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Field-Flow Fractionation of Microparticles in a Rotating Coiled Column for the Preparative Separation of Sorption Materials

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Abstract—It is demonstrated that field-flow fractionation using a rotating coiled column (RCC) can be used for the separation and purification of the polydisperse sorbents based on polystyrene–divinylbenzene. The behavior of the sorbent at different operating parameters of RCC was studied. The mobile phase composition, column rotation speed, and the initial flow rate of the mobile phase were selected, optimized for the isolation of a monodisperse fraction with the particle size 4.5 μm and the separation of particles 0.5–2 μm in size, particle fragments, and residues of sorbent synthesis. It is shown that a 15-mL analytical RCC ensures the efficient fractionation of 100 mg of the sorbent in one experiment taking less than one hour with the yield of the desired fraction greater than 95%. The perspectives for scaling up the fractionation in RCC are evaluated; the possibility of using the method for the preparative separation of particles of different nature is discussed.

Keywords: field-flow fractionation, rotating coiled column, chromatographic sorbent, microparticles, preparative separation

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The efficiency of a chromatographic column, defining the broadening of the zones of separated substances, depends largely on the size of sorbent particles and the uniformity of their packaging. The sorbent particle size also affects the permeability of the column, determining the pressure required to pass the mobile phase through the column at a predetermined rate. The permeability substantially decreases in the presence of even a small number of particles significantly smaller than the main fraction. To ensure high performance of columns, it is desirable to use spherical microparticles with a narrow size distribution. Preparation of such particles, in particular, polymer, is a challenge in some cases [1, 2]; it seems advisable therefore to fractionate a polydisperse synthesized material in order to isolate particles of a required size.

Various methods are used for the fractionation of microparticles, such as sedimentation (either in the Earth's gravity or using a centrifuge), sequential membrane filtration (MF), split flow thin cell fractionation (SPLITT), and field-flow fractionation (FFF) [3]. Unfortunately, all of these methods have drawbacks that limit their application to the effective preparative separation of fractions of microparticles.

Sedimentation is time-consuming, particularly when sedimentation is performed in the gravitational field, and suitable for the fractionation of particles larger than 2 μm [3]. In some cases, it is necessary to introduce additional stabilizing agents preventing the aggregation of particles. In addition, the sedimentation method is characterized by low efficiency in the separation of fractions of particles similar in size.

Membrane methods are used for the fractionation of macromolecules, microorganisms, and colloidal and solid particles, and successfully used in analytical chemistry [3]. One of the advantages of membrane filtration is the possibility to operate with large samples [3], which is necessary for the preparative isolation of desired fractions. However, despite a number of indisputable advantages of this method, the results may be distorted by the aggregation and adsorption of particles on the membrane surface. These processes reduce both the efficiency of particle separation and fractionation performance due to clogging of membrane pores.

The SPLITT enables the fractionation of a large enough amount of sample, which makes it useful for the preparative separation of samples. SPLITT-systems are used for the fractionation and investigation of microparticles of different nature, including polymers [3]. Commercially available equipment is suitable for operating particles of 1 μm in size and above. The disadvantage of this method is that only two fractions can be separated in one cycle (for example, particles larger than 1 μm and less than 1 μm).

Field-flow fractionation gives a unique opportunity for the separation and evaluation of physical parameters of the samples differing in nature, from biopolymers and microorganisms to colloidal and solid particles in the range from 1 nm to 100 μm [3]. For example, sedimentation FFF may advantageously be used for the fractionation of polystyrene particles that are 6, 10, or 15 μm in size [4], and microthermal FFF offers the estimation of the average diameter and particle size distribution of the particles of chromatographic sorbents [5]. However, the method of field-flow fractionation has one significant limitation associated with the weight of the sample, which is usually less than 1 mg. This disadvantage keeps conventional FFF out of the preparative fractionation of samples.

Field-flow fractionation using rotating coiled columns of various sizes enables the elimination of this limitation and offers the isolation of the weight fraction of particles in a narrow size range for solving preparative problems. Rotating coiled columns were successfully used in the fractionation of nano- and microparticles of different nature, for example, particles of latex, quartz, silica, street dust, and volcanic ash [6–9].

The goal of this work is to develop further the method, to study the behavior patterns of particles of porous spherical sorbent based on polystyrene–divinylbenzene (PS–DVB) copolymer at different operating parameters of RCC, and to optimize condition for the isolation of a narrow size fraction of the sorbent particles.

EXPERIMENTAL

Equipment. We used a planetary centrifuge made at the Institute for Analytical Instrumentation, Russian Academy of Sciences (St. Petersburg, Russia), equipped with a vertically disposed cylindrical drum. The centrifuge is equipped with a single-layer spiral RCC, the revolution radius of which is $R = 10$ cm and the rotation radius is $r = 5$ cm. The column is a Teflon tube 1.5 mm in inner diameter, with a wall thickness of 0.75 mm. The total volume of the column is 15 mL. The centrifuge is operated at the rotation speeds in the range of 100–1000 rpm.

The mobile phase was fed into the inlet of the column with a MasterFlex peristaltic pump of Series L/S (Laboratory/Standard) (United States) with a capacity from 0.3 to 21 mL/min (when using an L/S 14 tube). To detect particles in the eluate of the column, a flow spectrophotometer with a variable operating wavelength was used, manufactured in the Institute for Analytical Instrumentation, Russian Academy of Sciences. The working wavelength is 254 nm. A Sapphire ultrasonic bath was used to disperse the sorbent samples and to destruct possible aggregates of particles. The sample and isolated fractions were examined by scanning electron microscopy (SEM) using a JEOL JSM-6700F microscope (Japan) with a cold cathode field emission and a high resolution.

Test samples. We explore the possibility of fractionation of a sample of sorbent based on PS–DVB with density 1.1 g/cm^3, which was synthesized by seeded suspension polymerization at the Faculty of Chemistry of the Moscow State University [1, 2].

Fractionation of the PS–DVB sorbent in RCC. A weighed portion of the sample was dispersed by shaking the sorbent in the mobile phase. The resulting suspension was sonicated to disrupt possible aggregates of particles. The sample was introduced into the RCC using a peristaltic pump at a flow rate of 15 mL/min; after that, the column was driven in rotation. Further, a fraction (or fractions) of particles were isolated at the initial flow rate of the mobile phase; then, the RCC was stopped; and the remaining fraction was recovered at the mobile phase flow rate of 21 mL/min. To wash out the particles from the column completely, a mixture of the mobile phase and air bubbles was used in some cases.

RESULTS AND DISCUSSION

Prior to the study of behavior of the sorbent particles in the RCC, the sample was characterized by SEM (Fig. 1). It is found that the sample consists of spherical particles ranging in size from 0.5 to 2 μm and particles of approximately 4.5 μm in size. In addition, the sample contains fragments of various sizes and residues of the synthesis of the sorbent.

The specific task of fractionation was set using the SEM data: the isolation of the desired fraction with the particle size of 4.5 μm by separating it from particles with sizes 0.5–2 μm, particle fragments, and residues of synthesis. The elution order of particles in the RCC corresponds to the conventional mode of the elution of particles in the FFF methods; that is, particles are eluted in the order of increasing size. To purify the sample, optimally, it should be divided into two factions: (1) particles less than 2 μm in size including fragments and residues of synthesis and (2) the desired fractions of particles 4.5 μm in size.

Behavior patterns of the PS–DVB sorbent in RCC. It is previously proven that the operating parameters of RCC (the rotation speed of the column and the flow rate and direction of the mobile phase) may significantly affect the process and, consequently, the results of fractionation of nano- and microparticles of different nature [6, 8, 10]. In the present study, we examined the behavior of the particles of PS–DVB sorbent taking into account their hydrophobicity and low density (approximately 1.1 g/cm^3) and selected the conditions for the fractionation of the sorbent, optimal for isolating a fraction of particles 4.5 μm in size.

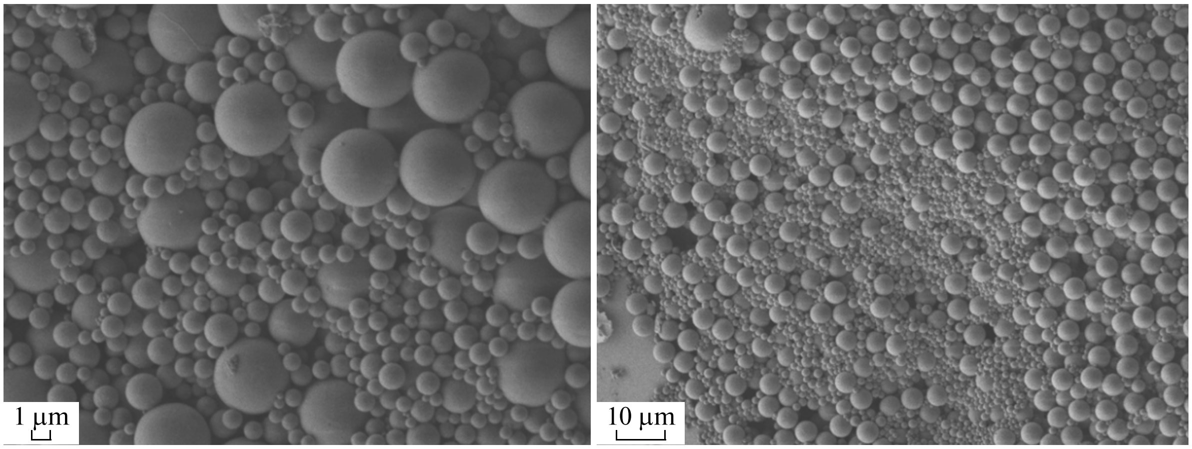

Fig. 1. SEM micrographs of the initial PS–DVB sorbent.

Effect of the mobile phase composition. The difference in the densities of the separated particles and the mobile phase is significant during fractionation in RCC and is a key parameter affecting the sedimentation that is one of the driving forces of separation in RCC. To increase the difference in densities, ethanol and acetonitrile are used as the mobile phase, the density of which is 0.789 and 0.786 g/cm^3, respectively. Preliminary experiments to assess the retention of particles in the column demonstrated similar results for the retention and separation using both solvents, which is quite logical, given the small differences in their densities. In further experiments, ethanol was used as the mobile phase as the most accessible and inexpensive solvent.

Effect of the operating parameters of RCC. It is shown that a high rotation speed of the planetary centrifuge (approximately 800 rpm) enables the fractionation of silica submicroparticles in RCC [8, 10]. For the fractionation of microparticles, lower rotation speeds of the centrifuge can be used [6]. Taking into account the low density of the sorbent (1.1 g/cm^3), the experiments on the effect of the initial flow rate of the mobile phase on the fractionation of the sorbent sample were initiated at a rotation speed of 800 rpm and the initial flow rate of the mobile phase in the range from 1.0 to 4.5 mL/min. The weight of the sample particles injected into the column was 10 mg (1 mL of suspension containing 10 g of particles per liter).

It is shown that the use of the initial flow rate of the mobile phase in the range of 1.0–2.5 mL/min ensures the isolation of fraction *1* (Fig. 2a) composed entirely of particles 0.5–2 μm in size and residues of synthesis of the sorbent (Fig. 3a). Target fraction *2* consisted of a mixture of particles 4.5 and 0.5–2 μm in size (Fig. 3b), indicating that the separation of the sample was incomplete.

Increasing the initial flow rate of the mobile phase to 3.5 and 4.5 mL/min leads to the elution of the first fraction with the particles size of 4.5 μm along with particles 0.5–2 μm in size. The peak of fraction *1* in Fig. 2b does not return to the base line, and its "tail" corresponds to the elution of 4.5-μm particles. Fraction *2*, separated when the column rotation was stopped and the flow rate of the mobile phase was

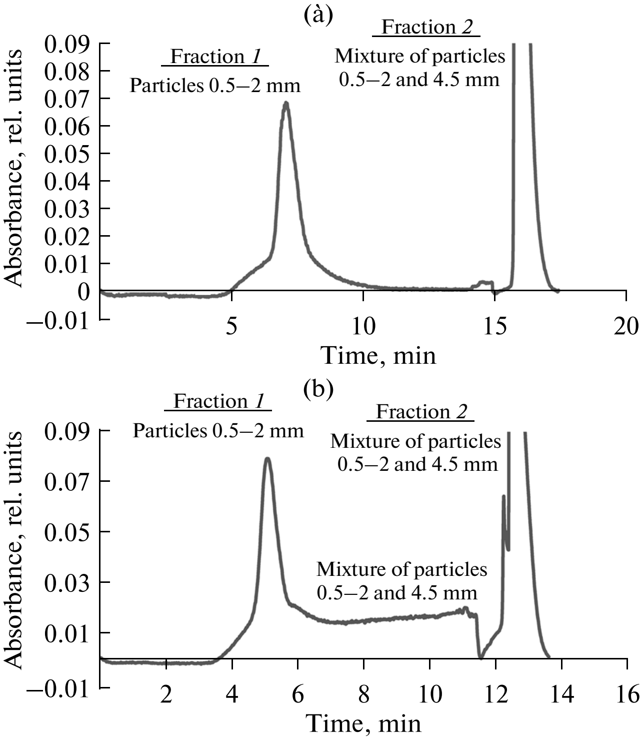

Fig. 2. Effects of the initial flow rate of the mobile phase (ethanol) on the fractionation of 10 mg of the PS–DVB sorbent at the column rotation speed of 800 rpm. Fraction *1*: the mobile phase flow rate, (a) 2.5 and (b) 3.5 mL/min; the column rotation speed, 800 rpm; and fraction *2*: the flow rate of a mixture of the mobile phase and air bubbles, 21 mL/min; the column rotation speed, 0 rpm.

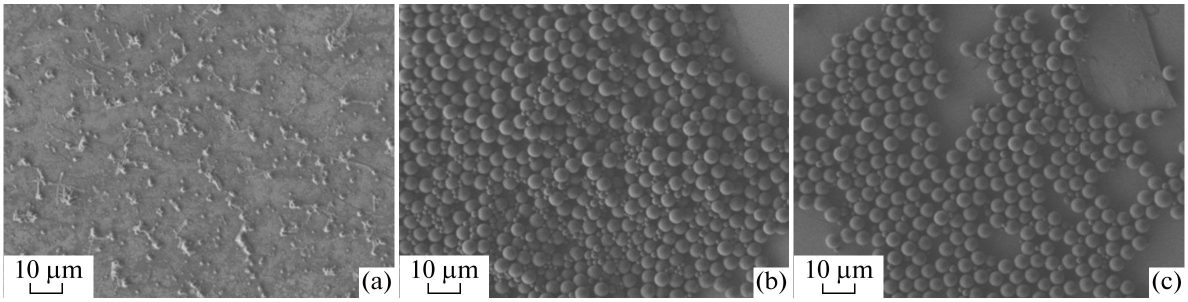

Fig. 3. SEM micrographs of isolated fractions of the PS–DVB sorbent; (a) fraction *1*; the column rotation speed, 800 rpm; the mobile phase flow rate, 2.5 mL/min; consisted of particles 0.5–2 μm in size; and fraction *2*; the column rotation speed, 800 rpm; the initial flow rate of the mobile phase, (b) 2.5 and (c) 3.5 mL/min; consisted of a mixture of particles 0.5–2 and 4.5 μm in size.

21 mL/min, also consists of a mixture of particles of 0.5–2 and 4.5 μm in size (Fig. 3c).

The proportion of particles 0.5–2 μm in size in the target fractions isolated at the initial flow rates of 2.5 and 3.5 mL/min was estimated by the SEM data (Figs. 3b and 3c). It is shown that the second (target) fraction obtained at the initial flow rate of the mobile phase of 3.5 mL/min contains fewer particles 0.5–2 μm in size; that is, it is more "pure" in terms of the task to solve. Despite this, the efficiency of the separation of fractions is not satisfactory under these experimental conditions.

Thus, it is found that the complete separation of fractions, that is, the isolation of a monodisperse fraction with the particle size of 4.5 μm, cannot be attained by increasing the flow rate of the mobile phase at the column rotation speed of 800 rpm. With the flow rate of the mobile phase increasing in the range of 1.0–4.5 mL/min, the proportion of 4.5-μm particles in the target fraction increases but does not reach the desired level. In addition, at an initial flow rate of the mobile phase above 2.5 mL/min, the particles 4.5 μm in size start to elute in the first fraction; that is, there is a loss of particles of the desired fraction.

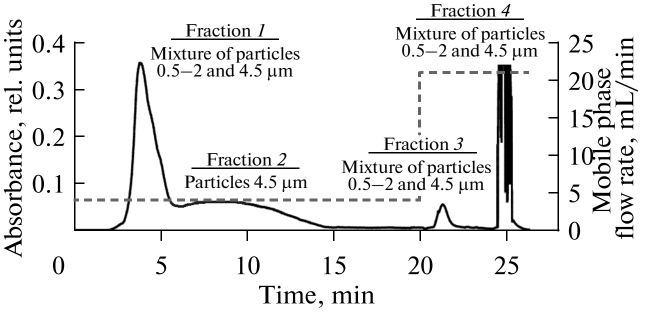

Fig. 4. Fractionation of a 25-mg portion of the PS–DVB sorbent. Fractions *1* and *2*: the flow rate of the mobile phase (ethanol), 4 mL/min, the column rotation speed, 800 rpm; fraction *3*: the mobile phase flow rate, 21 mL/min, the column rotation speed, 0 rpm; fraction *4*: the flow rate of a mixture of the mobile phase and air bubbles, 21 mL/min, the column rotation speed, 0 rpm; (—) elution curve and (- - -) the mobile phase flow rate.

Experiments on the fractionation of the PS–DVB sorbent in the RCC at the rotation speed of 800 rpm also showed that under these conditions, a part of the sample is retained in the column and is not eluted even from the stopped columns and at the mobile phase flow rate increased to 21 mL/min. For example, the fractionation of 25 mg of the sorbent (5 mL of suspension with a concentration of 5 g/L) at the column rotation speed of 800 rpm and the initial flow rate of the mobile phase of 4 mL/min yielded the separation of four fractions, which were further characterized by SEM (Fig. 4). The first two fractions were isolated at the mobile phase flow rate of 4 mL/min: one fraction contained particles 0.5–2 and 4.5 μm in size, and another fraction was almost monodisperse and consisted of 4.5-μm particles. The third fraction was isolated after the column was stopped and the flow rate of the mobile phase increased to 21 mL/min. Then, the residual fourth fraction was separated by a mixture of the mobile phase with air bubbles. The SEM data showed that the third and fourth fractions were a mixture of particles 0.5–2 and 4.5 μm in size. The isolated fractions were dried and weighed. It is found that about a quarter of the sample is retained in the column at a rotation speed of 800 rpm.

The presence of particles 0.5–2 μm in size in the first, third, and fourth (residual) fractions and their absence in the second fraction suggest the conclusion that at a high rotation speed of the column (800 rpm), the particles with the sizes of 0.5–2 μm are partially coprecipitated in the RCC with larger 4.5-μm particles at the beginning of separation. These coprecipitated particles 0.5–2 μm in size are "shielded" by particles of 4.5 μm and thus occur to be "cut off" from the flow and, respectively, out of the separation process. As a result, a part of the particles of 0.5–2 μm are eluted along with the fraction of particles 4.5 μm in size. Apparently, the main reason for this phenomenon is a high concentration of separated particles. However, an approach involving a decrease in the concentration of separated particles, in fact, a decrease in the weight of the sample, seems inappropriate, since the aim of the present study is the preparative fractionation and purification of sorption material. A possible way to reduce the effect of coprecipitation is a decrease in the transverse force field, in this case, by lowering the speed of column rotation. This decreases the sedimentation rate of the particles and enables the particles to remain longer in the flow, which contributes to their separation. In addition, the large particles are distributed along the length of the column more evenly by the effect of slow precipitation, forming a thin layer on the capillary surface, eliminating the "shielding" of smaller coprecipitated particles, here, the particles 0.5–2 μm in size. A decreasing rotation speed also prevents the formation of the "residual" fraction in the column.

Optimization of the fractionation of sorbent in RCC. It was found experimentally that to fractionate the sorbent, it is advisable to use the column rotation speed of 400 rpm. The fractionation of 25 mg of the sorbent (5 mL of suspension with a concentration of 5 g/L) was performed at different initial flow rates of the mobile phase: 0.5, 1.0, and 1.5 mL/min (Fig. 5). It is shown that the best separation of fractions of 0.5–2 and 4.5 μm is achieved at the initial flow rate of the mobile phase of 0.5 mL/min. As a result of fractionation at the column rotation speed of 400 rpm and the mobile phase flow rate of 0.5 mL/min, fraction *1* is isolated, consisting of particles 0.5–2 μm in size, fragments of particles, and residues of synthesis of the sorbent. Further, with the centrifuge stopped and the flow rate of the mobile phase increased to 21 mL/min, target Fraction *2* is obtained consisted of 4.5-μm particles. At the initial flow rates of the mobile phase of 1.0 and 1.5 mL/min, 4.5-μm particles are partially eluted in the first fraction; the second (target) fraction thus is almost monodisperse and contains only particles 4.5 μm in size. When using the initial flow rates of 0.5, 1.0, and 1.5 mL/min and the column rotation speed of 400 rpm, we mention the absence of the residual fraction in the column after the experiment.

When solving the problem, an increase of the initial flow rate of the mobile phase from 0.5 to 1.0 mL/min can be justified in terms of reducing the duration of the experiment (by more than 1.5-fold). The purity of the desired fraction remains the same and the elution of particles 4.5 μm size in the first fraction is negligible. By using the initial flow rate of the mobile phase of 1.5 mL/min, the duration of the experiment is reduced by 2.5 times as compared with the experiment at the initial flow rate of 0.5 mL/min, but the amount of 4.5-μm particles, eluted with the first fraction, increases. Therefore, by increasing the initial flow rate of the mobile phase from 0.5 to 1.5 mL/min, the yield of the desired fraction decreases.

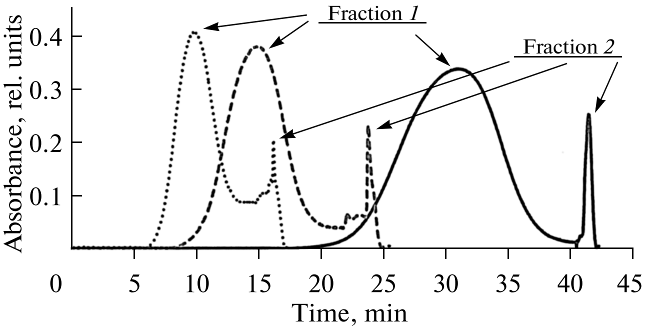

Fig. 5. Effect of the initial flow rate of the mobile phase (ethanol) on the fractionation of 25 mg of the PS–DVB sorbent at a column rotation speed of 400 rpm: fraction *1* isolated at the initial flow rates of the mobile phase indicated in the figure and the column rotation speed of 400 rpm and fraction *2* isolated at the flow rate of the mobile phase of 21 mL/min and the column rotation speed of 0 rpm; the initial flow rate of the mobile phase: (—) 0.5, (- - -) 1.0, and (···) 1.5 mL/min.

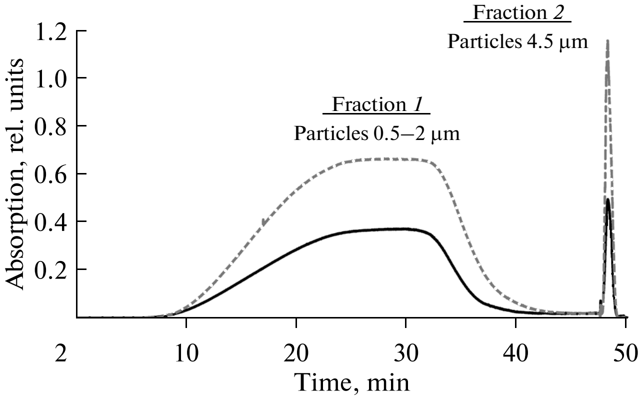

Fig. 6. Fraction patterns of (—) 50 and (- - -) 100 mg of the PS–DVB sorbent column, obtained at the column rotation speed of 400 rpm and the initial flow rate of the mobile phase of 0.5 mL/min. Fraction *1*: the mobile phase flow rate, 0.5 mL/min, and the column rotation speed, 400 rpm; fraction *2*: the mobile phase flow rate, 21 mL/min, and the column rotation speed, 0 rpm.

Based on the found patterns, an initial flow rate of the mobile phase of 0.5 mL/min and the column rotation speed of 400 rpm may be considered optimal for the fractionation of the sorbent in RCC. Under these conditions, 50- and 100-mg portions of the sorbent (10 mL of suspension at a concentration of 5 and 10 g/L, respectively) were fractionated (Fig. 6). In both cases, we obtained almost monodisperse fractions with the particle size of 4.5 μm (Fig. 7b). Furthermore, the fraction is separated consisting of particles 0.5–2 μm in size, fragments of particles, residues of the synthesis of the sorbent, and single particles 4.5 μm in size (Fig. 7a). It should be noted that the 50- and 100-mg portions of the sorbent were divided with the same efficiency; the yield of the desired fraction was greater than 95%.

Thus, it is demonstrated that a 15-mL analytical RCC ensures an efficient fractionation of up to 100 mg of the sorbent based on PS–DVB in one experiment

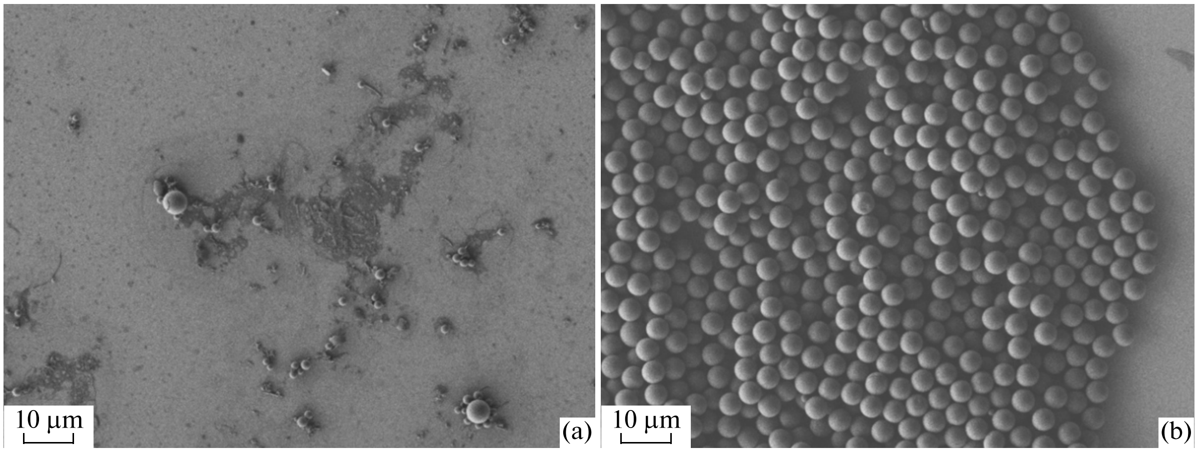

Fig. 7. SEM micrographs of the fractions of the PS–DVB sorbent, isolated under optimized conditions: (a) fraction *1*; the column rotation speed, 400 rpm; the mobile phase flow rate, 0.5 mL/min; consisted of particles 0.5–2 μm in size, particle fragments, residues of the synthesis, and single particles 4.5 μm in size and (b) fraction *2*; the column rotation speed, 0 rpm; the mobile phase flow rate, 21 mL/min; consisted of particles 4.5 μm in size.

run. An increase in the RCC volume by increasing its length and/or inner diameter opens up great possibilities for scaling up the process and for the application of the method to solving problems of the preparative separation of particles, in particular, of sorption materials. The theoretical background of the field-flow fractionation of particles in RCC [10] and the identified behavior patterns of particles of different nature under different structural and operational parameters of the RCC [6–9] are a good basis for selecting fractionation conditions and their optimization.

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