Optimization of the matrices structural parameters for the synthesis of restricted access media (RAM) adsorbents for chromatography

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Optimization of the matrices structural parameters for the synthesis of restricted access media (RAM) adsorbents for chromatography

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Abstract. The geometric parameters of a matrix for the synthesis of restricted access media adsorbents are optimized, using mathematical modeling based on the basis of a globular model with tetrahedral and octahedral pores. For calculations were used the distribution curves of pore sizes obtained on the basis of the desorption branches of isotherms obtained via low temperature nitrogen adsorption. In contrast to the model of cylindrical pores, the calculation model of globular pores gives realistic values of the surface area of the silica with grafted hexadecylsilyl groups, due to the change of ratio of the amounts of tetrahedral and octahedral pores in the model. The obtained value of the surface area of the silica with grafted hexadecylsilyl groups is in good agreement with elemental analysis data. The obtained refined size of the mouth of the pores makes it possible to choose correctly the particle diameter of the external protective coating.

Most restricted access media (RAM) adsorbents are chemically improved modified silica obtained through the covalent binding of molecules on hydroxylated silica surfaces and specially selected to be effective for the required application tasks [1]. The most significant chromatographic feature of RAM is their ability to differentially interact with components of a chromatographic sample depending on the size of its molecules.

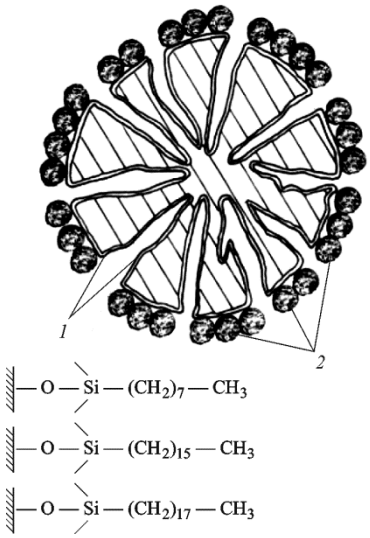

Fig. 1. Schematic structure of RAM adsorbent: (*1*) internal surface (alkyl groups); (*2*) external surface with a protective coating

The surface accessible to the macromolecules of the sample is inert to ensure the exclusion of macromolecules without separation and at a speed close to the speed of movement of the mobile phase. They quickly leave the chromatographic column, since they do not practically interact with the groups grafted on their external surface, while other components of the sample separation are implemented by one or more mechanisms through various interactions with the groups grafted on the inner surface, inaccessible to macromolecules. This allows us to inject complex samples, whose components differ substantially in molecular masses, into a chromatographic column [2,3].

About 70-80% of routine methods of analysis in high performance liquid chromatography (HPLC) are based on dominant method reversed-phase chromatography [1]. To use the existing methods it is necessary that the inner surface of RAM is allowed to work in the mode of reversed-phase chromatography, and the influence of external surface on the retention of low molecular weight components is minimal. It can be achieved through optimization of the structural parameters of the original silica matrix, which must be done as accurately as possible taking into account the chain length of grafted alkyl groups [2-4].

The structural and geometric characteristics of RAM adsorbents were controlled using a method of low-temperature nitrogen adsorption. Despite high informativeness this method, the data obtained should be used with care, since measurement conditions (gas phase; tempreture of 77 K) are far from conditions of RAM synthesis and use. Fig. 2 shows the adsorption isotherm of nitrogen on the silica with grafted hexadecylsilyl groups (*1*) and final RAM (*2*).

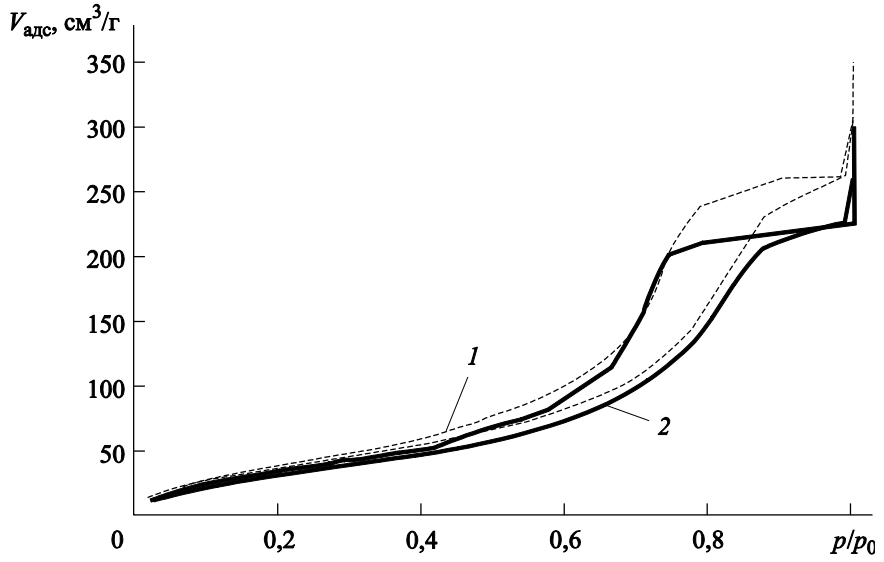

Fig. 2. Adsorption−desorption isotherms were obtained via low temperature nitrogen adsorption: (*1*) silica with grafted hexadecylsilyl groups; (*2*) final RAM adsorbent with human serum albumin on the external surface

There are three areas on the isotherm: a low-pressure area with no hysteresis; a medium-pressure area (0.4–0.9) with a hysteresis and a final area where adsorption increased considerably. The point near the origin of the hysteresis loop corresponds to the onset of capillary condensation in the narrowest pores. The lower closure point of hysteresis for nitrogen, corresponding to $p/p_0 = 0.4$, is responsible for cylindrical pore diameter of about 3.6

nm. We can see that both isotherms (Fig. 2) are almost identical up to values of $p/p_0 = 0.75$. Isotherms therefore differ less if better silica is used. The changes in high-pressure area are associated with the influence of the wide pores geometry coating on the RAMs external surface.

Commonly used to estimate the structural parameters of the silica is the model of cylindrical pores. Fig. 3 shows three curves of pore size distribution, obtained from the desorption branches of the isotherms; pore size distributions were found with the capillary condensation equation for cylindrical pores.

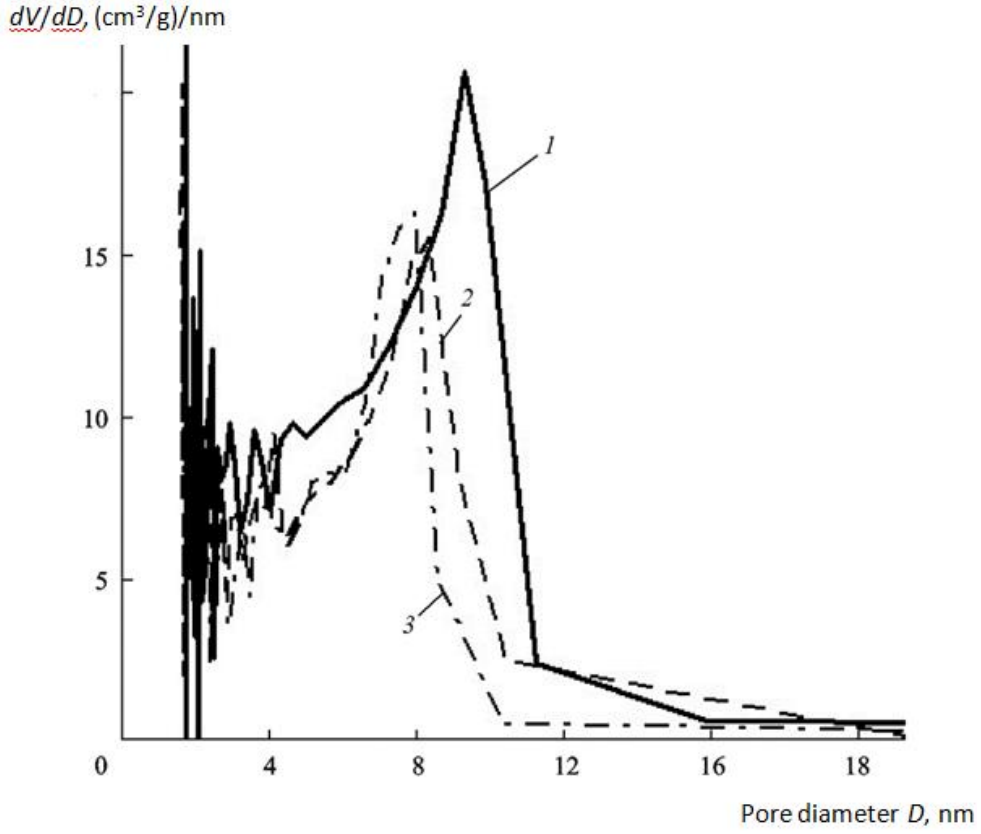

Fig. 3. Differential curve of pore diameter distribution: (*1*) original silica; (*2*) silica with grafted hexadesylsilyl groups; (*3*) RAM

However, it does not convey the true geometry of the silica matrix. Silica is known to have globular structure and space of the pores formed by the voids between the globules of fused silica [1]. Therefore, the use of the model of cylindrical pores could hinder the correct selection of a source of silica and lead to a significant deterioration of the chromatographic properties of the finished RAM.

Application of a globular model, in contrast to the model of cylindrical pores, gives the real size of the mouths of the pores, effectively allowing you to compare the silica matrix parameters with the size of inert macromolecules to sample particles. Fig. 4 shows a representation of the octahedral (*1*, *2*) and tetrahedral (*3*, *4*) pores. The surface (*A*), marked in blue, is not modified silica, available for the nitrogen molecules. Between the blue and green

areas there is a modified surface (*B*), partially accessible for the nitrogen molecules. In this region the layers of the modifier are closed, impeding the diffusion of adsorbate. In red (*D*) it is shown the mouth of the pores at the contact of the globules of silica; green (*C*) represents modified surface of silica globules fully accessible to the adsorbate.

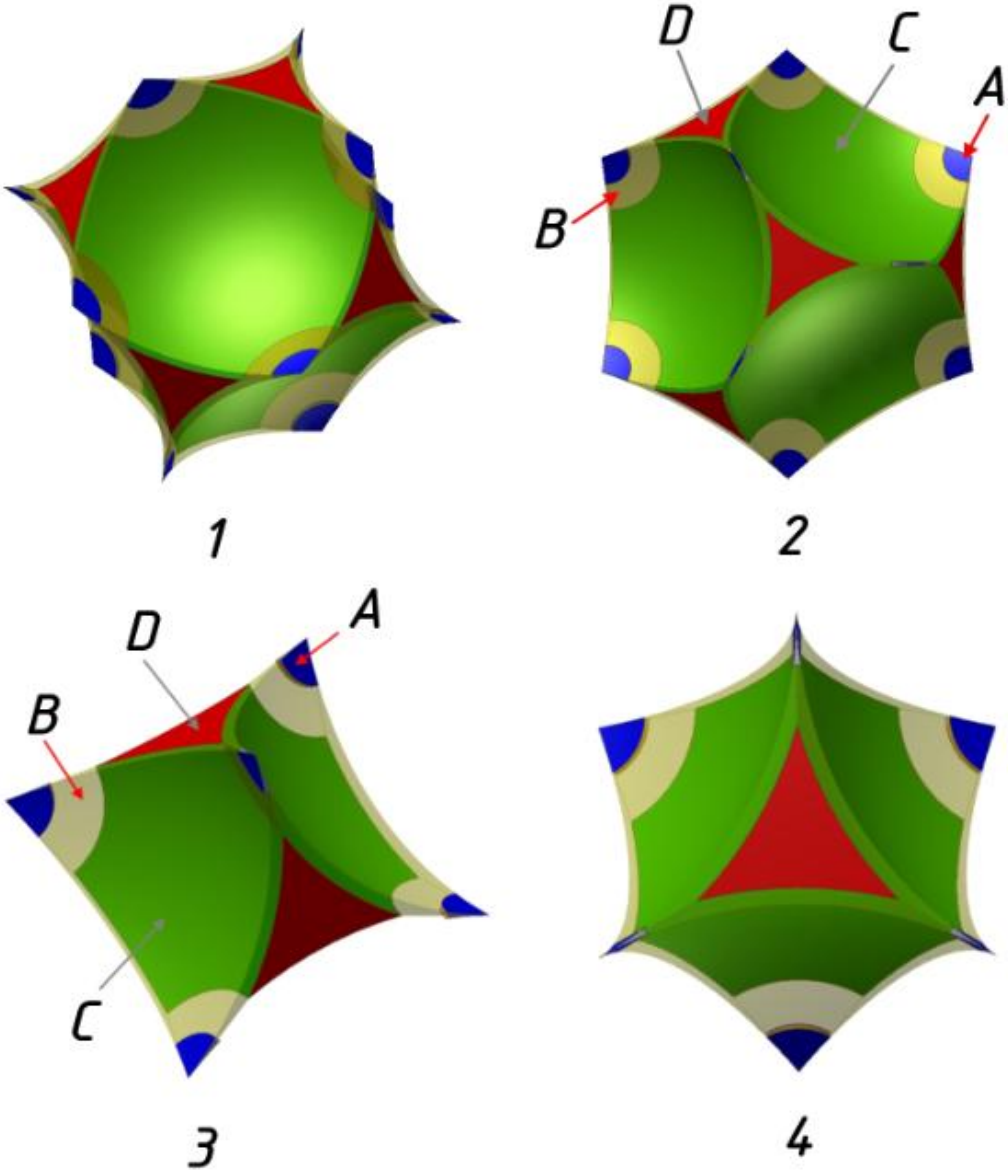

Fig. 4. Representation of the octahedral (*1*, *2*) and tetrahedral (*3*, *4*) pores. Blue color (*A*) shown is not modified silica, (*B*) shows modified surface, partially accessible for the nitrogen molecules; in red (*D*) is shown the mouth of the pores; green (*C*) represents modified surface of silica globules

Globular proteins (e.g., albumin [2-4], which is modeled by ellipsoid is about 8.5 nm long [5]) or particles of inorganic nature with a modified surface modeled by a sphere of known diameter can be used as shielding particles. The shielding coating must not penetrate

into the mouth of tetrahedral and octahedral pores of silica matrix formed by the contact of the globules of the same diameter. It allows us to calculate the size of the shielding particles on the basis of the diameter of the globules of silica. For example, for shielding the external surface with human or bovine serum albumin the desired maximum distribution of the pore sizes for the matrix obtained is close to 5 nm.

It is optimal to use a method of low-temperature nitrogen adsorption for the experimental evaluation of the structural parameters of unmodified and modified matrix. The best match between estimated and actual values of the diameters of the orifices of the pores is obtained by using the distribution curves of pore sizes obtained on the basis of the desorption branches of isotherms. The presence of one maximum on the curve makes it easy to interpret it on the basis of a globular model with tetrahedral and octahedral pores. By changing the ratio of the amounts of tetrahedral and octahedral pores in the model it is possible to achieve satisfactory agreement with the experimental shape of the distribution curve. In contrast to the model of cylindrical pores, the calculation model of globular pores gives realistic values of the surface area of the silica with grafted hexadecylsilyl groups: 131 m^2/g instead of 183 m^2/g. Film thicknesses were calculated from the obtained surface area for initial and modified silica and determined the number of layers of the modifier. The obtained value 1.41 is in good agreement with elemental analysis data.

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