaOH in 300 ml water). To that mixture, a solution of 44.2 g of dimethylmaleic acid anhydride in 300 ml of dimethylacetamide is added dropwise with stirring. The solutionis heated at 90 °C and, after 1.5 h, 175 ml of aqueous hydrochloric acid (2N) are added. The solution is cooled to room temperature and stirring is switched off. The crystalline product which has precipitated is filtered off, washed with water and dried in vacuo at 60 °C. Yield: 73.6 g (85.7%). Melting point: 230–231 °C. 73.6 g of this intermediate are suspended in 700 ml of dry toluene. 32 ml of thionyl chloride are added dropwise to this suspension at 70 °C. As soon as the evolution of gas has ceased (approximately 2 h), the temperature is increased to 80 °C for 2 h. After cooling, the solution is concentrated using a rotary evaporator. The solid residue is recrystallized from toluene and then dried at 60 °C. Yield 88%. Melting point: 199–200 °C. ^{1}H NMR (CDCl3): 2.08 (s, CH3), 7.68 (d, phenyl), 8.20 (d, phenyl).

2.2.4. Preparation of 4-[(2,5-dihydro-3,4-dimethyl-2,5-dioxo-pyrrol-1-yl)methyl]-benzoic acid chloride (cross-linking spacer C)

100 g (0.66 mol) of 4-(aminomethyl)-benzoic acid are dissolved in sodium hydroxide solution (26.4 g of NaOH in 300 ml water). To that mixture, a solution of 83.3 g of dimethylmaleic acid anhydride in 500 ml of dimethylacetamide is added dropwise with stirring. The solution is heated at 90 °C and, after 1.5 h, 330 ml of aqueous hydrochloric acid (2N) are added. The solution is cooled to room temperature and stirring is switched off. The crystalline product which has precipitated is filtered off, washed with water and dried in vacuo at 60 °C. Yield: 155 g (90%). Melting point: 182–183 °C. 120 g of this intermediate are suspended in 1000 ml of dry toluene. 50 ml of thionyl chloride are added dropwise to this suspension at 70 °C. As soon as the evolution of gas has ceased (approximately 2 h), the temperature is increased to 80 °C for 2 h. After cooling, the solution is concentrated using a rotary evaporator. The solid residue is recrystallized from toluene and then dried at 60 °C. Yield: 155 g (80%). Melting point: 98–99 °C. ^{1}H NMR (CDCl3): 1.98 (s, CH3), 4.72 (s, CH2), 7.45 (d, phenyl), 8.06 (d, phenyl).

2.2.5. Reaction of cellulose with acid chlorides (cross-linking spacers A, B or C)

In a brown glass reactor, 3 g of low molecular weight cellulose prepared above are dried for 4 h at a bath temperature of 125 °C with nitrogen flushing. The dried cellulose is suspended in 120 ml of dry pyridine and mixed with one of the above acid chloride derivative (cross linking spacers A, B or C) in the presence of catalytic amount of 4-(dimethylamino)-pyridine (0.2 ml). 151 ml triethylamine were then added dropwise to the suspension. The mixture is stirred for 24 h at 80 °C. After cooling, the mixture is poured in 1 l methanol and the cellulose ester derivative is isolated by filtration and washed with methylene chloride. Various degrees of substitution have been obtained depending on the amount of acid chloride added. The products have been characterized by elemental analysis.

2.2.6. Esterification of the residual hydroxyl groups of the intermediates of cellulose obtained from cellulose and the cross-linking spacers 1–3 with 4-methylbenzoyl chloride

2.57 g of the intermediate derivative of cellulose are suspended 86 ml of dry pyridine and 22 ml of triethylamine in the presence of 10 mg of 4-(dimethylamino)-pyridine. 20 ml of 4-methylbenzoic acid chloride were added dropwise to this suspension and the mixture was stirred under nitrogen for 42 h at 60 °C. After cooling, the mixture was poured into 200 ml methanol and the precipitate is filtered off. The crude product is dissolved in 200 ml of methylene chloride and the solution is filtered. The product is precipitated by addition of methanol to this solution. The precipitate is filtered off, washed with methanol and dried under high vacuum. The products have been characterized by elemental analysis.

2.4. Coating of the cellulose derivatives

Two methods were used for coating, namely the evaporation and the precipitation techniques, which have been described in details in a previous paper [18].

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