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Quantitative characterization of pore structure of cellulose gels with or without bound protein ligand

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Abstract

Structural properties of cellulose gels Perloza MT, materials designed for the preparation of chromatographic adsorbents and immobilized biocatalysts, having a different content of polymer were investigated using a batch solute exclusion method. A homologous set of dextrans with a wide range of molecular weights was used to probe the pore accessibility of the gel particles. It was found that all gels possessed a bimodal pore structure where macropores were fully accessible to all dextrans whereas the solute partitioning depending on the molecule size occurred in the micropores of the swollen polymer network. The macropore and micropore fractions of the gels were estimated from the masses of total water and water accessible to the largest solute. The macropore fraction decreased with the gel polymer content. It was 0.57 at the gel containing 8% of polymer but only 0.22 at the gel with 38% of polymer. The micropore fraction varied from 0.38 to 0.47. The mass of accessible water for each solute was used to calculate the particle and gel-phase partition coefficients. The dependence of the latter quantity on the solute hydrodynamic radius was successfully fitted with the Ogston model. Bovine serum albumin that was used as a model protein ligand blocked almost all gel-phase pores of the gel with the highest polymer content whereas it little affected the accessibility of other materials.

Keywords: Batch solute exclusion method; Equilibrium partitioning; Gel structure; Cellulose gel; Dextran; Partition coefficient; Ogston model; Protein ligand

1. Introduction

Cellulose is a widespread natural polymer, which has found applications in a broad range of bioprocesses due to its low cost and possibility of various forms of appearance. Cellulose gels have frequently been used as carriers in biocatalysis and chromatography [1–3]. One of the most investigated application areas of cellulose gels is affinity chromatography where ligands of very different nature have been applied [4–6].

The textural properties of cellulose gels such as porosity, accessible surface area, mean pore size or the pore size distribution directly influence their transport properties or equilibrium partitioning of solutes and therefore they have frequently been investigated [3,7–14]. The batch solute exclusion method has often been used to characterise the gel structure as it allows the experiments being performed in wet state what is in many cases of highest importance for the preservation of gel integrity [15]. This method utilizes the obstruction effect of gel pores to the solutes with different size. The measured quantity is the gel pore volume accesible to the solute which can be transformed into the partition coefficient. The dependence of the relative mass of non-accesible water versus the solute size represents the cumulative function of apparent pore size distribution. The true mean pore sizes are about 2.5–3 times larger than the apparent ones [3,16].

Several theories have been developed to describe the solute distribution within the gel pores. Giddings et al. [17] used a statistical mechanics approach and derived several models for the calculation of the pore partition coefficient in dependence from the size and shape of the solute molecules

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for different unimodal pore structures. The model suggested for spherical molecule was widely used for its simplicity [15,16,18–20]. Cassasa et al. [21–23] sugessted a more complex model for partitioning of a random-coil macromolecular solute in pores of various shape based on the theory of Brownian motion. The pore partition coefficient can be integrated through pore-size distribution function, mostly represented by the log-normal distribution, to provide the particle partition coefficient [15,17,24]. Although such an approach could provide good characterization of many gels, it was not generally accepted [25].

Several models which do not require an assumption about the geometrical shape of the pore and mathematical function of pore size distribution have been suggested. Giddings et al. derived [17] a relationship for the partition coefficient of a molecule of arbitrary shape in a nonuniform network represented by random planes. An equation for solute partitioning inside macropores where the diameter of pores is much greater than the diameter of molecule, was presented by Cassasa et al. [21–23]. Their relationship can be transformed into an equation proposed by Gorbunov et al. [24] by substituting the radius of the solute molecule with its effective chromatographic radius and employing the ratio of the pore surface to the pore volume instead of the pore radius.

The often used Ogston model [16,26,27], which considers pores as spaces between randomly arranged rigid rods representing polymer fibers, includes the obstruction efftect of three dimensional gel matrix. Consequently, the interpretation of the particle partition coefficient is different. While the partition coefficient in the models of Giddings, Cassasa and Gorbunov characterizes only the accesibility of the liquid filled pores of gel material, the particle partition coefficient in the Ogston model is related to the total volume or mass of gel.

The main objective of this study was to determine the pore structure of cellulose gels with different content of polymer and with or without bound protein model ligand. The size exclusion data of homologous set of dextrans were evaluated using the Ogston model.

2. Experimental

2.1. Materials

Three kinds of bead cellulose Perloza were used in the experiments. MT 50 was from Iontosorb (Ústí nad Labem, Czech Republic), MT 100 and MT 500 from Lovochemie (Lovosice, Czech Republic). The mean bead sizes were 0.11 mm at MT 50 and MT 100 and 0. 27 mm at MT 500). Perloza MT 50 and MT 100 were used for binding bovine serum albumin (BSA) (fraction V powder, Sigma Chemical Co., St. Louis, MO, USA). BSA was immobilized on the cellulose materials which were first derivatized with cyanuric chloride. The derivatization was made according to the method of Kay and Lilly [28] with a slight modification [29]. Wet cellulose (40 g) was resuspended in 233 ml of water and, under stirring, 100 ml of 3 M NaOH were added slowly during the first 15 min. The suspension was then stirred for additional 30 min. Then, small portions of cyanuric chloride solution (5 g dissolved in 100 ml of acetone) were added during 15 min and the suspension was stirred for 15 min. After that, 60 ml of water was added and the suspension was stirred again for another 15 min.

The cellulose-chlorotriazine material was washed on a sintered glass filter by 100 ml of acetone–water (1:1) mixture and by 500 ml of water and used immediately for the BSA binding. For that, 20 g of wet derivatized particles were resuspended in 100 ml of acetate buffer (0.1 M, pH 4.6) with 0.2 g of BSA and stirred at ambient temperature for 2 h. After this time, the preparative was washed with water, 1 M KCl and finally again with water.

Dextrans (Fluka Chemika AG, Buchs, Switzerland and Sigma Chemical, St. Louis, MO, USA) with the relative molecular weights 1500, 6000, 9300, 17,500, 40,000, 60,000, 70,000, 110,000, 200,000 and 2,000,000 were used as solutes. For comparison, polyethylene glycols (PEGs) with the relative molecular weights 200, 400, 600, 1000, 1500, 2000, 3000, 6000, 12,000, 17,500, 20,000 and 35,000 (Fluka Chemika AG, Buchs, Switzerland) were used as solutes in the experiments with Perloza MT 100 and MT 500. Both kinds of solutes were dissolved in re-distilled water to the concentration of 5 g/L. The solute hydrodynamic radius, r_s, was calculated from the relative molecular weight, M_r, using the Mark–Houwink–Sakurada equation

$$r_s = kM_r^a \quad (1)$$

The parameters k and a were adopted from the paper of Broek et al. [30] and they had the values of 0.027 and 0.5 for dextrans and 0.087 and 0.4 for PEGs.

2.2. Experimental procedure

The distribution coefficients for dextrans and PEGs were determined by the batch solute exclusion method. Approximately 0.5 g of wet cellulose gel (accurately weighed) was brought into the contact with approximately 1 g of solution with the dextran or PEG concentration of 5 g/L. Hermetically sealed vials with the suspension of gel and the solution were placed in a water bath with a reciprocate shaker and kept for 24 h at the temperature of 25 °C. Afterwards, the gel particles were separated from the equilibrated solution by filtration, rinsed three to four times with water, air-dried at ambient temperature and weighed for the determination of polymer content in the gel sample. The solute concentration was determined using a differential refractometer of a chromatography set-up from Knauer GmbH (Berlin, Germany). Each exclusion experiment was made in triplicate.

2.3. Data evaluation

The total mass of the water imbibed by the gel, m_w, was calculated as the difference between the masses of wet and dry samples, m_d. These values were used to calculate the total cellulose gel particle porosity, ε_p, which was distributed into two parts: macropore fraction, ε_m, and micropore (gel porosity) fraction, ε_g,

$$\varepsilon_p = \varepsilon_m + \varepsilon_g = \frac{m_w/\rho_w}{(m_w/\rho_w) + (m_d/\rho_d)} \quad (2)$$

where ρ_w and ρ_d are the densities of water and solid cellulose (1.48 g/mL [31]). The distribution of the porosity into two fractions was based on the determination of the mass of water accessible to the largest solute used, dextran with the relative molecular weight of 2,000,000. It was assumed that this value was equal to the mass of water imbibed in the macropores, m_m. The macropore fraction was then calculated as follows,

$$\varepsilon_m = \varepsilon_p \frac{m_m}{m_w} \quad (3)$$

The determination of partition coefficients was based on the slightly modified procedure of Kremer et al. [15]. The particles with the total mass of imbibed water, m_w, were contacted with the solution with the mass of m_s and solute concentration of c_0. After equilibration, the solute concentration in the solution decreased to the value of c_{eq} due to the solute distribution between the solution and particles. The degree of distribution depended on the molecular size of the solute. It was characterized by the mass of accessible water, m_a, that was evaluated from a simple mass balance,

$$m_a = \left(\frac{c_0}{c_{eq}} - 1\right) m_s \quad (4)$$

For each solute, the total imbibed water was thus distributed into non-accessible water with zero concentration of solute and the accessible water with the concentration equal to that of the bulk solution.

The mean pore partition coefficient, K_d, was calculated as the ratio of the accessible and total water masses,

$$K_d = \frac{m_a}{m_w} \quad (5)$$

Obviously, the mean pore partition coefficient ranges from 0 at totally excluded solutes to 1 at solutes that can access the whole pore volume. The particle partition coefficient, K_{av}, scales the accessible pore volume to the total particle volume so it can be easily calculated from K_d as follows,

$$K_{av} = K_d \varepsilon_p \quad (6)$$

The gel-phase partition coefficient, K_g, was obtained from the particle partition coefficient if the effect of macropores was eliminated from both solute partitioning (Macropore partition coefficient equal to 1) and particle volume,

$$K_g = \frac{K_{av} - \varepsilon_m}{1 - \varepsilon_m} \quad (7)$$

3. Results and discussion

Dextrans and polyethylene glycols (PEGs) are the most common molecular probes used in size-exclusion methods in aqueous phase. Dextrans are branched polysaccharides, which are available in a very broad range of molecular weights up to tens of millions. They tend to behave more like a flexible coiled polymer rather than a molecule with rigid globular conformation [32,33]. On the other hand, PEGs have linear molecules and act as an "expanded random coil of hydrated polymer" in aqueous solutions [34]. Compared to dextrans, the commercial preparations of PEGs have much narrower size distributions but, unfortunately, their upper size is limited by the value of the molecular weight of about 50,000. Both dextrans and PEGs were demonstrated to have negligible adsorptivity on cellulose [35] which would make them suitable solute probes for the investigation of textural properties of Perloza gels.

As Perloza MT 100 and MT 500 were reported to be macroporous materials [1,36], dextrans had to be used. Nevertheless, we considered it useful to make control experiments with both types of solutes in the lower range of molecular weights so that the assumptions of size-exclusion methods were the subject of the double control. Fig. 1a and b show the dependence of the mass of accessible water obtained from Eq. (4) on the solute relative molecular weight and solute radius, respectively, for both dextrans and PEGs in Perloza MT 100. Obviously, a very good match was reached between the results obtained with these two solutes if besides the experimental error of the size-exclusion method, the accuracies of the approximation equations for the hydrodynamic radius were considered. As dextrans covered a broader range of molecular weights, they were used in the further presentations shown below. At fitting with the Ogston model, the partition coefficients of dextrans (the lower limit of molecular weight of 1500) were supplemented by the data for the PEGs with the lowest molecular weights.

The difference of mass of accessible water for the lowest-size solute (Fig. 1) and the total water content in the gel (Table 1) was less than 1% at MT 100. All gel water was thus accessible to the PEG with the molecular weight of 200. Following the hydrodynamic radius of this molecule, it can be concluded that the minimum size of the pores of the gel-

Table 1
Porosities of investigated cellulose gels

	m_w/m_d	ε_p	ε_g	ε_m
MT 50	1.47	0.684	0.467	0.217
MT 100	7.63	0.919	0.416	0.502
MT 500	13.7	0.953	0.380	0.573

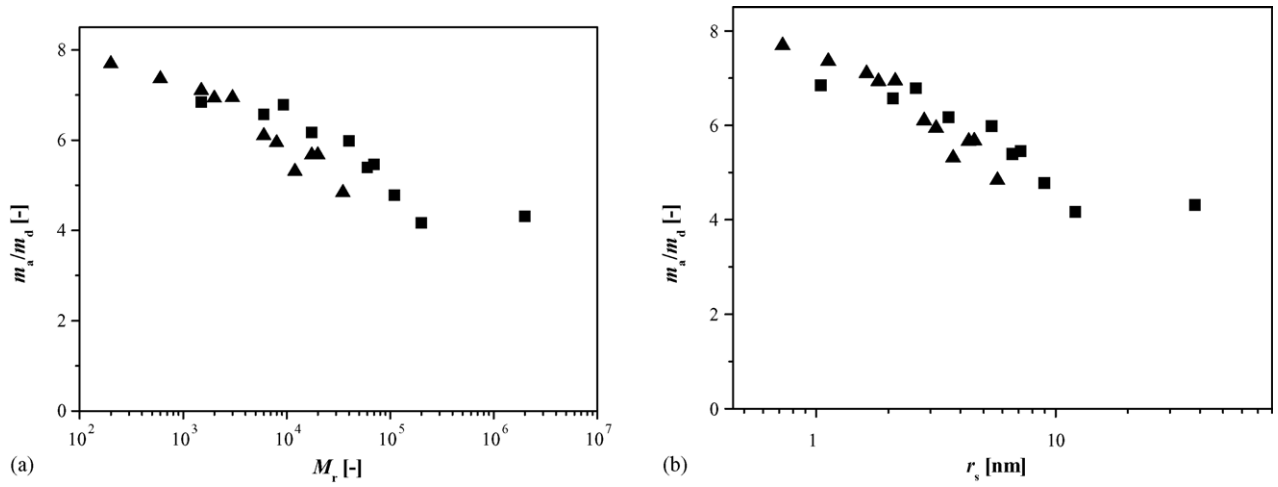

Fig. 1. Comparison of the behaviour of dextrans (■) and PEGs (▲) in the batch size-exclusion experiments using the gel Perloza MT 100. The graphs express the dependence of the mass of accessible water per unit mass of dry gel vs. (a) relative molecular weight and (b) solute radius.

phase was about 1 nm. These results supported the reliability of the batch size exclusions measurements. Similar observations were made also at MT 50 and MT 500 (Fig. 2 versus Table 1). The absolute values of the total water content confirm that MT100 and MT500 are typical highly-swollen hydrogels whereas Perloza MT 50 is a more rigid material with the polymer content of 40%. Fig. 2 clearly demonstrates the decrease of the mass of accessible water with the polymer content.

Figs. 1 and 2 demonstrate one important advantage of the plotting of mass of accessible water instead of the more common mass of non-accessible water. In the latter case, the formation of a plateau or asymptotic trend of the relationship at the largest solutes can lead to overlooking of the bimodal character of the porosity of cellulose gels. On the contrary, Figs. 1 and 2 clearly show that even the largest solute was only partly excluded from the gel. At MT 50, it is less apparent due to the scale used in Fig. 2 therefore Fig. 3 demonstrates better the presence of macropores.

The total particle porosity, ε_p, was calculated from the masses of wet and dry gels (Eq. (2)). The values of ε_p given in Table 1 were obtained as a mean from about 40 samples, which resulted in a low error of estimation. The presented values are essentially identical with the data of producer [36]. It has been mentioned in Section 2 that the accessibilities of cellulose gels for the dextran with the relative molecular weight of 2,000,000 (hydrodynamic radius of 38.2 nm) were chosen as a threshold value for the discrimination between the microporosity and macroporosity. Fig. 2, however, shows that the mass of accessible water either did not decrease or decrease only very slightly above the solute hydrodynamic radius of 10 nm. The size of the most micropores was below this limit which can be interpreted so that the microporosity represents a gel-phase of swollen polymer network of chains

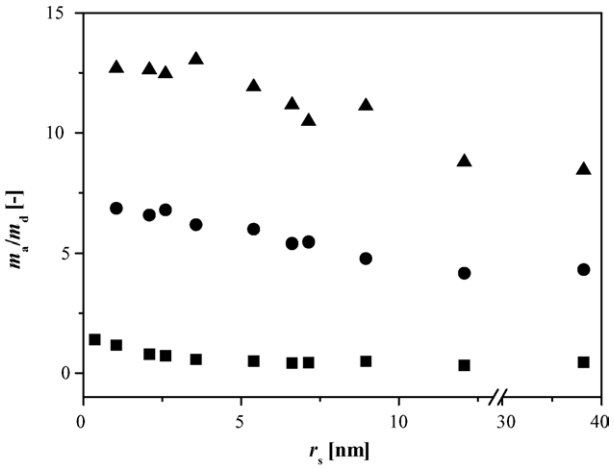

Fig. 2. Accessibilities of cellulose gels for dextran solutes; Perloza MT 50 (■), MT 100 (●) and MT 500 (▲).

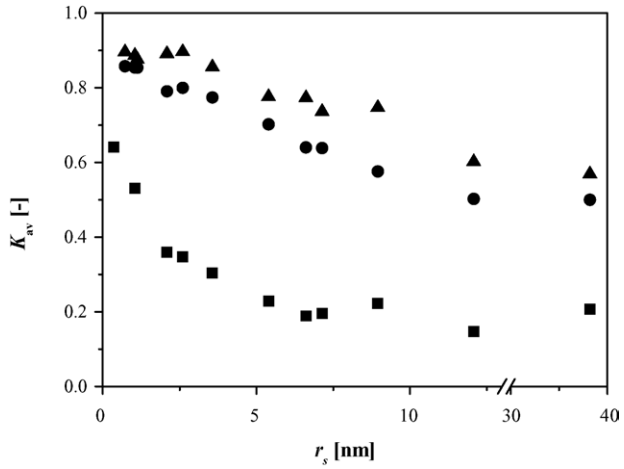

Fig. 3. Particle partition coefficient of dextrans, K_{av}, in cellulose gels; Perloza MT 50 (■), MT 100 (●) and MT 500 (▲).

of cellulose macromolecules. The minimal changes of the mass of accessible water in the range of solute sizes 10–40 nm document that the size of most macropores must have been well above 50 nm. The macropores are thus intraparticle voids free of polymer.

The macropore fraction, ε_m, of individual gels was calculated from Eq. (3). Table 1 shows that at macroporous gels MT 100 and MT 500, it was 55 and 60%, respectively, of the total pore volume. At MT 50, it was 30% which was a surprisingly high value as this gel is recommended for the small protein separation or buffer exchange by the producer [36]. This could be explained by the gel radial inhomogeneity where the macropore frequency and/or macropore size could increase towards the particle centre, which would not be surprising at the methods of the preparation of cellulose gels [1].

The best comparison of the accessibility of investigated cellulose gel particles in respect to solute molecule size (their static size-exclusion separation efficiency) is made in Fig. 3. The particle partition coefficient, K_{av}, is defined as the fraction of the total particle volume available for the permeation of solute of given size. Fig. 3 also shows the distribution of the total porosity between the microporosity and macroporosity fractions. The mentioned solute-size effect relates only to the microporosity fraction. Perloza MT 50 having a high polymer content and low total porosity provided a sharp size-separation for the solutes with the effective radius up to 5 nm. Perloza MT 50 would thus be suitable for desalting or low molecular compound removal from protein solutions by size-exclusion chromatography in the biotechnological production processes. On the other hand, the increased fraction of macroporosity in Perloza MT 100 and MT 500 and their wider distribution of pore sizes in the gel-phase made the size-exclusion effect of these two supports less distinct.

As follows from the presented results, only the pore-size distribution in the gel-phase could be quantitatively characterized. In order to do that, the partitioning of solutes in the gel-phase had to be deconvoluted from the partitioning in the whole particle. As the relatively clear boundary between the microporosity and macroporosity was observed, it was a good assumption to consider the partition coefficients of all solutes in the macropores equal to 1. Fig. 4 presents the gel-phase particle partition coefficients K_g (Eq. (7)) for all three gels. It is evident that the dependencies of particle partition coefficients followed a similar trend which could mean that their gel-phases had a similar structure but a different polymer content.

From the models discussed in the Introduction, the Ogston model [37] was selected to describe the partitioning of solutes in the gel-phase of the Perloza gels. The Ogston model (Eq. (8)) was designed for sherical rigid solute molecules dispersed in a polymer matrix represented by randomly arranged straight rigid rods.

$$K_g = \exp\left(-\pi L(r_s + r_r)^2\right) \quad (8)$$

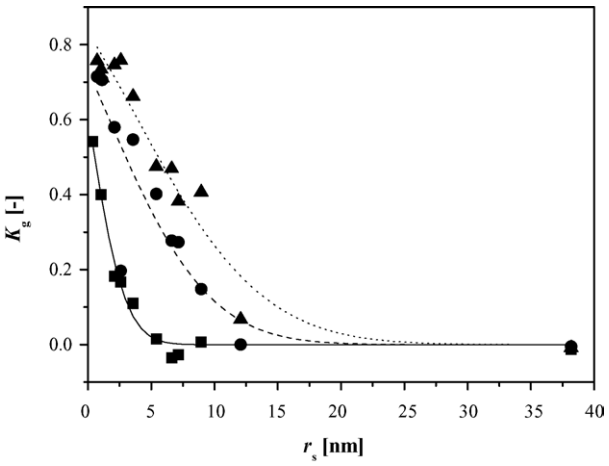

Fig. 4. Gel-phase partition coefficients of dextrans and low-molecular PEGs in cellulose gels; Perloza MT 50 (■), MT 100 (●) and MT 500 (▲). The lines are the fits of experimental data with the Ogston model; solid line is for MT 50, dashed line for MT 100, and dotted line for MT 500.

In Eq. (8), r_r is the radius of the rod representing a polymer chain and L is the rod concentration defined as their total length per unit volume. The Ogston model provided a very good fit of our experimental results (Fig. 4) and the values of estimated parameters of Eq. (8) are given in Table 2. When the change of the parameters with the polymer content was assessed, only L exhibited a meaningful dependence. The rod concentration increased with the polymer content as could be expected. The decrease of the rod radius, r_r, with the polymer content cannot however be substantiated. It is evident from the form of Eq. (8) that the value of r_r is always characterized by the interval of the solute radius where the transition from the fully accessibity to complete exclusion occurs. Therefore, r_r will be always higher for less dense gels which is an inherent problem of the Ogston model.

Interesting results were obtained when the accessibilities of the cellulose gels with a bound model protein ligand, BSA, were measured. Fig. 5 shows that BSA, having the hydrodynamic radius of 3.6 nm [38], almost completely blocked the micropores of MT 50. The molecules with the hydrodynamic diameter larger than 1 nm were almost completely excluded from the gel structure. This property of MT 50 was used in an investigation of equilibrium and kinetics of affinity sorption that was assumed to take place on the surface of the gel [39]. On the contrary, the mass of imbibed water in the macropores remained unchanged. Fig. 6 illustrates that the accessibility of MT 100 was little affected by binding BSA. This is understandable since more than 50% of the imbibed water was in macropores and the mean micropore size was

Table 2
Parameters of the Ogston model (Eq. (8)) with their 95% confidence intervals

	L/nm^{-2}	r_r/nm
MT 50	0.0209 ± 0.0035	2.719 ± 0.333
MT 100	0.0026 ± 0.0012	6.161 ± 2.159
MT 500	0.0017 ± 0.0004	5.908 ± 1.185

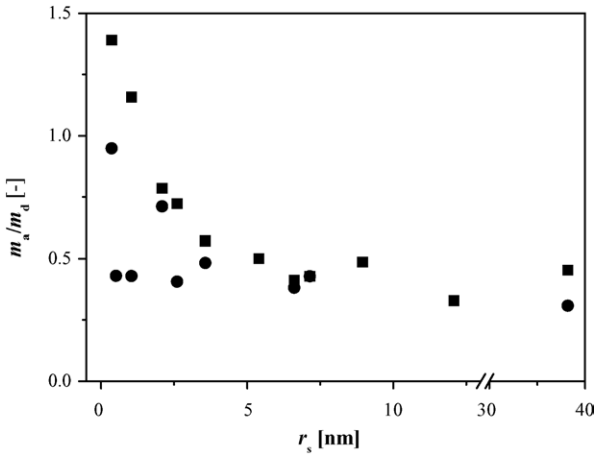

Fig. 5. Comparison of the accessibilities of Perloza MT 50 (■) and Perloza MT 50 with bound BSA (●).

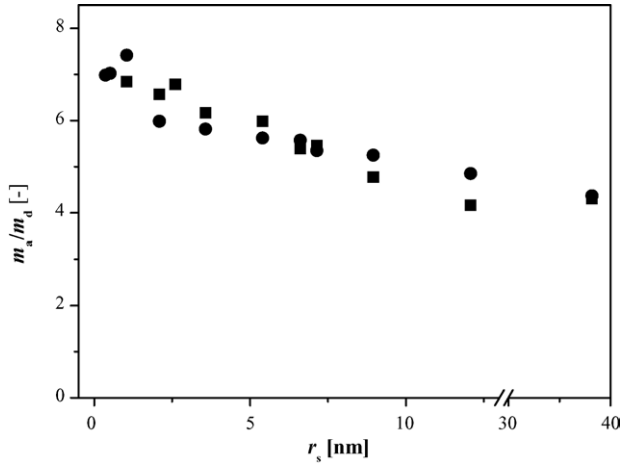

Fig. 6. Comparison of the accessibilities of Perloza MT 100 (■) and Perloza MT 100 with bound BSA (●).

several times larger than that of MT 50 (see Fig. 4). MT 100 and MT 500 thus provide a large accesible pore volume to biological macromolecules which is not significantly reduced by binding of macromolecular ligands. This makes them suitable for the use in the protein chromatographic, purification processes using different chemistry of functionalization of internal surface of gel particles including, especially affinity chromatography [5].

4. Conclusions

Three kinds of commercial cellulose gel with different content of polymer were investigated by evaluating the accessibility of the gels for a set of dextrans of varying molecular weight. A bimodal structure was observed in all examined gels with macropores for which the partition coefficient was equal to one, and micropores where the solute partitioning took place according to the size of a solute and pore size. The sizes of most micropores were below 10 nm. The apparent mean pore sizes were estimated between 3 and 5 nm. On the contrary, the sizes of the macropores could not be estimated by this measurement method but they had to be well above 50 nm. The cellulose gels with the different polymer content were found to differ mainly in the distribution of the total porosity between the micropore and macropore fractions. The fraction of macropores that was about 60% at the gel with the lowest polymer content, decreased to 30% of the total porosity at the gel with the highest content. The experiments with gels having bound protein showed that only more macroporous gels are suitable for further use in affinity chromatography as an enormous pore blockage occurred at the gel with a high polymer content.

5. Nomenclature

a, k	parameters of Eq. (1)
c_0, c_{eq}	solute concentration before and after contacting with gel (g/L)
d_s	solute diameter (nm)
K_{av}	particle partition coefficient
K_g	gel-phase partition coefficient
K_d	mean pore partition coefficient
L	concentration of the polymer rods in the gel (nm^{-2})
m_a	mass of accessible water (g)
m_d	mass of dry gel (g)
m_m	mass of macropore water (g)
M_r	relative molecular weight
m_s	mass of solution (g)
m_w	total mass of water in the gel pores (g)
r_r	radius of the polymer chain (nm)
r_s	solute radius (nm)
w_p	polymer fraction

Greek letters

ε_p	particle porosity
ε_g	micropore fraction
ε_m	macropore fraction

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