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Methacrylate-based monolithic layers for planar chromatography of polymers

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ABSTRACT

A series of macroporous monolithic methacrylate-based materials was synthesized by *in situ* free radical UV-initiated copolymerization of functional monomers, such as glycidyl methacrylate (GMA), butyl methacrylate (BuMA), 2-aminoethyl methacrylate (AEMA), 2-hydroxyethyl methacrylate (HEMA) and 2-cyanoethyl methacrylate (CEMA), with crosslinking agent, namely, ethylene glycol dimethacrylate (EDMA). The materials obtained were applied as the stationary phases in simple and robust technique – planar chromatography (PLC). The method of separation layer fabrication representing macroporous polymer monolith bound to the specially prepared glass surface was developed and optimized. The GMA–EDMA and BuMA–EDMA matrixes were successfully applied for the separation of low molecular weight compounds (the mixture of several dies), as well as poly(vinylpyrrolidone) and polystyrene homopolymers of different molecular weights using reversed-phase mechanism. The materials based on copolymers AEMA–HEMA–EDMA and CEMA–HEMA–EDMA were used for normal-phase PLC separation of 2,4-dinitrophenyl amino acids and polystyrene standards.

1. Introduction

Nowadays, rigid macroporous copolymers synthesized by bulk method and known as *polymer monolithic materials* [1] are widely used as efficient sorbents for fast HPLC separations [2], high-speed affinity chromatography [3,4], capillary electrochromatography [5], gas chromatography [6], solid phase extraction [7], as well as high-throughput solid phases for flowing enzymatic reactors [8–10] and platforms for microarrays [11–13]. The arise of interest to polymer monoliths is stimulated by convection-controlled interphase mass exchange resulting from high permeability of such materials and dramatically elevating the speed of a process, their mechanical and chemical stability, as well as the easiness of monolith preparation. Enormous number of current publications are devoted to the various modes of chromatographic separations of biological objects (proteins, peptides, oligonucleotides, DNA, viruses) using monolithic sorbents [14–16]. However, the chromatography of synthetic macromolecules on monoliths is still not thoroughly investigated and, moreover, practically used.

The general separation technique widely applied for polymer analysis is size-exclusion or gel-permeation, chromatography (SEC or GPC, respectively) based on a difference of molecular size and, accordingly, different ability to diffuse into porous space of sorbent particle. This method allows determination molecular weight (M_w) and molecular weight distribution (MWD) of synthetic polymers. On the other hand, SEC does not enable to give any information on chemical composition of studied polymer. Therefore, the separation of macromolecules with close hydrodynamic radii but different composition seems to be not a possible task for this case. Usually, a modern analysis of synthetic polymers is carried out by a combination of SEC with adsorption modes of liquid chromatography (two-dimensional separation) [17–19]. As to the monolithic stationary phases with their high speed advantage, the development of separation methods for synthetic macromolecular compounds represents very important scientific and practical interest.

There are only a few early publications concerning polymer separation, namely, the chromatography on monolithic columns at gradient elution conditions of styrene oligomers [20] and some examples of polymers [20–23]. For example, Petro et al. used styrene–divinylbenzene, glycidyl methacrylate–ethylene dimethacrylate and dihydroxypropyl methacrylate–ethylene dimethacrylate monolithic columns for HPLC of commercial polystyrene, poly(methyl methacrylate), poly(vinyl acetate) and polybutadiene standards [20,21]. It was shown that monolithic columns provided fast and efficient determination of molecular weight parameters of synthetic polymers and constituted a viable, less expensive, much faster alternative to the more expensive and slower conventional packed columns.

Rapid and sufficiently robust method of polymer fractionation is a well-known planar chromatography (PLC) [24]. The first results on this topic were published by Inagaki et al. and Belenkii et al. [25,26]. The advantages of PLC based on a difference in adsorption energy of analytes are the simplicity of equipment, low cost and high speed of separation process. Additionally, planar chro-

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matography can be considered as a rapid method for a selection of conditions for HPLC mode, namely, found at PLC eluent composition providing appropriate separation can be transferred to the column packed with a sorbent of the same chemistry.

The stationary phase in planar chromatography usually represents porous inorganic particles (silica gel) applied as a thin layer on a plate. The bead size of a sorbent and particle size distribution defines an efficiency of separation. The eluent flow is realized due to capillary forces influencing the diameter of interparticle channels.

In 2002, monolithic silica materials in a shape of thin layers were developed and offered for PLC. Such inorganic phase is characterized by bimodal pore size distribution that means its skeleton consists of large transport macropores with a diameter of 1–2 μm and a network of mesopores with size of a few nm. The advantage of such solid phase in PLC was proved by significant increase of adsorption capacity, speed and chromatographic efficiency [27].

Polymer monoliths have not yet found wide application in this separation format. The papers concerning planar separation of peptides and proteins using MALDI-TOF-MS detection and poly(butyl methacrylate-*co*-ethylene dimethacrylate) monoliths as separation of phases have to be mentioned here [28,29]. Recently, Woodward et al. [30] have demonstrated the ability of thin monolithic layers to be used in planar electrophoresis and pressurized electrochromatography for rapid separation of peptides and oligonucleotides.

The presented work describes the development of monolithic methacrylate-based layers with different surface functionality that allows application of different mechanisms of adsorption at PLC separations. The plates were tested in separation of substances of different classes and molecular masses. Thus, the general goal was the preparation of planar polymer monolithic supports of various functionalities and study of their behavior in separation of small compounds and synthetic polymers in different adsorption chromatographic modes.

2. Experimental

2.1. Materials

The microscope glass slides (75 mm × 25 mm, 1 mm thick) were obtained from MiniMed (St. Petersburg, Russia). Chromatographic chamber with dimensions 150 mm × 20 mm × 80 mm and glass capillaries for sample spotting were from Lenchrom (St. Petersburg, Russia).

2.2. Chemicals

Glycidyl methacrylate (GMA, 97% pure), ethylene glycol dimethacrylate (EDMA, 98% pure), butyl methacrylate (BuMA, 99% pure), 2-aminoethyl methacrylate (AEMA, 90% pure), 2-hydroxyethyl methacrylate (HEMA, 98% pure), (CyOH, 99% pure), 2-methoxy-2-phenylacetophenone (99% pure), *p*-aminoazobenzene (98% pure), 1-dodecanol (DoOH, 98% pure), 1,4-butandiol (1,4-BD, 99% pure), N-methyl-2-pyrrolidone (NMP, 99% pure), *p*-aminoazotoluene (97% pure), methyl red (ACS reagent), N-(2,4-dinitrophenyl)-DL-aspartic acid (DNP-aspartic acid), N-(2,4-dinitrophenyl)-L-leucine (DNP-leucine), N-(2,4-dinitrophenyl)-L-tryptophan (DNP-tryptophan), N-(2,4-dinitrophenyl)-β-alanine (DNP-alanine) were purchased from Sigma–Aldrich Rus (Moscow, Russia). 3-(Trimethoxysilyl)propyl methacrylate, polyethylene glycol with $M = 200$ (PEG-200, standard for GPC) were from Fluka AG (Buchs, Switzerland). 2-Hydroxy-2-methylpropiophenone (Darocur-1173, 97% pure), methanol (MeOH, 99.8% pure for liquid chromatography LiChrosolv®), tetrahydrofuran (THF, 99.9% pure for liquid chromatography LiChrosolv®), propan-2-ol (isopropyl alcohol, 99.8% pure for HPLC), were purchased from Merck KGaA (Darmstadt, Germany). 2-Cyanoethyl methacrylate (CEMA, 97% pure) was provided by Yarsintez (Yaroslavl', Russia). N,N-dimethylformamide (DMF, 99% pure), toluene (98% pure), acetone (98% pure), were purchased from Vekton (St. Petersburg, Russia). Acetonitrile (AcN, 99,95% pure), n-hexane (97% pure) were from Cryochrom (St. Petersburg, Russia).

2.3. Samples

Polystyrene (PS) samples with molecular masses (M_w) 154,000, 500,000 and 960,000 were purchased from Fluka AG (Buchs, Switzerland). The value of sample polydispersity was in a range of 1.02–1.05. The synthesized by free-radical polymerization and characterized samples of poly-N-vinylpyrrolidones (PVP) with molecular masses (M_w) 14,400, 94,700 and 1,065,000 were kindly donated by Dr. I.I. Gavrilova (IMC RAS).

2.4. Instruments

The Philips 125-W mercury lamp (Philips, Netherlands) of wide spectrum and constant intensity of irradiation was used for free-radical copolymerization of chosen monomers. The mean pore size and specific surface area were estimated using ThermoQuest Pascal 440 porosimeter (Rodano, Italy). The morphology of the polymer samples obtained was investigated with JEOL JSM-35 CF scanning electron microscope (Tokyo, Japan).

2.5. Methods

2.5.1. Preparation of monolithic layers for PLC

The glass plates were etched using paraffin mask with 11 M hydrochloric acid for 30 min to provide formation of operative cell on a glass slide surface. Fig. 1 represents a scheme of monolithic layer manufacturing. The size and depth of cells obtained were 60 mm × 20 mm and 200 μm, respectively. After that, the slides were washed with water, boiled with 0.1 M NaOH for 40 min, then with water again. The plates were dried at 100 °C for 1 h.

To introduce double bonds into the cell surface for further polymer layer preparation by triple free radical copolymerization, the plate was immersed into 15% toluene solution of 3-(trimethoxysilyl)propyl methacrylate. The reaction was allowed to proceed for 12 h at room temperature [31].

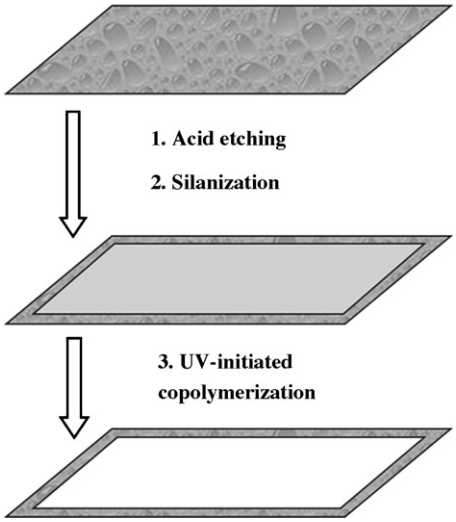

Fig. 1. Scheme of the monolithic layer fabrication.

Table 1
Composition of varies of polymerization mixtures used for the preparation of the monolithic layers for PLC.

Monolith	Composition of polymerization mixture (%, w/w)											
	BuMA	EDMA	GMA	AEMA	HEMA	CEMA	DoOH	CyOH	1,4-BD	NMP	DMF	PEG-200
M1[a]	–	16.0	24.0	–	–	–	–	60.0	–	–	–	–
M2[b]	24.0	16.0	–	–	–	–	28.0	32.0	–	–	–	–
M3[b]	24.0	16.0	–	–	–	–	16.0	44.0	–	–	–	–
M4[b]	24.0	16.0	–	–	–	–	11.0	49.0	–	–	–	–
M5[b]	24.0	16.0	–				39.5	–	20.5	–	–	–
M6[b]	24.0	16.0	–	–	–	–	–	29.0	31.0	–	–	–
M7[b]	24.0	16.0	–	–	–	–	–	41.0	19.0	–	–	–
M8[a]	–	14.0	–	14.0	12.0	–	18.0	–	–	42.0	–	–
M9[a]	–	14.0	–	14.0	12.0	–	18.0	–	–	–	42.0	–
M10[a]	–	14.0	–	14.0	12.0	–	30.0	–	–	–	30.0	–
M11[a]	–	14.0	–	14.0	12.0	–	24.0	–	–	–	36.0	–
M12[a]	–	16.0	–	–	8.0	16.0	25.5	–	–	–	–	34.5
M13[a]	–	16.0	–	–	8.0	16.0	38.0	–	–	–	–	22.0
M14[a]	–	16.0	–	–	8.0	16.0	60.0	–	–	–	–	–
M15[a]	–	16.0	–	–	8.0	16.0	28.0	32.0	–	–	–	–
M16[a]	–	16.0	–	–	8.0	16.0	40.0	20.0	–	–	–	–

[a] Mixture included 1% 2-methylpropiophenone (Darocur-1173).
[b] Mixture included 1% 2-methoxy-2-phenylacetophenon.

The photochemical destruction of 3-(trimethoxysilyl)propyl methacrylate was prevented by covering the reactor with aluminum foil. Functionalized by such a manner plates were washed with toluene, acetone, and ethanol and dried for 1.5 h at 35 °C. The modified glasses were kept in a dark.

Polymerization mixtures of different compositions (Table 1) were used for preparation of polymer layers. The ratio of components in reaction phase was chosen as 6:4 regarding to functional monomer/cross linker, and 4:6 for monomers/porogens pairs. The concentration of initiator was 1.0% from the mass of monomers. 2-Methoxy-2-phenylacetophenon was selected as the initiator for BuMA–EDMA copolymers preparation. In all other cases, 2-methylpropiophenone (Darocur-1173) was used. The solutions were purged with nitrogen for 5 min before polymerization. The operation cell on glass plate was filled with polymerization mixture and exposed to UV light for 20 min at room temperature. The plates with polymer monolithic layers were washed for 4 h by methanol to remove residual monomers and porogenic solvents. Finally, the layers were dried in vacuum at room temperature for several hours.

2.5.2. Sample solutions

The solutions of dyes, namely, *p*-amino-azo-toluene, *p*-amino-azo-benzene and methyl red, with concentration of 1 mg/ml were prepared by dissolving in ethanol. 2,4-Dinitrophenyl amino acids were dissolved in acetone (2.5 mg/ml). The solutions of poly-N-vinylpyrrolidones (concentration 25 mg/ml) and polystyrenes (concentration 10 mg/ml) were obtained by their dissolving in good studied polymers solvents, such as ethanol and THF, respectively.

2.5.3. Chromatographic procedure

The volume of chromatographic chamber was saturated with a vapor of solvents for 30 min before the plate was placed inside. The samples were spotted on monolithic layer in a 5 mm distance from down edge using glass capillaries. Loaded sample volume was 1 μl for low molecular mass compounds and PVP, whereas in the case of PS, this volume was increased up to 5 μl. The plate was dried for several minutes and then was placed into a chamber. When the solvent front was raised to 50 mm, the plate was removed and dried in the air. The detection of colored substances (dyes) was carried out visually. To visualize PVP zones, dried plate was placed into a chamber saturated with iodine vapors. The layers with separated PS spots were sprayed with 1% I_2 solution in methanol.

Every separation experiment was reproduced five times at the plates from different manufacture batch. To estimate a reproducibility of monolithic layers, the standard deviation (RSD) for R_f values was calculated. RSD values are presented in figure captions.

3. Results and discussions

3.1. Synthesis of monoliths appropriate for preparation of PLC plates

The first step of presented research was to develop and optimize the methods of synthesis of methacrylate monolithic phases with porous structure and surface area appropriate for planar chromatography. Obviously, the initial testing of fabricated sorbent layers regarding the adsorption mechanism mode assumed the use of low molecular mass substances of various chemistry.

Taking into account our previous experience in synthesis of methacrylate-based monoliths [31–33] as well as analysis of existing publications [13,28] the following copolymers have been selected:

- Copolymer GMA–EDMA represents a well-known monolithic sorbent used as a base for separation by different modes of adsorption liquid chromatography of a wide range of substances, in general, biological (macro)molecules. Its chemical structure provides appropriate balance of hydrophilic and hydrophobic material properties that allows assuming realization of separation mechanism based on hydrophobic interactions between a substance to be separated and sorbent surface.
- Copolymer BuMA–EDMA contains more hydrophobic butyl group (C4) in its chemical structure. Hence, this sorbent has to be a more suitable for separation by reversed-phase mechanism.
- Terpolymers AEMA–HEMA–EDMA and CEMA–HEMA–EDMA, containing functional amino- and cyano-groups, as well as more hydrophilic HEMA in comparison to GMA monomer, represent hydrophilic materials that allow assuming their use in chromatographic separations by normal-phase adsorption mode.

As it was mentioned above, the first important step of this work was to optimize the procedure of synthesis of polymer monolithic phase with a homogeneous interstitial structure and narrow pore size distribution. In particular, the influence of nature and ratio of porogenic solvents on porous characteristics of final products were thoroughly investigated.

Table 1 demonstrates the compositions of tested polymerization mixtures, whereas Table 2 collects the data of mercury intru-

Table 2
Porous characteristics of the developed monoliths.

Sample	Chemical structure	Average pore size (μm)	Specific surface area ($m^2 g^{-1}$)
M1	GMA–EDMA	1.50	30
M2	BuMA–EDMA	0.16	43
M3	BuMA–EDMA	0.10	67
M4	BuMA–EDMA	0.10	56
M5	BuMA–EDMA	1.80	3
M6	BuMA–EDMA	1.80	6
M7	BuMA–EDMA	1.60	53
M8	AEMA–HEMA–EDMA	0.14	23
M9	AEMA–HEMA–EDMA	0.13	28
M10	AEMA–HEMA–EDMA	1.36	43
M11	AEMA–HEMA–EDMA	1.31	44
M12	CEMA–HEMA–EDMA	1.02	44
M13	CEMA–HEMA–EDMA	1.54	40
M14	CEMA–HEMA–EDMA	0.72	64
M15	CEMA–HEMA–EDMA	1.43	41
M16	CEMA–HEMA–EDMA	1.02	37

sion porosimetry for 16 synthesized samples of rigid macroporous copolymers. The conditions of GMA–EDMA synthesis in a thin layer design by UV-initiated free-radical polymerization has been previously optimized in our group [33]. In particular, it was shown that the use of cyclohexanol as an individual porogen led to obtaining the material (sample M1) with average pore size of about 1.5 μm and specific surface area – 30 m^2/g.

The analysis of porous structure of copolymers BuMA–EDMA (samples M2–M7) showed that the use of porogen mixtures of cyclohexanol and dodecanol in various ratios gave also unsatisfactory results. The samples obtained are characterized by small mean pore size (about 100 nm). Application of hydrophilic alcohol (such as butanediol) instead of dodecanol led to the positive effect. For example, the samples M5 and M6 have an average pore size of 1.8 μm. However, the surface area appears to be only a few m^2/g that indicates the absence of mesopores in these structures. In contrast, sample M7 already has the required average pore size and surface area value. This monolith has been chosen for further examinations.

Monomer 2-aminoethyl methacrylate (AEMA) was selected for synthesis of functional material containing amino groups. This monomer represents crystalline substance that is added as a solution into a mixture of two comonomers, namely, HEMA and cross-linker ethylene dimethacrylate (EDMA). The solvents used for AEMA dissolving (dimethylformamide and N-methyl-2-pirrolidone) automatically become the components of the porogenic system and contribute to the formation of porous structure. As a result, varying the polymerization conditions, a series of copolymers (samples M8–M11) with 14 wt% of amino monomer was obtained.

From the results presented in Table 2 it is obvious that the use of DMF/dodecanol system in a ratio 30:30 and 24:36 wt% enables to obtain the samples with satisfactory pore size and high surface area. To prepare the plates for PLC, we used the conditions of synthesis developed for sample M11.

To obtain the sorbents with cyanogroups, the ternary copolymer CEMA–HEMA–EDMA was synthesized. The amount of 2-cyanoethylmethacrylate introduced into a polymerization mixture was reached 16 wt%. Hydrophobic alcohols, such as cyclohexanol and dodecanol, as well as hydrophilic PEG 200 were used to form the porous structure of discussed medium. The use of dodecanol as individual porogen led to sufficient average pore size and surface area of polymer material. The combination of dodecanol with cyclohexanol in different ratios allowed increasing pore size up to 1.43 nm for the ratio of 28:32 wt% (sample M15). Similarly to porogenic system mentioned above, the mixture of PEG and dodecanol provided formation of structure with satisfactory pore characteristics (sample M13).

Fig. 2 presents some examples of porous structure images of synthesized monoliths. Obviously, the samples M1, M7 and M11 demonstrate the most homogeneous morphology both for surface of cross-section. In contrast, the surface of CEMA–HEMA–EDMA monolith obtained using a porogen PEG-200/DoOH mixture (sample M13) looks denser in comparison with internal porous space. Moreover, the microglobules forming a polymer matrix are very small. The similar situation was observed for all CEMA–HEMA–EDMA monoliths obtained in a presence of PEG-200 as porogenic agent. Therefore, sample M16 synthesized with cyclohexanol and dodecanol in 20:40 wt% ratio was used as separation media for PLC. According to the data from intrusion mercury porosimetry, polymer samples M1, M7, M11, and M16 possessed a bimodal porous structure with narrow pore size distribution (Fig. 3).

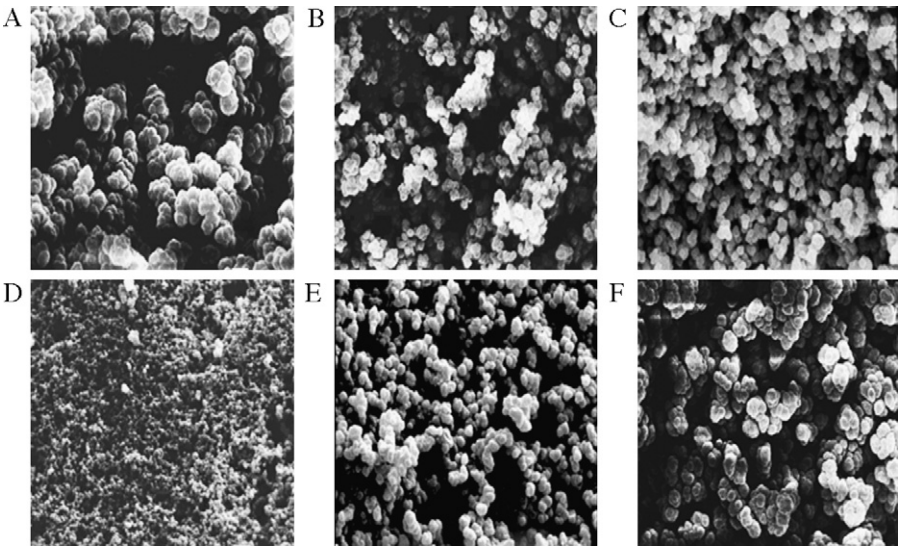

Fig. 2. SEM micrographs of porous structure of monolithic layers. (A) GMA–EDMA, surface, sample M1, (B) BuMA–EDMA, surface, sample M7, (C) AEMA–HEMA–EDMA, surface, sample M11, (D) CEMA–HEMA–EDMA, surface, sample M13, (E) CEMA–HEMA–EDMA, cross-section, sample M13, and (F) CEMA–HEMA–EDMA, surface, sample M16.

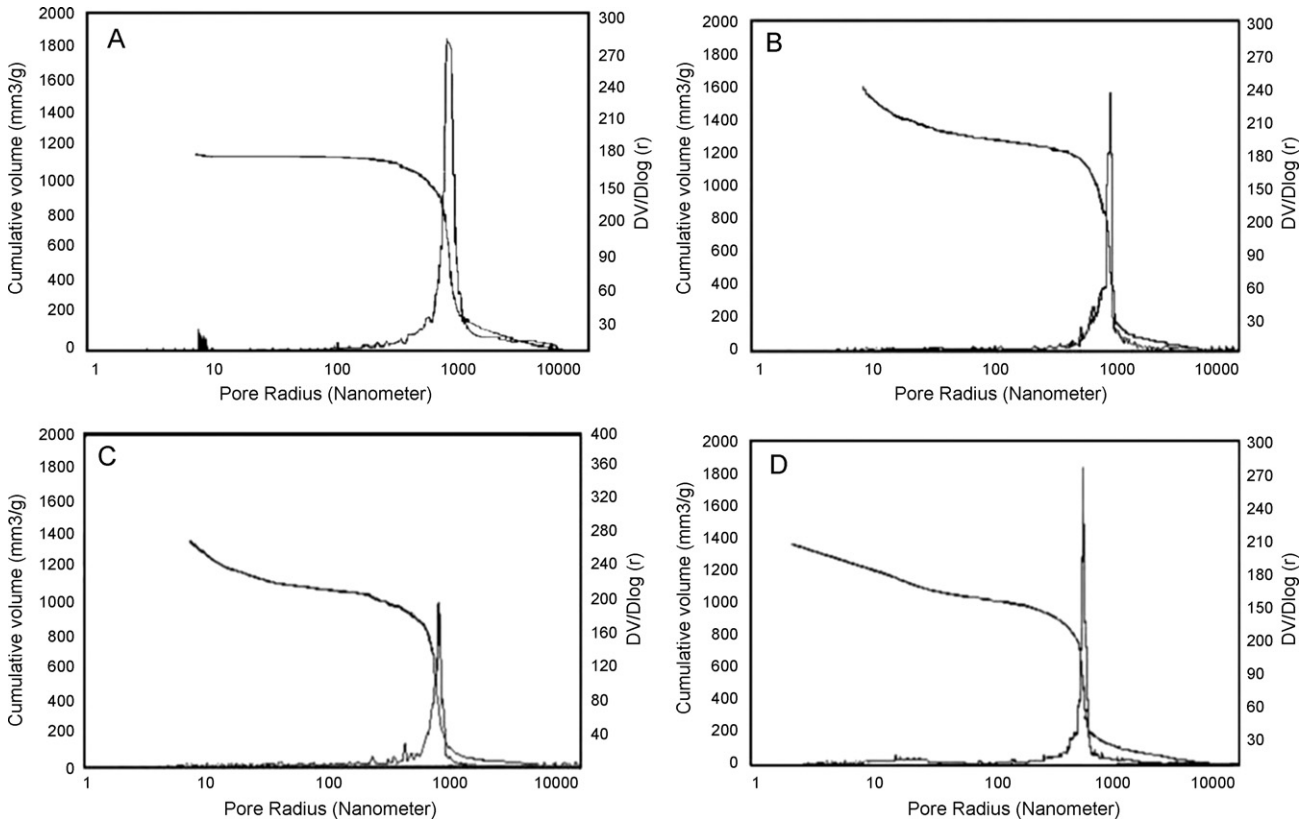

Fig. 3. Pore size distribution curves (MIP analysis) of GMA–EDMA, BuMA–EDMA, AEMA–HEMA–EDMA and CEMA–HEMA–EDMA monolithic materials used in PLC. Samples: (A) M1, (B) M7, (C) M11, and (D) M16.

3.1.1. Chromatography of colored substances

The developed stationary phases were examined using low-molecular mass substances to be separated by PLC. These were dyes to test a separation ability of BuMA–EDMA and GMA–EDMA sorbents, as well as DNP-amino acids to check the separation on AEMA–HEMA–EDMA layers. Fig. 4 shows the chromatograms of ternary dyes mixture on BuMA–EDMA and GMA–EDMA monolithic plates. The dyes *p*-aminoazobenzene, *p*-aminoazotoluene and methyl red were chosen as adsorbates. All substances are related to the class of aromatic azo-compounds and differ only by the nature of substituting groups in aromatic ring. In all cases, the separation was achieved in 7 min using the mobile phase ethyl acetate–ethanol–water in a ratio 7:8:7.5 (v/v/v). The use of BuMA–EDMA layers enables separation of the mixture of these three components with good resolution (Fig. 4A). When PLC of the same mixture was performed on GMA–EDMA monolith, the separation can be considered as unsatisfactory because of insignificant difference of retention between used dyes (Fig. 4B and C). The variation of solvent ratio of a mobile phase did not lead to improvement of selectivity. This fact clearly confirms the predominance of much more hydrophobic BuMA–EDMA copolymer for RP-separation mode over relatively hydrophobic GMA–EDMA stationary phase.

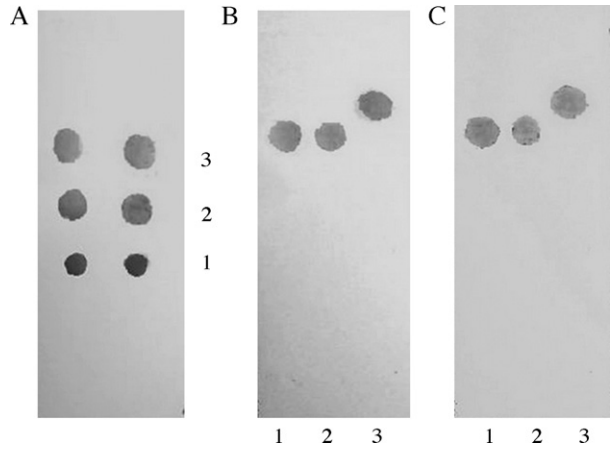

Fig. 4. PLC separation of dyes. Substances to be separated: *p*-aminoazotoluene (1), *p*-aminoazobenzene (2) and methyl red (3). (A) Stationary phase: BuMA–EDMA; eluent: ethyl acetate–ethanol–water 7:8:7.5 (v/v/v); R_f (1) = 0.4, R_f (2) = 0.6, R_f (3) = 0.8; RSD values were in interval from 3.4 to 4.1%. (B) Stationary phase: GMA–EDMA; eluent: ethyl acetate–ethanol–water 7:8:7.5 (v/v/v); R_f (1) = 0.54, R_f (2) = 0.6, R_f (3) = 0.7; RSD values were in interval from 3.1 to 3.6%. (C) Stationary phase: GMA–EDMA; eluent: ethyl acetate–ethanol–water 6:4:3 (v/v/v); R_f (1) = 0.8, R_f (2) = 0.8, R_f (3) = 0.9; RSD values were in interval from 2.8 to 3.7%.

3.1.2. Separation of 2,4-dinitrophenyl amino acids

Yellow 2,4-dinitrophenyl amino acids are obtained by reaction of free amino acids with 2,4-dinitrofluorobenzene (DNFB). Adsorption maximum of these derivatives is equal to 370 nm that allows visual detection of zones on a chromatogram. To separate ether-soluble DNP-leucine, DNP-aspartic acid, DNP-tryptophan and DNP-alanine, the normal phase PLC on AEMA–HEMA–EDMA layers was used. The mobile phase consisted of a mixture of hexane:chloroform:acetic acid (33:64:3). The time of analysis was 7 min. Fig. 5 demonstrates a good separation of selected substances. R_f values increase in a range of DNP-aspartic acid – DNP-tryptophan – DNP-alanine – DNP-leucine, while the polarity increases in reverse order. The result obtained confirms the normal-phase mechanism of PLC separation using chosen sorbent and elution conditions.

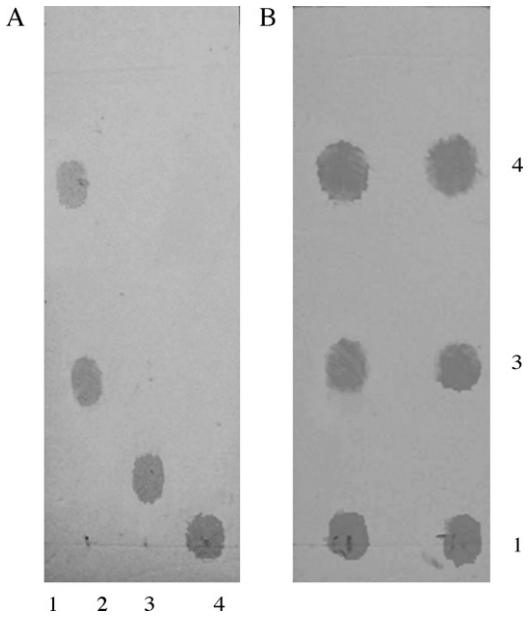

Fig. 5. PLC of DNP-amino acids. Stationary phase: AEMA–HEMA–EDMA; eluent: hexane–chloroform–acetic acid 33:64:3 (v/v/v). (A) Samples: DNP-derivatives of aspartic acid (1), tryptophan (2), alanine (3), leucine (4); R_f (1) = 0, R_f (2) = 0.2, R_f (3) = 0.4, R_f (4) = 0.8; RSD values were in interval from 3.0 to 5.3%. (B) Mixture of aspartic acid (1), alanine (3) and leucine (4); R_f (1) = 0, R_f (3) = 0.4, R_f (4) = 0.8; RSD values were in interval from 3.0 to 4.0%.

3.2. PLC of polymers

GMA–EDMA, BuMA–EDMA and CEMA–HEMA-EDMA monoliths were successfully used in PLC experiments for separation of synthetic polymers. Hydrophilic PVP and hydrophobic PS have been chosen as model macromolecular objects.

It is known that unlike the separation of small molecules, the chromatography of polymers is more complicated process because of multivariable chemical structure that defines polymer chain conformation and its ability to be adsorbed on a sorbent surface. Low diffusion coefficients have to be also taken into consideration.

The influence of a mobile phase composition on the mobility of PVP zones, as well as on PLC system selectivity was studied. The separation upon PVP molecular masses was not achieved when single-component eluent was used. Therefore, the mobile phase consisting of water mixed with organic solvents (methanol, acetonitrile, isopropanol) in various ratios was applied in further experiments.

Increasing part of organic component led to increasing of mobility of PVP zones but, simultaneously, to decreasing of separation selectivity. The compromising alternative of separation conditions for PVP samples in terms of zone mobility and appropriate selectivity was achieved using a mixture of water and the above listed solvents, namely, 30 vol% methanol, 15 vol% acetonitrile and 10 vol% isopropyl alcohol. PLC-chromatograms of PVP samples with different molecular masses obtained are shown in Fig. 6. The best result which is characterized by compaction of polymer zones and, respectively, maximum selectivity of separation, was achieved using the mobile phase water–isopropanol (90/10). Thus, we can conclude that the transition from methanol to acetonitrile and, further, to isopropanol provides a better separation when reducing the concentration of organic solvent in a mobile phase. Obviously, the chromatography of PVP on GMA–EDMA layers obeys the reversed-phase mechanism because the displacement ability of competitors in RP HPLC exactly corresponds to the following order: methanol < acetonitrile < ethanol < isopropanol.

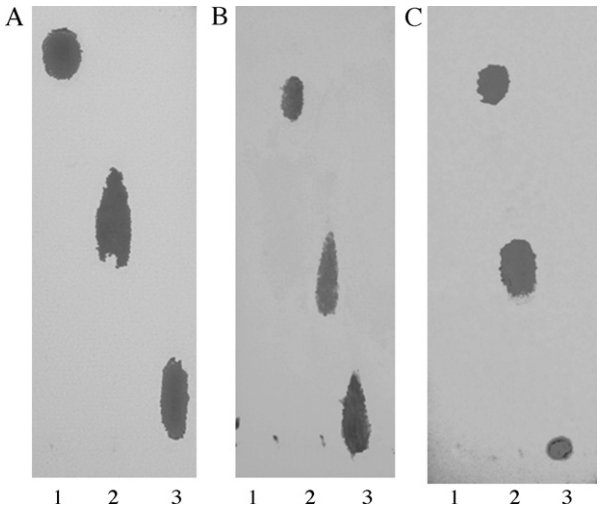

Fig. 6. PLC of poly-N-vinylpyrrolidones with M_w of 14,400 (1), 94,700 (2) and 1,065,000 (3). Stationary phase: GMA–EDMA; eluents: (A) water–methanol 70:30 (v/v), R_f (3) = 0.1, R_f (2) = 0.5, R_f (1) = 0.9, RSD for R_f values were in interval from 2.7 to 6.3%; (B) water–acetonitrile 85:15 (v/v), R_f (3) = 0.1, R_f (2) = 0.6, R_f (1) = 0.9, RSD for R_f values were in interval from 2.9 to 6.1%; (C) water–isopropyl alcohol 90:10 (v/v), R_f (3) = 0, R_f (2) = 0.45, R_f (1) = 0.8, RSD for R_f values were in interval from 2.4 to 4.3%.

It is known, that homopolymers and oligomers of styrene of different molecular masses can be separated by normal-phase and reversed-phase liquid chromatography modes (NPLC and RPLC, respectively). In the first case, silica sorbents and a mobile phase consisting of a mixture of hexane and THF are used [34]. RP separations are carried out on silica with grafted hydrophobic phase using a mixture of methanol and THF [34] acetonitrile and THF [35], acetonitrile and dichloroethane [36] as mobile phases.

In our study, the retention behavior of polystyrenes was investigated in experiments with BuMA–EDMA and CEMA–HEMA–EDMA monolithic layers as stationary phases, as well as AcN–THF and n-hexane–THF mixtures as mobile phases. The composition of a mobile phase was optimized in each case in order to achieve the

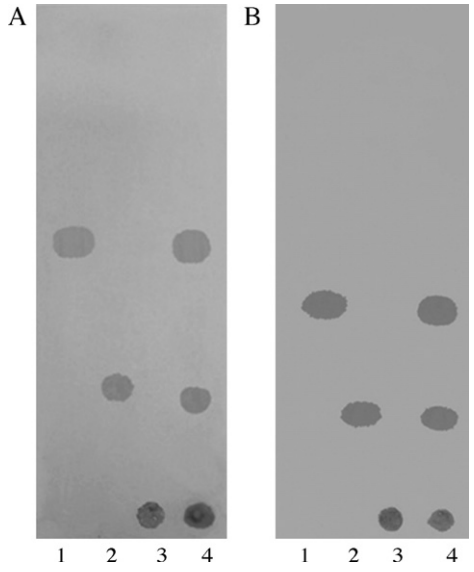

Fig. 7. PLC of polystyrenes with M_w of 154,000 (1), 500,000 (2), 960,000 (3) and their mixture (4). (A) Stationary phase: BuMA–EDMA; eluent: AcN–THF 5:5 (v/v); R_f (3) = 0, R_f (2) = 0.4, R_f (1) = 0.8; RSD for R_f values were in interval from 2.4 to 3.7%. (B) Stationary phase: CEMA–HEMA–EDMA; eluent: *n*-hexane–THF 6:4 (v/v); R_f (3) = 0, R_f (2) = 0.25, R_f (1) = 0.5. RSD for R_f values were in interval from 2.7 to 3.8%.

best difference in R_f values. The separation was achieved in 8–9 min. Fig. 7A and B shows PLC results for PS separation obtained according to reversed- and normal-phase mechanisms using AcN–THF and n-hexane–THF as mobile phases, respectively.

4. Conclusions

The present paper devoted to the development and preliminary testing of thin methacrylate-based monolithic layers collects the results regarding to appropriate pore and surface formation for their use in planar liquid chromatography (PLC). The main focus of this research is concentrated on variation of surface chemistry, as well as hydrophobic/hydrophilic properties of synthesized stationary phase to apply them for different modes of adsorption mechanisms. Though the general goal is to start the series of experiments on novel topic in monoliths application, namely, the separation of synthetic polymers, the plates obtained were tested using both small and macromolecules to choose (1) optimal morphology of developed materials that could be appropriate for PLC, (2) optimal surface chemistry that could provide realization of different adsorption mode, and (3) optimal compositions of mobile phases allowing achievement the best separation of different classes of analytes using the same PLC mode. To reach the goals, different UV-initiated free radical polymerization approaches based of variation of monomers and porogens were developed and optimized. The results obtained can be considered as the first impact to further investigation of chromatography on monoliths of polymers with molecular behavior differed significantly from that known and thoroughly studied of proteins, oligonucleotides, plasmid DNA and viruses.

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