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Macroporous monolithic columns modified with cholesterol-containing glycopolymer for cholesterol solid-phase extraction

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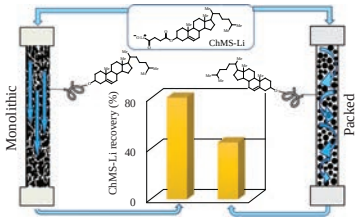
The macroporous monolithic stationary phase was elaborated for the efficient solid-phase extraction of cholesterol from aqueous media. The advantages of the developed monolithic materials as compared with the bead-based columns of the same functionality were demonstrated.



The development of methods for the efficient extraction of hydrophobic compounds from biological liquids is of significant importance for biochemistry, medicine and biotechnology.[1,2] Among the hydrophobic compounds in biological liquids the cholesterol is of special interest due to the potential risk of the appearance of cardiovascular diseases. One of the most practically useful methods to remove the hydrophobic compounds from a solution is solid-phase extraction.[3] The method is based on the adsorption of a component of interest from a solution onto the solid support, its accumulation and further desorption.[4] A series of studies on cholesterol extraction with the use of molecularly-imprinted polymer sorbents,[5–8] as well as cyclodextrin-containing solid phases[9] were reported. Also, it is known that cholesterol molecules are capable of undergoing self-complexation caused by the hydrophobic interactions in aqueous media.[10]

The goal of this research was the development of novel macroporous monolithic sorbents for the effective solid-phase extraction of cholesterol from aqueous media. Being introduced as HPLC stationary phases, macroporous monolithic materials are widely used for different dynamic processes based on an interphase mass exchange, such as liquid,[11] gas[12] and electrochromatography,[13] solid phase extraction[14] and high flow-through heterogeneous biocatalysis.[15] A key feature of macroporous monolithic matrices is the convective mechanism of interphase mass transfer favoured to the more effective distribution of a solute between liquid and solid phases compared to the packed columns with dominant diffusion mass transport. Moreover, monolithic matrices are characterized by elevated mechanical and chemical stability. Being highly cross-linked, they practically do not swell in both water and organic media.

Recently, the preparation of the cholesterol-containing macroporous monoliths was reported.[16,17] In particular, capillary monolithic columns based on copolymer of colesteryl methacrylate and trimethylolpropane trimethacrylate [P(ChMA-co-TRIM)] and copolymer of colesteryl methacrylate, 4-methylstyrene, vinylbenzyl chloride and divinylbenzene were developed and applied as stationary phases in reversed-phase mode of liquid chromatography for separation of different low molecular hydrophobic compounds[16] and proteins.[17] The successful application of the former for separation of cholesterol structure analogues, *viz.* steroid hormones (estriol, testosterone, estrone, β-estradiol, progesterone), using the columns prepared from polymerization mixture containing 12.5 wt% of ChMA and 27.5 wt% of TRIM was demonstrated.[18]

Here, we suggested another way to prepare the cholesterol-containing monolith and applied it for cholesterol solid-phase extraction. A general scheme illustrating the developed approach is presented in Figure 1. At the first step, the copolymer of glycidyl methacrylate and ethylene dimethacrylate [P(GMA-co-EDMA)]

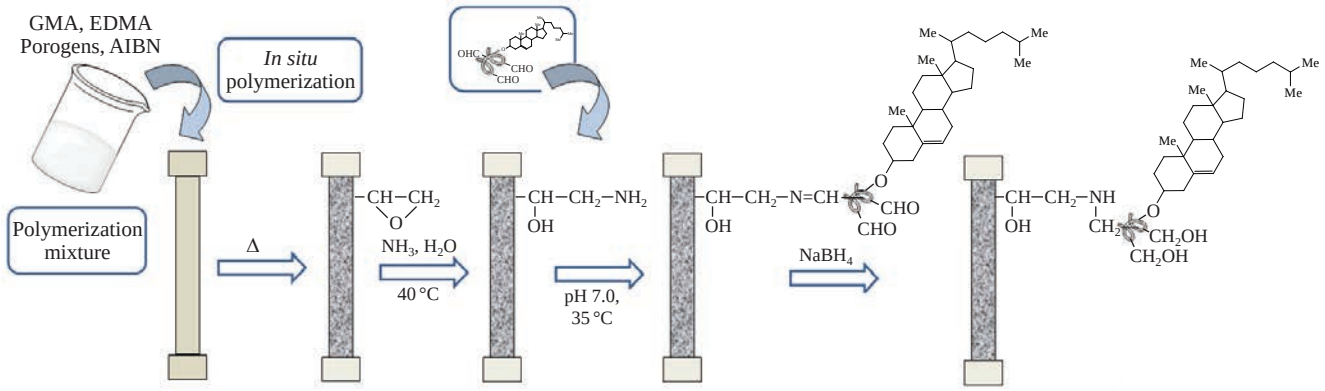


Figure 1 Preparation of cholesterol-containing macroporous monoliths.

macroporous monolithic matrix was synthesized using free radical thermo-initiated polymerization (for details of synthesis, see Online Supplementary Materials). The surface of solid phase was aminated *via* the conversion of epoxy groups and then covalently modified with biocompatible water-soluble polymer containing cholesterol residues.

The copolymer of 2-deoxy-2-methacrylamido-D-glucose (MAG) and cholesteryl methacrylate (ChMA) was synthesized and used as a functional vector capable of interacting and binding hydrophobic compounds such as cholesterol.[19] Being a glycopolymer [or poly(vinylsaccharide)], PMAG is water-soluble and biocompatible.[20] The saccharide units that present in a side-chain can be easily oxidized with IO_4^- to form highly reactive aldehyde groups convenient for covalent immobilization, cross-linking or conjugation processes.[21]

To generate the macroporous structure in the process of copolymerization of GMA and EDMA, cyclohexanol, dodecanol and toluene, which are poor solvents for the formed polymer, were applied as porogens (for details of synthesis, see Online Supplementary Materials). The pore size and porosity of monolithic matrices were calculated according to the data on hydrodynamic permeability determined as described earlier.[22]

To keep the balance between the good hydrodynamic properties, high mechanical stability, as well as low back pressure, the mean pore size of monolithic materials has to be in the range of 1–2 μm.[23] The optimization of porous structure was realized by varying the composition of porogenic solvents in polymerization mixture. According to the data given in Table 1, the introduction of toluene into polymerization mixture allowed one to increase the mean pore size of resulted materials. The porogen ratio, *e.g.* cyclohexanol : dodecanol : toluene = 6 : 2 : 2, was counted to be optimal. The materials prepared with the use of this porogenic system had the following characteristics: permeability $B = 0.5\times10^{-13}$ m^2, mean pore size d = 1960±70 nm, and porosity 63±3%.

The copolymer of MAG and ChMA was used for the functionalization of monolithic surface (Scheme 1, for more details, see Online Supplementary Materials). Moreover, the homopolymer of MAG was used to compare the properties of solid

Table 1 Influence of porogenic mixture composition on the characteristics of macroporous monolithic materials.[a]

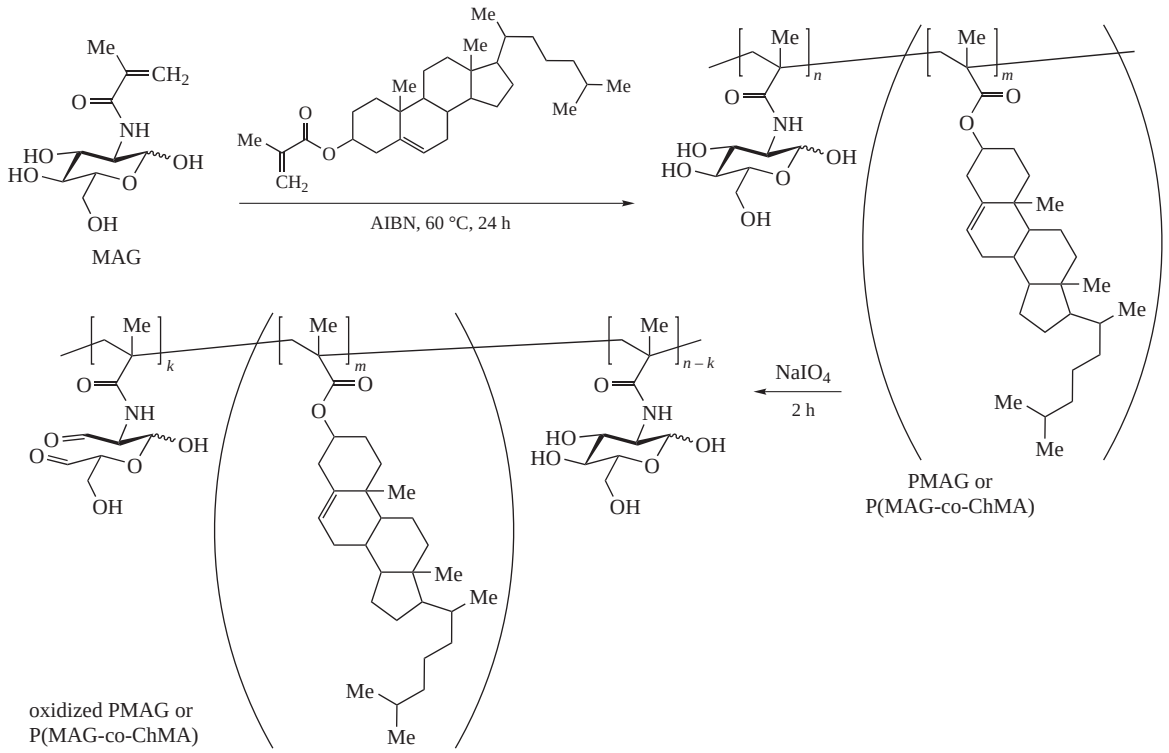
Entry	Composition of porogens (vol%)			Characteristics of monolithic materials	
	Cyclohexanol	Dodecanol	Toluene	Mean pore size/nm	Porosity (%)
1	40	60	-	650	60
2	30	60	10	1160	73
3	25	55	20	1480	78
4	25	60	15	1850	43
5	20	60	20	1960	63

[a] Conditions of *in situ* polymerization: GMA : EDMA (60 : 40 v/v); 1.0 wt% of AIBN, 70 °C, 8 h; stainless steel cartridge of 4.6 mm i.d. × 50 mm was used.

phase extractors. MAG and ChMA were synthesized as described elsewhere.[24,25] The molecular weights of PMAG and P(MAG-co-ChMA), estimated by the Mark–Kuhn–Houwink equation derived for the MAG homopolymer,[24] were found to be around 15 000. In the case of P(MAG-co-ChMA), the calculated ChMA content was 4 mol% or 6 wt%.

The covalent immobilization of both PMAG and P(MAG-co-ChMA) on the surface of monolithic materials was carried out using the reaction between aldehyde and amino groups preliminary introduced into polymers and polymer matrix, respectively. For that, aldehyde groups were generated in PMAG *via* oxidation of glucose units with sodium periodate (see Scheme 1) as described.[24] The quantity of aldehyde groups was determined using the reaction with Schiff's reagent. Both oxidized PMAG and P(MAG-co-ChMA) contained 56±4 mol% of aldehyde groups.

To introduce the amino groups into the P(GMA-co-EDMA) monoliths, the amination with aqueous ammonium solution was performed (for more details, see Online Supplementary Materials). According to the elemental analysis, 26±1% of epoxy moieties was converted into the amino groups. To compare the extraction efficiency of macroporous monoliths with that of conventionally packed columns, the P(GMA-co-EDMA) macroporous beads



Scheme 1 Synthesis and oxidation of PMAG or P(MAG-co-ChMA).

(~200 μm) were modified using the same way. The amounts of polymers bound to the monolithic matrix and bead-based sorbent were 1.3±0.1 and 2.0±0.2 mg per gram of stationary phase, respectively.

Taking into account that cholesterol is water-insoluble compound, to study its extraction from aqueous media, the water-soluble cholesterol derivative, *viz*., its lithium monosuccinate salt (ChMS-Li) was applied (Figure S1, Online Supplementary Materials). The ability of prepared sorbents to extract the water-soluble cholesterol form was studied under the dynamic conditions. To evaluate the effect of column material on possible cholesterol extraction, the unmodified and aminated monolithic columns were also tested. ChMS-Li extraction was carried out from 0.01 M Na-phosphate buffer solution (pH 7.0) at a flow rate of 0.5 ml min^{-1}. After that the columns were washed with water, methanol–water (1:1) and final desorption was performed with methanol. The ChMS-Li extraction was studied using both zonal elution and recirculation modes. The former is characterized with the loading of fixed small amount of the solution with a high concentration, whereas the latter is based on the continuous passing of a solution through a column in a cyclic manner.

In the first case, tested solution (0.1 ml, 1.2 mg ml^{-1}) was loaded on both monolithic and packed columns. The ChMS-Li adsorption on the surface of monolithic and packed materials was comparable and did not exceeded 10% for matrixes containing epoxy groups [initial P(GMA-co-EDMA)], 24% for amino-bearing solid phases [aminated P(GMA-co-EDMA)] and 34% for materials modified with oxidized PMAG [Figure 2(*a*)]. As expected, the highest recovery (72%) was achieved for monolithic column modified with P(MAG-co-ChMA). The column packed with particles bearing oxidized P(MAG-co-ChMA) demonstrated only 45% recovery. These results can be related to the mentioned difference in mass transfer mechanism of packed and monolithic columns, *viz*., the absence of 'dead'-volume and domination of convection over diffusion in monoliths.

Thus, the introduction of cholesterol residues into the sorbent structure provides the binding of hydrophobic compounds in aqueous media, while the application of macroporous monoliths improves the extraction efficiency.

As compared to the zonal elution mode, which is basically used as analytical regime, the recirculation mode has an advantage of accumulation of target compounds from large volumes. For the extraction of ChMS-Li the recirculation of a 2.0 ml of solution (0.3 mg ml^{-1}) was carried out. It was found that after first cycle about 50% of ChMS-Li, was retained [Figure 2(*b*)], whereas the recovery of target compound after three cycles was close to 90%. Therefore, the recirculation can be considered as an efficient approach to extract cholesterol with the use of developed monolithic solid phase.

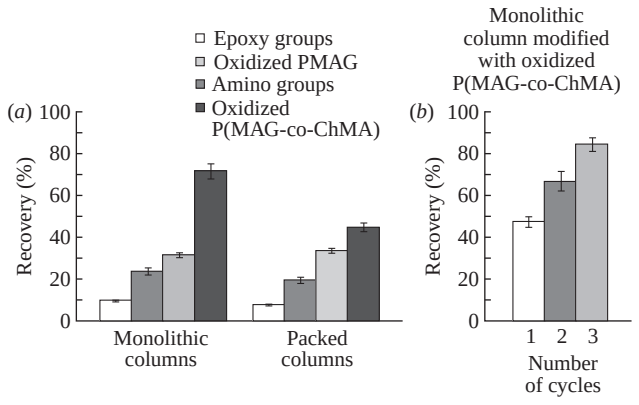


Figure 2 Effect of the solid phase surface functionality on ChMS-Li extraction on the monolithic and packed columns (*a*) in the zonal elution and (*b*) recirculation modes.

The repeatability and stability of a column during the solid-phase extraction process are critical in the evaluation of developed sorbents. The day-to-day repeatability was studied according to cholesterol recovery. The RSD value (n = 5) for the developed cholesterol-bearing monolithic column was found to be 5.2% for the operation in zonal elution mode and 4.6% in recirculation mode. As for the stability study, the obtained cholesterol-containing monolith was used approximately in 20 cycles of adsorption/desorption during a month. No decrease in the extraction efficiency was observed after both column usage and storage up to three months.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2018.05.038.

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