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Cholesterol-based polymeric monolithic columns for capillary liquid chromatography

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ABSTRACT

A novel, cholesterol-based polymeric monolithic stationary phase for capillary liquid chromatography, was prepared by thermally initiated *in-situ* polymerization. Cholesteryl methacrylate (CholMA) was used as a functional monomer and trimethylolpropane trimethacrylate (TRIM) was a cross-linker, while azobisisobutyronitrile (AIBN) was an initiator. Isooctane and toluene were chosen as "poor" and "good" solvent, respectively, as constituents of the porogen solvent. Isocratic elutions of alkylbenzenes and separation of the testing mixture of *o*-terphenyl and triphenylene were conducted for all of the monoliths to assess their hydrophobicity and planar selectivity characteristic for cholesterol-based stationary phases. The synthesized columns demonstrated efficiency exceeding $N = 10{,}000$ plates and a plate height of ca. $H = 30\ \mu m$. Column preparation was found to be highly reproducible; the relative standard deviation (RSD) values ($n = 3$) for day-to-day and column-to-column were less than 4.08 and 2.02%, respectively, based on retention factor of alkylbenzenes.

1. Introduction

The requirements of modern analytical chemistry towards fast, cheap and simple analysis makes it necessary to improve the existing and create new materials as the stationary phases for chromatographic columns. There are quite many stationary phases used in contemporary liquid chromatography. Some of them (like popular C_{18} materials) appeared to be more "universal" than others, however, there have been still many effort paid to the development of tailored phases for particular applications. Most of the present LC materials are silica-based stationary phases. Silica gel can be modified, e.g., with hydrophobic hydrocarbon chains of various lengths (C_2, C_8, C_{18}, C_{22}, C_{30}) or polar group (–NH_2, –CN, –DIOL) to achieve the desired chromatographic properties [1,2]. Special attention of scientists has been devoted to cholesterol chemically immobilized on silica, as the selective material for chromatographic columns. It should be noted here that cholesterol-based silica stationary phases, introduced by Pesek et al., were initially studied as an example of liquid crystal stationary phases [3]. However, later interest in these materials was rather connected with the role of cholesterol as a basic component of biological membranes, so such a phase was used in simulation of the selective permeation of drugs through the biological membrane, and, as a result, the scientists considered the creation of so-called immobilized artificial membrane (IAM) stationary phases [4]. The most important advantage of the cholesterol stationary phase is that it can be successfully used in both normal- and reversed-phase liquid chromatography. The compounds resolved include, e.g., antibiotics [5], benzodiazepines [6], flavonoids [7], polycyclic aromatic hydrocarbons (PAHs) [1,8–10], steroids [11–13], beta-blockers [14,15] and different xenobiotics [4]. Additionally, commercially available cholesterol-based chromatographic columns (*Cosmosil Cholester* and *Cogent*^{TM} *UDC*-cholesterol) were used in other applications, e.g., separation of catechins, saikosaponins, carotenes, vitamin K isomers [16,17].

The most important methods of modification of silica with cholesterol are silanization and hydrosilation procedures [5]. During hydrosilation procedure a silica hydride surface leads to the formation of direct Si-C bond [7,18]. Throughout the silanization procedures, silica surface can be modified by organo-silanes. The cholesterol molecule may be directly attached to the Si-H group [12], silanization procedures or to the different linkage molecules, e.g. amino- [8] or diamino-bonded ligand [19]. Attachment of cholesterol molecules can be performed using a variety of derivatives in which the cholesterol hydroxyl group is changed, e.g. cholesteryl chloroformate [2,4,9] or cholesteryl-10-undecenoate [6,10].

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Most of the cholesterol-based columns listed in the above-mentioned references are packed columns. As far as the column packing process itself is not very complicated, it requires a lot of experience. An additional difficulty when it comes to capillary columns packing is the use of internal frits protecting the bed from extrusion. The frits are also suspected to cause formation of gas bubbles in capillary electrochromatography [20].

The above problems have been solved by developing monolithic columns [21,22]. A driving force in their development was the need to achieve high efficiency together with high permeability (low column back pressure) and to improve the stability compared to packed bed columns. It is generally believed that the most important advantages of the monolithic columns are much easier procedure of their preparation, no frits are needed [22,23], as well as possibility of more or less easy adjusting of their porosity [24]. Generally, the monolithic columns are divided into two main groups: organic and inorganic materials [25]. It is difficult to specify which types of monoliths are better and the differing features include mechanical strength, the efficiency of the columns or resistance against organic solvents or solutions of extreme pH, surface area [26], and applicability in separation of macromolecules such as proteins or nucleotides [27]. Also the synthesis of silica-based monoliths is much more complicated and time consuming [24,28–31] in comparison to a single step (usually) polymerization of functional monomers and cross-linking reagents in the presence of a porogen solution and an initiator [25]. Initiation of polymerization can occur under the influence of heat, UV or γ radiation and chemical reaction [21]. Morphology of the obtained polymeric monolith depends on the method of initiation, temperature, the composition of the polymerization mixture and the type of porogen solution [32]. Although the polymeric monoliths are usually characterized by lower surface area the hyper-cross-linking process enable to increase surface area of the polystyrene polymers significantly [27].

Although the cholesterol stationary phases based on the silica–gel support have been used in liquid chromatography since 1990s, in the available literature there are no reports on synthesis and characterization of cholesterol-based polymeric monolithic columns. In this work, we present the preparation and characterization of novel cholesterol monolithic capillary columns. As a functional monomer, cholesteryl methacrylate was used, and trimethylolpropane trimethacrylate (TRIM) as a cross-linking monomer. The obtained monoliths were successfully used for separation of such compounds as alkylbenzenes and mixture of *o*-terphenyl and triphenylene.

2. Experimental

2.1. Chemicals and reagents

Trimethylolpropane trimethacrylate (TRIM), 3-(trimetho xysilyl)propyl methacrylate (γ-MAPS), methacryloyl chloride, 2,2,4-trimethylpentane (isooctane), *o*-terphenyl, triphenylene, cholesterol, triethylamine, hydroquinone, cyclohexane were purchased from Sigma-Aldrich (Steinheim, Germany). The radical polymerization initiator, 2,2′-azoisobutyronitrile (AIBN), was from Fluka (Buchs, Switzerland). Toluene, chloroform, ethyl acetate, tetrahydrofuran, xylene, methanol, ethanol, acetone, *n*-hexane, sodium hydroxide, hydrochloric acid, thiourea (all of analytical grade) were purchased from Polskie Odczynniki Chemiczne (POCh, Gliwice, Poland). Acetonitrile (HPLC ultra gradient grade) was from J.T. Baker (Witko, Łódź, Poland). Deionized water was produced in our laboratory using a Milli-Q ultrapure water system (Millipore, Bedford, MA, USA). Polyimide-coated fused silica capillaries of various internal diameters were purchased from Polymicro representative CM Scientific Ltd. (Silsden, United Kingdom).

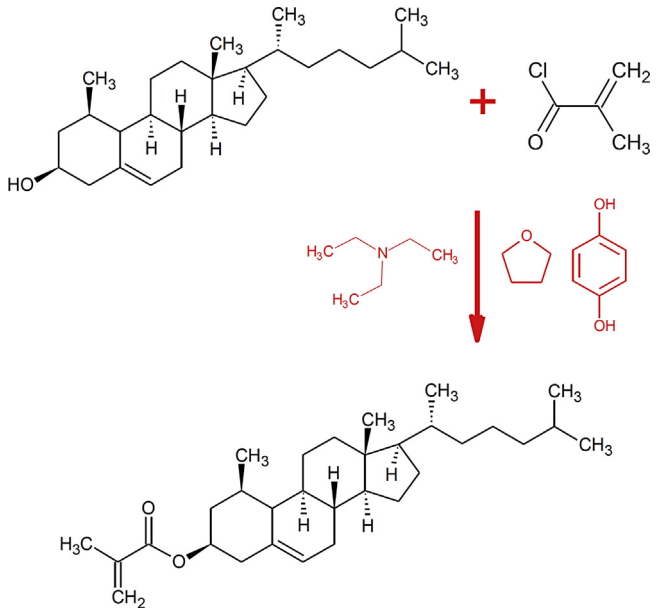

Fig. 1. Schematic illustration of the synthesis of cholesteryl methacrylate.

2.2. Capillary liquid chromatography

All chromatographic measurements were performed on a capillary LC system consisting of a pump delivering the mobile phase (Agilent 1260 cap pump with degasser, Agilent Technologies, USA), 10-port valve with a microelectric actuator model C72MX-4694EH (Vici Valco Instruments Inc. Co., Houston, TX, USA) with a 50 nl capillary loop, a set of connecting capillaries (TSP capillaries of various diameters from Polymicro Technologies). On-column detection was performed using the Spectra-100 (Thermo Separations Products, San Jose, CA, USA) detector. The chromatographic system was controlled and the data were collected by the Clarity software (Data Apex, Prague, Czech Republic).

The pressure versus flow relationship during initial studies was measured using an air-driven constant pressure HPLC pump from Knauer (Knauer GmbH, Berlin, Germany) which was also used for column flushing after synthesis.

2.3. Synthesis of cholesteryl methacrylate monomer (CholMA)

The cholesteryl methacrylate (CholMA) monomer is not commercially available, so there is a need to synthesize it for the purpose of this research. In the literature the number of available publications on CholMA synthesis is very limited [32–40]. The available procedures are based on the same substrates, and the differences relied on conditions of carrying out the synthesis which, according to the authors, influenced the obtained yield in the range of 52–94%. The scheme of the process is schematically presented in Fig. 1.

The cholesteryl methacrylate monomer was synthesized following the procedure proposed by Zhou and Kasi [33] with some modifications. Cholesterol (8.0 g; 0.02 mol), triethylamine (30 mL; 0.22 mol), tetrahydrofuran (100 mL) and hydroquinone (0.00386 g; 0.035 mmol) were placed in a round-bottom flask (the reactor) equipped with a magnetic stir bar and a reflux condenser. The mixture was stirred and heated at reflux (72 °C) until complete dissolution of the components occurred (clear, transparent solution was observed). Then, methacryloyl chloride (3.3 mL; 0.68 mol) was slowly added dropwise to the mixture over a period of 30 min. Addition of this substrate resulted in the precipitation of a white