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NEW CHROMATOGRAPHIC MATERIALS FOR ENZYME SEPARATION

The simple method of evaluation of polymer matrix pore size distribution is presented. The method allows us to estimate some changes in structural porosity of terpolymer of 2-hydroxyethyl methacrylate, dodecyl methacrylate and ethylene glycol dimethacrylate. It is shown that an increase of dodecyl methacrylate concentration results in reduction of both distribution parameters: average pore diameter and standard deviation. However, at the same time the presence of dodecyl monomer affects a gel porosity. The effects of side-chain length of modifier as well as monomers dilution ratio are also discussed.

1. INTRODUCTION

Today when the environment is affected by the excessive occurrence of various pollutants, the creation of novel, nature-compatible technologies appears to be the real challenge to chemical engineers. Among several possibilities, biotechnology seems to exhibit the best features. In biotechnology, we deal with natural reactants and imitations of natural processes allowing production of required items. As a result, the amount of side-products is limited and some new methods for utilization of pollutants and wastes are developed. Due to biotechnology the degradation of our ecosystem can be controlled. There is a clue to rapid development of biotechnology in the last decades. However, in this field, the development of new separation methods is necessary. Beside reliability and low cost, such methods satisfy special requirements. The specific activity of separated species should not be altered during the processes. It seems that chromatography fully meets these requirements.

Evaluation of the structure of polymeric chromatographic packing materials becomes a crucial issue in the determination of some important parameters for

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synthesis of matrices and/or their modifications. In order to describe better a polymer matrix, one should supplement the commonly used parameters, such as average pore diameter and total porosity, with the following characteristics: pore size distribution (PSD) function, pore polydispersity and specific pore surface area [1]. Such characteristics provide also a convenient way of the evaluation of the effects of some synthesis parameters on the structure of polymers.

Gel chromatography porosimetry (GCP) may be considered as the simple analytical method allowing determination of PSD function [2]. Having this function known one is able to calculate average pore diameter or pore polydispersity. GCP is also valuable for its simplicity and possibility to be used in laboratories with standard liquid chromatography equipment. In several approaches published so far [3]–[6], the calculations of GCP function were based on the well-known chromatographic equation:

$$V_e = V_0 + V_p K \tag{1}$$

where V_e, V_0 and V_p are the volume of solute retention and volumes of mobile and stationary phases, respectively. K is the distribution coefficient of a solute.

The coefficient K is related to size and type of pores, size and shape of solute molecules and some interactions between solute and matrix surface [2], [7]. Taking into account the fact that pores in polymer matrices are highly polydispersed one is able to redefine equation (1) to the following form:

$$V_e = V_0 + V_p \langle K \rangle \tag{2}$$

where the average distribution coefficient is expressed as:

$$\langle K \rangle = (1/V_0) \int_0^\infty K F_v(R) dR \tag{3}$$

where $F_v(R)$ is the volume distribution function.

More convenient way allowing one to evaluate the PSD function can be used by making the following assumptions:

1. The coefficient K is not affected by the solute–sorbent interface interactions. The coefficient results only from size exclusion mode.

2. Pore volume distribution $F(R)$ may be expressed by PSD function $f(R)$

$$F_v(R) = Vf(R). \tag{4}$$

3. PSD function has the logarithm-normal shape

$$f(R) = [1/(s\sqrt{2\pi})] \exp [-(\ln R - M)^2/2s^2] \tag{5}$$

where M and s are the mean value and standard deviation of the distribution.

Finally, equation (3) takes the form

$$\langle K \rangle = \int_0^\infty K\,[1/(\sqrt{2\pi})]\,\exp\,[-(\ln R - M)^2/2s^2]\,dR. \tag{6}$$

According to HALASZ [3], [4] the molecules of solute with the coil diameter of a_i are totally accessible or inaccessible to matrix pores. Hence, in this approach, the coefficient K can take only 0 or 1 values:

$$\begin{aligned} K &= 1 \quad \text{for} \quad a_i < R, \\ K &= 0 \quad \text{for} \quad a_i \geq R. \end{aligned} \tag{7}$$

For this simplification, equation (6) gives

$$\langle K \rangle = \int_0^{a_i} [1/(\sqrt{2\pi})]\,\exp\,[-(\ln R - M)^2/2s^2]\,dR \tag{8}$$

where integration is carried out to $R = a_i$.

GORBUNOV [2] has estimated the PSD function with restriction given by equation (7). He concluded that for sorbents with narrow or monodisperse pore size distribution the use of approximation (7) resulted in obtaining too wide distribution functions. He did not evaluate, however, the possibility of using this approach to calculation of distribution functions when pores were highly polydispersed. Hence, it may be assumed that the approach (7) can be used in the evaluation of these polymer sorbents, which display polydispersity. The paper presented shows the simple way of porosity structure evaluation. It may serve as the recognition tool in selection and tailoring of polymer matrices with desired properties.

2. MATERIALS AND METHODS

2.1. POLYMER MATRICES

Polymer matrices were prepared by suspension copolymerization of the following reactants: 2-hydroxymethyl methacrylate (HEMA), dodecyl methacrylate (DOMA) (or butyl acrylate (BA)) and ethylene glycol dimethacrylate (EGDMA) at various monomer ratios in the presence of 0.5 wt.-% of azoisobutyronitrile (AIBN) as the initiator. Compositions of organic phase are presented in table 1. All syntheses were performed according to the same procedure. Mixture of HEMA with gradually increasing amount of DOMA (or BA) and crosslinking agent (50 wt.-% of EGDMA) containing AIBN and porogens was added to the aqueous phase consisting of 1 wt.-% of polyvinyl alcohol (in respect to the organic phase) and 4 wt.-% of calcium chloride. Suspension was stirred and heated as follows: 60 °C – 1 h, 85 °C – 2 h and 90 °C – 8 h. After synthesis polymer was extracted with hot toluene, dried and sieved to obtain 8 : 100 mesh fraction.

Table 1

Composition of organic phase used in the synthesis of polymer mixture. Monomer # 1 HEMA, crosslinking agent EGDMA (50 wt.%), porogens mixture: in the dodecane to 2-ethylohexanol ratio equal to 1:1

Matrix	Monomer #2		Porogens:monomers ratio
	Kind	Amount [mmol/g]	[vol:vol]
DM003/1	DOMA	0.03	1:1
DM009/1	DOMA	0.09	1:1
DM027/1	DOMA	0.27	1:1
DM009/2	DOMA	0.09	2:1
BA009/1	BA	0.09	1:1
DM081/1	DOMA	0.81	1:1
DM162/1	DOMA	1.62	1:1

2.2. GEL CHROMATOGRAPHY POROSIMETRY

GCP measurements were performed using liquid chromatography system consisted of Shimadzu 6LC pump (0.2 cm^3/min), Rheodyne 7725 injector (20 μL loop), RIDK-102 detector (ECOM) and integrating unit APEX 3.0 (Data Apex). Columns 200 × 3 mm were loaded with the investigated matrices just before measurements. Retention volume of narrow fraction of dextrane standards (concentration 2.5 mg/cm^3) was detected. Each experiment was triplicated. Properties of dextranes are summarized in table 2.

Table 2

Dextrane standards used in this study

Dextrane	Coil radius* [nm]	Supplier
Glucose	0.33	Polfa, Kutno
T1.5	1.11	Polfa, Kutno
T5	1.76	Polfa, Kutno
T10	2.50	Polfa, Kutno
T15	3.00	Polfa, Kutno
T40	5.00	Pharmacia, Uppsala
T70	6.61	Pharmacia, Uppsala
T110	8.87	Polfa, Kutno
T500	17.67	Pharmacia, Uppsala
T2000	35.36	Pharmacia, Uppsala

* Dextrane coil radius was taken from [9].

2.3. WATER REGAIN

Water regain (WR) was calculated according to common procedure [9]. Water-swollen samples were centrifuged, weighed (m_s), air dried and weighed again (m_d).

$$WR = (m_s - m_d)/m_d. \tag{9}$$

3. DISCUSSION

The method applied in this paper is based on the normalization of equation (8)

$$\langle K_i \rangle = \int_0^{z_i} [1/(\sqrt{2\pi})] \exp [-z^2/2]\, dz \tag{10}$$

where subscript i is applied to the dextrane molecule of a_i radius. The variable z is the normalization parameter

$$z = (\ln R - M)/s. \tag{11}$$

The $\langle K \rangle$ value was calculated directly from experimental data

$$\langle K_i \rangle = (V_e - V_0)/V_p = (V_e - V_0)/(V_m - V_0) \tag{12}$$

where V_m and V_0 were the retention volumes of glucose and T2000 dextrane, respectively.

The experiments guaranteed the set of $\langle K_i \rangle$ values from which the normalization factors z_i were found by means of the Standard Cumulative Distribution Table. Taking into account equations (7), (8) and (11) one is able to estimate average pore radius and standard deviation of PSD function. It is recommended to use the least square method for calculation of linear regression

$$Z_i = (1/s) \ln a_i - (M/s). \tag{13}$$

The proposed procedure of estimation of the PSD function may be shown in the form of a flow-chart (figure 1). The procedure was used to calculate PSDs for matrices

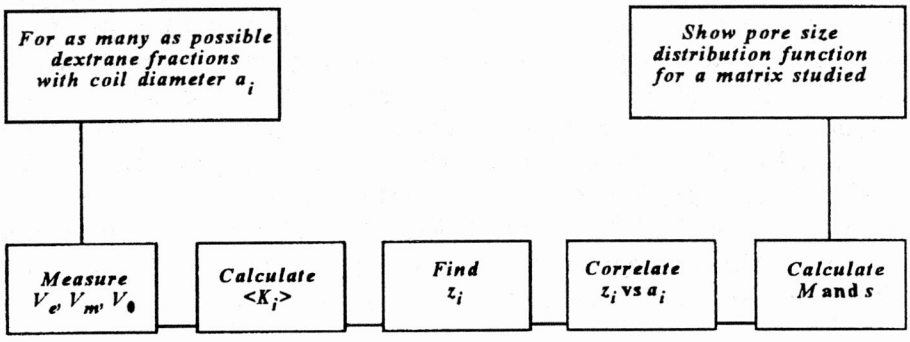

Fig. 1. Flow-chart of PSD function calculation

studied. The data is collected in table 3. Additionally, in the two last columns, matrix porosity is presented.

Table 3

Calculated mean pore radius, standard deviation, water regain and structural porosity of matrices studied

Matrix	Average pore [nm]	Standard deviation	Water regain [g/g]	Porosity* [%]
DM003/1	13.52	1.4101	2.21	44.5
DM009/1	6.18	0.9061	2.23	28.4
DM027/1	4.74	0.6971	2.41	14.2
DM009/2	7.13	0.8646	3.46	41.6
BA009/1	7.42	0.952	2.52	38.2
DM081/1	0.94	0.5312	1.91	9.4
DM162/1	N/D**	N/D	0.98	N/D

* Porosity was calculated from equation $P = (V_e - V_0)/(V_c - V_0)$ where V_c is the volume of column.

** N/D – not determined.

For more fruitful discussion it is convenient to assume that total porosity of the polymer matrix is the sum of gel and structural porosities. Hence, water regain may be considered as a specific measure of total porosity, while the structural porosity shows only the part of matrix which forms pores accessible to small solute molecules (i.e., glucose). The comparison of these two parameters for matrices prepared with increasing amount of DOMA (DM003/1, DM009/1, DM027/1, DM081/1 and DM162/1) shows the constant decrease in the structural porosity. For better presentation of the DOMA effect on structural porosity, the volume distribution function, $F(R)$, was calculated according to equation (4). It is presented in figure 2. The decrease in pore diameter and standard deviation indicates that pores become smaller with narrowing distribution. In the same time, water regain reaches maximum value for the DM027/1 matrix. Taking into account the dramatic drop in structural porosity and the fact that water regain is governed by both porosities and matrix hydrophilicity one may conclude that presence of dodecyl methacrylate significantly affects gel porosity. When monomer mixture reveals more hydrophobic character (an increasing concentration of dodecyl methacrylate) the porogens became more compatible and acted as *sol*-solvents. As a consequence the gel region was actively formed [10]. Additionally, it seems possible that monomer with long alkyl chain acts as a specific surface active agent and affects the morphology of a matrix synthesized. To evaluate these effects DOMA was replaced by butyl acrylate. Comparison between matrices DM009/1 amd BA009/1 reveals the vital contribution of alkyl chain length to the

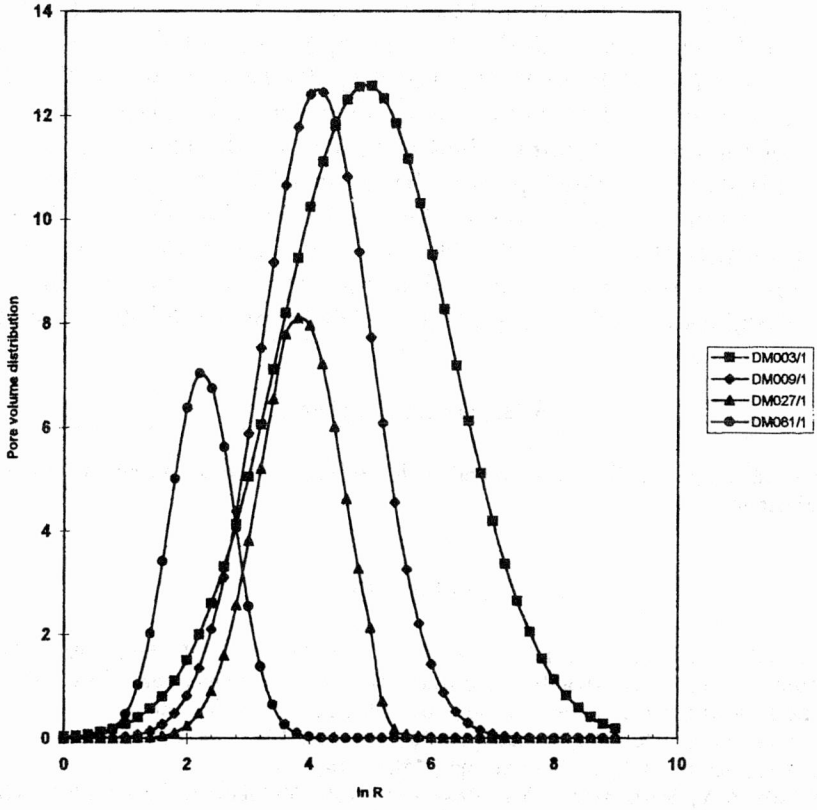

Fig. 2. Volume distribution functions for DOMA matrices

morphology of the matrix obtained. Higher structural porosity with pores of larger diameters was obtained for monomer with shorter side chain.

For *nosol* type of solvents, volume of porogens used in the synthesis was one more parameter to be mostly involved in creation of structural porosity [10]. The comparison between matrices DM009/1 and DM009/2 shows that in the polymerization process studied, the porosity may be controlled by monomer dilution. Average pore diameter increases and the matrix has larger pores. Having both synthesis variables under control, length of monomer side chain and monomer dilution ratio, one can note that porogens are mostly responsible for creation of higher gel porosity.

4. CONCLUSIONS

The presented method of evaluation of pore size distribution can actively furnish a useful characterization of structural porosity. It makes some synthesis parameters

available to control and tailors a requested matrix. In the case of terpolymer of 2-hydroxyethyl methacrylate, dodecyl methacrylate and ethylene glycol dimethacrylate, it was shown that structural porosity decreases dramatically when the concentration of dodecyl monomer in the copolymer is raised. Moreover, the properties of the matrix obtained depend mostly on the length of side chain of modifier. Matrices with wider pore distribution and larger pore dimension are obtained using modifier with shorter side chains. Similar effect can be observed when the porogens ratio increases. The matrix becomes more porous with higher contribution of pores with larger diameter. The efficiency of the matrices obtained in separation of several enzymes will be presented in the next paper.

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NOWE TWORZYWA CHROMATOGRAFICZNE DO ROZDZIELANIA ENZYMÓW

Przedstawiono prostą metodę oceny rozkładu wielkości porów matrycy polimerowej. Umożliwia ona określenie zmian w porowatości strukturalnej terpolimeru metakrylanu 2–hydroksyetylu, metakrylanu dodecylu i dwumetakrylanu glikolu etylenowego. Wykazano, że wzrost stężenia metakrylanu dodecylu powoduje zmniejszenie obu parametrów rozkładu – średniej średnicy porów i odchylenia standardowego. Jednocześnie obecność monomeru dodecylu wpływa na porowatość żelu. Opisano także wpływ długości łańcucha bocznego modyfikatora i stosunek rozcieńczania monomerów.

НОВЫЕ ХРОМАТОГРАФИЧЕСКИЕ ВЕЩЕСТВА ДЛЯ РАЗДЕЛЕНИЯ ЭНЗИМОВ

Представлен простой метод оценки расположения размеров пор полимерной матрицы. Он дает возможность определения изменений в структурной пористости тройного полимера

метакрилата 2–гидроксиэтила, метакрилата додецила и диметакрилата этиленгликоля. Было обнаружено, что повышение концентрации метакрилата додецила вызывает понижение обоих параметров разложения – среднего диаметра пор и стандартного отклонения. Одновременно наличие мономера додецила влияет на пористость желе. Описаны также влияние длины боковой цепи модификатора и отношение разбавления мономеров.