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Review

Regulation of cellulose-based adsorbent granule morphology

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Abstract

The hydrophilic nature, biocompatibility and absence of nonspecific adsorption render cellulose-based adsorbents an attractive chromatographic medium for the isolation and purification of biopreparations. The analysis of the formation methods of cellulose gels for liquid chromatography revealed potential possibilities to regulate the morphological structure of cellulose granules. New methods of the preparation of cellulose adsorbents including those which enable one to regulate granule morphology are developed and discussed in more detail. The chromatographic behaviour of adsorbents of different morphological structure is also analysed.

Keywords: Reviews; Adsorbents; Cellulose-based adsorbents; Stationary phases, LC; Size exclusion chromatography, inverse

Contents

1. Introduction

The results of the development of column liquid chromatography (CLC) for biocompounds are very dependent on the design of packing materials. The traditional evaluation of stationary phases for CLC, which illustrates the state of art in this field of research, is based on a classification of adsorbents

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according to the chemical origin of their matrix. As may be seen from recent results in the design of more sophisticated packing materials for perfusion CLC [1,2], the organisation of the porous space in the adsorbent's granule is of special importance.

The soft polysaccharide gels have had an outstanding role in applications for the separation of biopolymers. According to [1] approximately 90% of currently known proteins are chromatographically separated and isolated by the use of polysaccharide gels, especially cross-linked dextran and agarose bioadsorbents. Because of their mechanical and chemical stability, porous cellulose adsorbents have been increasingly used in the last decade for chromatographic separations of biopreparations [3].

The objective of this study is to understand the morphology of adsorbent granules and the possibilities of influencing it. The granule morphology should be understood as a higher organization level rather then the porous structure. Not an average pore, but the whole granule has to be taken into consideration. Following the gradation of the porous polymer structure into the molecular, fibrilar-globular and microstructure, the granule morphology is attributed to the microstructural level of the polymeric object. Systematic changes in the granule structure across the particle cross-section can be defined as a heterogeneous morphology. As a rule, heterogeneous morphology manifests itself by a denser outer layer and may be called a surface barrier. Usually, this feature is undesirable. However, sometimes heterogeneous morphology may be successfully used for extremely selective size-exclusion chromatography (SEC) separations and, in fact, extends the common concept of SEC.

The absence of a more detailed study in the field of regulation of the particle morphology poses a serious problem for the design of suitable adsorbents. This situation was caused in part by the absence of a simple and convenient method for the evaluation of the granule morphology. The use of sorbents not characterized concerning their microstructure, causes the difficulties observed in chromatographic applications. The chromatographic behaviour of an adsorbent will be more predictable, if the matrix morphology and/or its changes in the process of modification (especially in coating procedures) are taken into account. The application of heterogeneous morphology granules, with limited outer surface permeability, may cause a split peak effect in SEC mode and, as was shown in [4], restricted capacities and unfavourable kinetics of adsorption in other modes. Therefore, it is extremely important for obtaining an efficient resolution to choose appropriate adsorbents with defined morphology.

Here we analyse the factors which govern the morphological structure of cellulose bioadsorbents synthesized by the use of different methods, and means of regulation of the porous space, for granules formed from a solution of cellulose material.

2. The production of cellulose adsorbents

2.1. Fibre cellulose bioadsorbents

The first attempt to separate proteins by using cellulose ion-exchangers was made by Hoffpauir and Guthrie [5]. Wide application of cellulose-based bioadsorbents became popular after the successful separation of proteins on fibre aminocellulose by Peterson and Sober in 1954 [6]. The main advantages of cellulose adsorbents as well as of dextran and agarose based packing materials for CLC are the hydrophilic biocompatible matrix, minimal non-specific adsorption and a high recovery of the biocompound activity.

Fibre cellulose ion-exchangers were synthesised by chemical modification of native cellulose [7]. For this reason, the morphology of the primary cellulose filament, i.e., the inhomogeneous porous structure, is characteristic for the product. In order to activate the cellulose, it is mercerized with concentrated sodium alkaline solution. Products with a higher content of ionogenic groups have a higher capacity. However, further substitution causes gelatinization and, finally, dissolution of the product. Fibre cellulose should be cross-linked initially to stabilize the porous structure.

The inhomogeneous morphology of fibres, low exclusion limit, specific particle shape and broad distribution of size, poor quality of column packing, high hydrodynamic resistance and, finally, poor chromatographic resolution were the reasons why

new attempts to develop more advanced forms of cellulose adsorbents were made.

2.2. Cellulose powder

Cellulose powder is the next phase in the design of cellulose adsorbents. It may be obtained by mechanical disintegration (grinding), chemical treatment (acidic or alkaline hydrolysis, oxidation) and high energy emission treatment (γ radiolysis of cellulose [8]). Cellulose disintegrated by a grinding procedure has a lower crystallization index and a less orderly porous structure, but the morphology has the same attributes as native cellulose.

More effective concerning its chromatographic properties is powder cellulose manufactured by hydrolysis, or so called microcrystalline cellulose as introduced by Battista [9–11]. Among the other fields of application it is used for chromatographic separations. Hydrolysis is usually performed by boiling cellulose fibres for 15 min in diluted hydrochloric (2.5 *M*) or sulphuric acid (1.25 *M*). Since destructive treatment is directed to the amorphous part of the cellulose, and because crystalline segments remain practically unchanged, the product is called microcrystalline cellulose. It has a higher crystallisation index, uniform particles, an accessible homogeneous porous space and a higher rate of filtration, but a relatively low exclusion limit, that is comparable with characteristics of cross-linked dextran gels [12].

A distinct kind of microcrystalline cellulose is the microgranular cellulose. It was developed by Knight [13,14] and is of special practical importance. Ion-exchangers based on microgranular cellulose have been produced by Serva (Germany) and Whatman (England) up to the present. Microgranular cellulose is obtained by the acidic hydrolysis of cotton fibres, followed by a treatment with sodium hydroxide (up to 46%, w/w). Preswollen cellulose has a high porosity. The cross-linking of the product stabilizes the porous matrix of the ion-exchanger in various pH media. The cross-linked microgranular cellulose adsorbent is used without additional grinding. Therefore, the packing obtained with this method has better hydrodynamic properties and a sufficiently high chromatographic resolution. The produced particles are morphologically homogeneous. Therefore, this method will not be further discussed in this paper.

2.3. The formation of adsorbents from a cellulose polymer solution

Formation from solutions is the third stage in the history of the designing of cellulose-based adsorbents. It is the most advanced, since it provides the possibility to build up directly the porous structure of granules. This method is completely different from the previous two methods, in which the native structure of fibre cellulose was not changed or just changed slightly.

Theoretically, the polymer structure formation from a solution should be suitable for the manufacture of cellulose adsorbents as well as for other kind of polymeric matrices. However, some peculiarities are characteristic for cellulose if compared with other polysaccharides, such as dextran or agarose. Cellulose is not soluble in water as well as in common organic solvents. That complicates the cellulose gel formation on one hand, but provides stability under chromatographic use conditions on the other hand. In terms of technology, cellulose beads cannot be formed by the simple gelation of a polymer solution in a classical water–oil emulsion. Cellulose derivatives or complex solvents of cellulose are taken in order to obtain solutions of cellulose material. Because of the complexity of systems used for the formation of cellulose gels, the analysis and classification of the numerous methods that have been developed is not a simple task. First of all, the analysis requires choosing a criterion according to which procedures should be classified. The task can be simplified if the driving force of the gelation process is taken as the decision criterion.

Common procedures of cellulose adsorbent synthesis consists of these consecutive stages:

- emulgation of a solution of cellulose material,
- gelation,
- washing, fractionation,
- chemical modification.

The gelation process in the droplets of the emul-

gated cellulose solution has a crucial influence on the structure of the formed granule. In accordance with this principle, gel formation processes may be divided into two groups:

- heterogeneous processes, in which gel formation is caused by mass transfer from one phase of the emulsion to the second,
- homogeneous processes, in which gelation of cellulose materials is not the result of mass transfer between the two phases.

Depending on the group of formation processes, a heterogeneous or a homogeneous granule should be expected.

There was no convenient method for the investigation of the morphology of soft gels until the inverse SEC (ISEC) was proposed for the evaluation of the granule microstructure [15]. It should be mentioned that the idea of using chromatography for investigation of the porous structures was intensively developed since the 1970s. The fundamentals of the theory and practice of ISEC were extensively reviewed by Gorbunov et al. [16]. The ISEC methods, including the classical ISEC method proposed by Knox and Scott [17], have had a focus to characterize the porous structure of the SEC packing material. The new possibilities of ISEC were found later, when different granule morphologies were investigated [15]. The ISEC method is based on the analysis of elution profiles of polymer standards and the calibration graphs of adsorbents. ISEC is applicable if there is no adsorption interaction between the packing material and the polymeric standard used and if the polymer retention process is entropy controlled. The other porosimetric methods (gas adsorption, mercury porosimetry, electron microscopy) require drying of the wet gel. This procedure affects the structure of the granules dramatically. Therefore, the interpretation of the results obtained is complicated. These circumstances had prevented the evaluation and regulation of the morphological structure of granules before the introduction of ISEC, as suitable method for the characterization of granule microstructure.

Determan et al. [18] reported the narrowing of pores on the granule's surface caused by drastic formation conditions of the granules. Others only presented average characteristics such as porosity, water retention or the specific volume in the column. However, even these characteristics together with the exclusion limit give information about the heterogeneity of the granule morphology. A high porosity and a low exclusion limit at the same time reflect a heterogeneous structure of gel particle. The granules can have a denser outer layer than the inner core. This structure is also called closed microstructure [15].

The processes resulting in the gelation of cellulose material, caused by mass-transfer between the two phases of an emulsion, can be classified into three groups:

(i) gelation is caused by the evaporation of the solvent of cellulose material.
(ii) the polymer structure is formed by adding to the emulsion a solvent which precipitates the cellulose material.
(iii) the polymer is gelated by the addition to the emulsion a chemical agent, which reacts either with the solvent or the cellulose material.

Table 1 gives a survey of methods representing these three groups.

(i) The evaporation of the solvent by heating an emulgated solution of cellulose derivative (for instance acetylcellulose in dichloromethane or in a mixture of dichloromethane, methanol and acetone [19]) results in the concentration of the solution and, finally, solidification of polymeric spheres. The next step is the chemical treatment of granules producing regenerated cellulose. One direction mass-transfer of the solvent from a polymer solution into the dispersion medium increases the viscosity of the polymer solution up to a threshold value, at which fluidity disappears. Consequently, produced granules have a low porosity. However, such an explanation does not reflect the dependence of the density of the final product on the concentration in the initial solution, which was observed [26]. Probably, the removal of solvent from the surface of the emulgated droplet causes a gradient of viscosity and concentration, resulting in differences in the porous structure between the outer and inner layer of the sphere. Experimental results corroborate the predicted inhomogeneity of the particle morphology: although granules formed from cellulose material solution of a lower concentration have a higher porosity, they have practically the same exclusion limit.

To obtain a more porous product, the evaporation

Table 1
Survey of cellulose gel formation in emulsions

Group of methods	Characteristic result of gel formation	Reference
Evaporation of the solvent	High density/low porosity	[19–26]
Addition of a precipitator	Medium/high porous structure which is dependent on the solubility of the cellulose derivative in the added precipitator and the dynamics of its addition	[27–34]
Addition of a chemical agent	High porosity	[18, 35–45]

method can be modified by adding a porogenic material to the initial solution of the cellulose derivative [24]. After the removal of the porogene, the granule will have an additional pore volume.

(ii) Precipitation methods are based on the decrease of the solubility of the cellulose material in the employed solvent by pouring the solution of the polymer into the precipitation bath or by adding a precipitator to the prepared emulsion. The formation of the porous structure depends on the precipitation conditions, i.e., by how much the concentration of the precipitator exceeds the limit at which the solid-phase of the polymer appears in the system of the polymer–solvent–precipitator. The regulation of gel formation conditions is possible (a) by using a diluted precipitator or (b) by controlling the concentration of the precipitator in the emulsion via controlled addition. With the two methods, the diffusive character of the precipitator transport through the interphase surface into the inner part of emulgated droplet results in differences in the structure across the granule. Therefore, a more or less expressed heterogeneous morphology of the granules should be expected.

Interesting products have been described in the patent of Fetisov [28]. A solution of diacetylcellulose was pulverized directly into a pure precipitator. Spongy granules with cavities of 10^4–10^5 nm were obtained. The result may be explained by the intensity of the mass-exchange between the phases of the emulsion, which causes fluctuations of concentration, thermal effects of molecular interaction and local changes in the surface tension. As the result, convection and vortices appear spontaneously. Large anisometric cavities are formed under these conditions.

(iii) In these procedures, which are based on the reaction of an added chemical agent with the cellulose material, or the solvent of the cellulose material, mass-transfer between the two phases of the emulsion occurs. Therefore, the organization of the morphological structure of the granules is due to the combination of heterogeneous processes affecting the gelation of the polymer material, as in the case of precipitation methods. In each method, the build up of the porous granule depends on the synthesis conditions. Employing chemical agents with reduced reaction velocity, the reducing of the concentration of the chemical reagent and the controlled addition of the reagent may be used to obtain milder conditions for the formation of the gel and a better homogeneity of the granule microstructure. Another characteristic feature of this group of methods is the predominating mass transfer direction into the emulgated phase of the cellulose material solution. It results in a high porosity of the product. For instance, the average porosity of synthesized spheres is up to 96% (v/v) [43] and exclusion limits for dextrans up to M_r 10^7 [18,35,44,45].

2.4. Homogeneous gelation

Homogeneous gelation methods essentially differ from the above described ones. The solidification of the polymeric material occurs practically without mass transfer between the two phases of the emulsion. These methods are not very numerous and as a rule are based on thermal treatment. In most of these methods sodium cellulose xanthate is used as the cellulose derivative [46–50], that is dissolved. After thermal treatment and partial saponification of the

xanthate, the solubility of the polymer material changes and the gelation of spheres occurs.

Another cellulose gel formation method was proposed by Kuga [51,52]. It is based on the cooling of the cellulose solution, which is prepared by the heating of low molecular mass microcrystalline cellulose in an aqueous solution of calcium rhodanide. Granules can be obtained in two ways: by grinding cellulose gel block after cooling the solution or by forming spheres by emulgation of a solution in hot *o*-dichloroethane and subsequent pouring of the emulsion into cold methanol. According to the evaluation of the formed gel by inverse SEC, the cellulose spheres have a heterogeneous microstructure in contrast to the ground cellulose granules. Ground cellulose granules are homogeneous and, in fact, show a better chromatographic resolution. This phenomenon may be explained by possible interphase mass transfer in the dispersion system, when emulsion is mixed with cold methanol.

3. The regulation of the granule morphology

Summarizing the analysis of data on the synthesis of cellulose adsorbents, it can be said, that the build-up of the porous structure has a tendency to form highly porous uniform particles of the cellulose adsorbent. The regulation of porous structure is limited by the changing of the formation conditions in order to produce granules of different porosity and exclusion limits. Up to the present there has been no systematic analysis of the formation the granule microstructure.

3.1. Heterogeneous morphology

Granules with a restricted permeability of the particle surface Granocel-4, -8, -14, have been developed in our laboratory [53]. The manufacturing procedures are based on the saponification of diacetylcellulose (DAC), emulgating a solution of the DAC in acetone (Phase I) in an alkaline water–glycerine medium (Phase II). The characteristics of the average porosity, such as water retention, the specific volume in the column and the porosity of the granule are governed mainly by the concentration of DAC in Phase I and by the concentration of the saponification agent, sodium hydroxide, in Phase II. Products of a higher porosity and exclusion limit have been formed from more diluted DAC solutions at a higher concentration of sodium hydroxide in the dispersion medium.

Since this method is based on a heterogeneous formation process of polymeric granules, a product with heterogeneous morphology is obtained, i.e., the granule has a so-called closed porous structure with a denser surface layer. In a previous article [15] chromatographic properties of Granocel-8 have been discussed. The SEC process on Granocel-8 was evaluated as a non-equilibrium process. The non-equilibrium state results in a split peak phenomenon for a single analyte under common SEC conditions. By the inverse SEC method and by electron-microscopic studies, the inhomogeneity of the granule microstructure has been confirmed.

For the investigation of the mass transfer in the dispersion system, a model emulsion without DAC was formed [54]. After equilibration, Phase I was analysed. As is evident from the data presented in Table 2, the volume of Phase I decreases and the volume fraction of acetone in this phase is a reciprocal function of the volume fraction of water in the dispersion medium (Phase II) and is directly proportional to the sodium hydroxide concentration in Phase II. The concentration of sodium hydroxide in Phase I is dependent on the volume fraction of water in the dispersion medium. The mass-transfer from Phase I into Phase II predominates. In a real system the contraction of Phase I manifests itself in a decrease of the volume of the product, especially, if the concentration of sodium hydroxide is low and the saponification of DAC is slower. On the other hand, mass transfer in the opposite direction (from Phase II, that is not a solvent for DAC, into Phase I) causes precipitation at the outer layer of the emulgated droplet in drastic conditions. Therefore, milder conditions for gelation may be obtained at higher concentrations of sodium hydroxide. For instance, granular cellulose Granocel MB-8 is formed at a higher concentration of sodium hydroxide in the dispersion medium if compared to the Granocel-8 manufacturing process (other conditions are similar). This product (Granocel MB-8) is characterized as a

Table 2
Composition of model system phases

Initial composition of the water–glycerine phase (Phase II)		Characterization of the acetone phase (Phase I) after the equilibrium is reached		
Glycerine–water(v/v)	Sodium hydroxide (g/l)	ΔV[a]	Volume fraction of acetone	Sodium hydroxide (g/l)
1:0.64	20	53%	86%	0.40
1:0.38	20	32%	91%	0.08
1:0.21	35	20%	93%	0.03
1:0.64	40	21%	86%	0.25
1:0.38	40	17%	91%	0.08
1:0.21	70	12%	93%	0.03

Initial phase ratio Phase II/Phase I (v/v)=1.35.
[a] ΔV=volume contraction of Phase I (1−volume at equilibrium/initial volume)×100.

membranous packing material which separates polymers in the SEC mode into two groups [15]. In addition, the chromatographic process takes place under equilibrium conditions. In terms of morphology, the granule of the Granocel MB-8 adsorbent has a surface barrier. In contrast to Granocel-8, the denser film on the Granocel MB-8 granule is permeable enough to ensure an equilibrium of the chromatographic process. In terms of chromatographic use, membranous packing Granocel MB-8 is of special interest, because it fractionates injected analytes into two groups that correspond to chromatographic zones that can be attributed to the dead (V_0) and the total (V_t) volume of the column. The membranous adsorbent Granocel MB-8 shows a resolution (R_s) of 1.82 for analytes with small differences in their molecular mass (Dextran fractions of weight-average molecular mass M_w 63 000 and 158 000) in contrast to 0.33 for Toyopearl HW-55F {in accordance with inverse SEC [16] Toyopearl HW-55F has a mean pore diameter (D_s) of 11.3 nm and a polydispersity of pores (U) of 2.15}. This observation shows, that not in every case are heterogeneous granules less effective than homogeneous granules. As shown in Fig. 1, a membranous adsorbent might exhibit an extremely high selectivity. According to the theory of SEC, the best selectivity is characteristic for adsorbent granules of homogeneous morphology containing pores of uniform diameter. The fractionation range of macromolecules in such a case is very narrow, i.e., selectivity of fractionation is highest, and the range width is dependent exclusively on the mobile phase used and the characteristics of the analyte. For

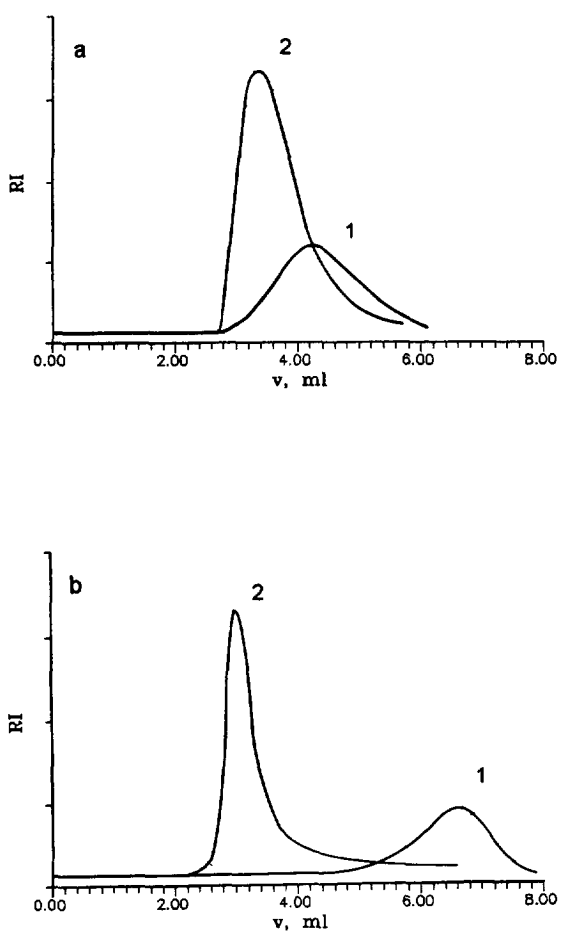


Fig. 1. Comparison of the resolution of two standard dextrans with M_w: (1) 63 000 and (2) 158 000 on (a) conventional gel Toyopearl HW-55F and (b) membranous Granocel MB-8 [15]. Column 250×6 mm I.D.; flow-rate=0.1 ml/min; detector: refractometer; eluent: distilled water.

instance, in the case of dextrans in water, the fractionation range of the standard dextrans corresponds to 1.46 orders of magnitude of molecular mass. As mentioned above, it is possible to separate dextrans, differing in their molecular mass only in 0.4 orders of magnitude, on membranous granules with maximal selectivity (peaks correspond to chromatographic zones of total exclusion and total permeation). In addition, the separation limit for membranous packing is adjustable. These advantages distinguish membranous packings from other SEC gels as a morphologically defined group that can be employed for very selective separations.

The survey of the literature concerning the application of heterogeneous morphology cellulose granules shows that often adsorbents of unregulated or indefinite morphology have been used for the chromatographic separations investigated. The absence of the control of the microstructure in characterization of the heterogeneous granules poses additional problems if compared to the homogeneous morphology packings, because of restricted thermodynamic characteristics and the non-equilibrium of the chromatographic process involved. As it can be seen in Fig. 2 kinetics of protein adsorption on the open, morphologically homogeneous anion-exchanger DEAE Granocel-500 differ essentially from kinetics shown by the morphologically heterogeneous anion-exchanger DEAE Granocel-8 of closed porous structure. The kinetics of the adsorption on a homogeneous morphology anion-exchanger is very rapid. On DEAE Granocel-8 kinetics is much slower, although the mean pore diameters of matrices are very close. Mean pore diameter (D_s) for ground Granocel-8 is 27 nm, for Granocel-500 28 nm. The polydispersity of pores (U) is even higher for ground Granocel-8 (U is equal to 1.1 and 2.1 for Granocel-500 and ground Granocel-8 correspondingly).

The higher permeability of the granule surface of Granocel MB-8 is caused by milder formation conditions. Similar products were obtained when methyl ethyl ketone was employed as solvent for Phase I [55]. Milder conditions for the gel formation in this case were obtained because of a lower methyl ethyl ketone transfer into the dispersion medium compared to the mass-transfer of acetone into the dispersion medium. The formation of the denser surface, compared to the core of the granule, can be attributed to precipitation.

3.2. Homogeneous morphology

In order to form morphologically homogeneous granules, the precipitation of cellulose material at the boundary of two phases of emulsion should be eliminated.

One possibility in order to modify the dispersion medium and to prevent precipitation of the cellulose polymer is to add acetone to Phase II. This approach is limited by the low solubility of acetone in the

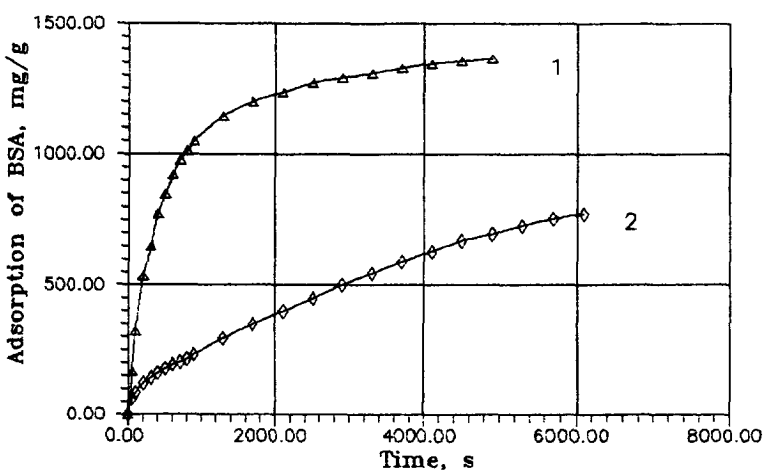


Fig. 2. Kinetics of adsorption of bovine serum albumin, fraction V (Merck) on different adsorbents: (1) DEAE Granocel-500 and (2) DEAE Granocel-8, fraction of the particles of both adsorbents 60–100 μm. Conditions of adsorption: 20°C, 0.5 mg/ml of protein in 40 ml Tris·HCl buffer (50 mM, pH 8.3).

dispersion medium (an aqueous solution of glycerine and sodium hydroxide). Therefore dimethyl sulfoxide (DMSO) and *N,N*-dimethylformamide (DMF) were chosen to prepare a range of different dispersion media which do not precipitate DAC and swells the final product, regenerated cellulose. It must be taken into account that the cellulose polymer is changed during the saponification, i.e., the chemical composition is not constant and depends on the rate of saponification. Therefore, the solubility of cellulose material is dependent on the reaction time.

Fig. 3 shows chromatograms of dextran standards obtained with packing materials prepared with emulsions that contain 20–30% DMF or DMSO in the dispersion medium. According to inverse SEC, the formed granules have a homogeneous morphology. The increase of the volume fraction of DMF or DMSO in the dispersion medium above 30% causes the collapse of the emulsion and results in the formation of one phase and the further solidification of the cellulose material as a block.

Another possibility for the synthesis of particles of

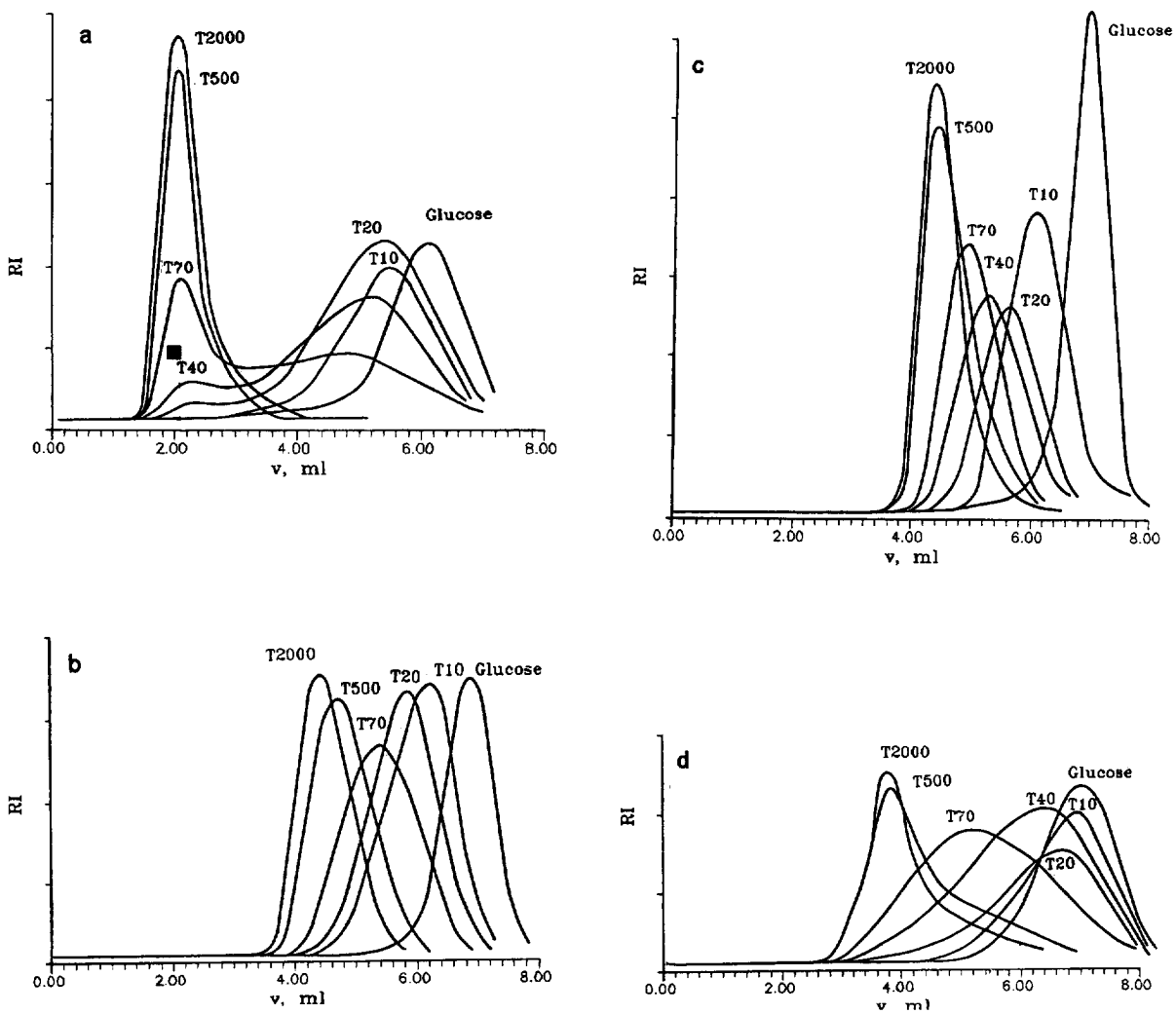


Fig. 3. Chromatograms of dextrans performed by use of modified and unmodified Granocel-8 packings. Packing materials are formed in (a) original dispersion medium [15] and with addition of (b) 20% DMF, (c) 30% DMF and (d) 20% DMSO. Column: 250×6 mm I.D.; flow-rate 0.1 ml/min; detector: refractometer; sample 0.3 ml, 2 mg/ml; eluent: distilled water, standard Dextrans as in [15].

an open morphological structure is the homogeneous formation process [56,57]. If a chemical agent, which causes the saponification and gelation of acetylcellulose is added to the cellulose polymer solution and the incubation period of gelation is used to form an emulsion in an inert dispersion medium (no mass transport from the polymer solution into the dispersion medium and vice versa), it can be expected that spheres of homogeneous morphology are obtained. The method may be simplified by the solidification of the solution of acetylcellulose in a gel block. A required fraction of morphologically homogeneous granules may be obtained by disintegration of the gel block and subsequent fractionation of the ground granules. The homogeneous formation process is very flexible and permits tuning of the mean pore diameter. Fig. 4 shows the dependence of permeation and exclusion limits for dextran standards of the formed gel on the concentration of the saponification agent and on the concentration of the DAC in the polymeric solution. All of the products obtained by the homogeneous formation method have a homogeneous morphology.

Table 3 presents the variety of used cellulose regeneration agents [57]. The products differ in their average pore diameter as well as in their pore size distribution, but not in the morphology. These gel-like porous structure granules [particles with mean pore size polydispersity (U) equal to unit] are of excellent selectivity in the SEC mode.

Macroporous products with broader pore size distribution and higher exclusion limit may be used as efficient matrices for other modes of chromatography of macromolecular biopreparations: ion-ex-

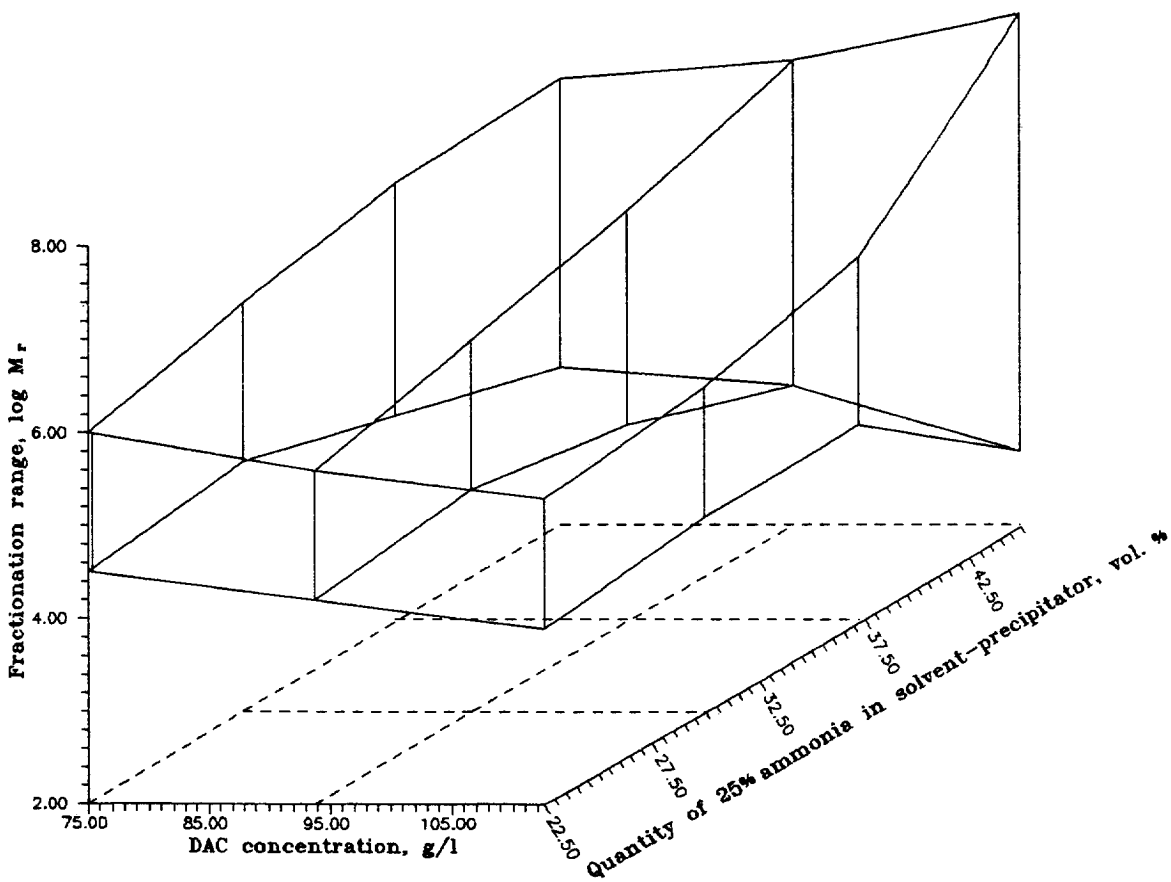


Fig. 4. Permeation and exclusion limits of homogeneous morphology cellulose gels Granocel-CD synthesized at different conditions [57].

Table 3
The homogeneous formation of cellulose gel and porosimetric characteristics of the product [57]

N	Conditions of gel formation				Characteristics of the gel				
	Concentration of acetylcellulose (g/l)	Composition of solvent–chemical precipitator			Specific pore surface area (m^2/ml of pore volume)	Mean pore diameter (nm)	Pore size polydispersity parameter (U)	Fractionation range, expressed by diameter of macromolecules (nm)	Selectivity in SEC mode (%)
		Solvent	Chemical precipitator	Volume ratio (chemical precipitator–solvent)					
1	53 Serikose[a]	81.7% acetone in water	50% ethylendiamine in water	25.0:75.0	46	43	1.0	8.5–45.0	100
2	44 Serikose[a]	81.7% acetonein water	50% ethylendiamine in	37.5:62.5	50	40	1.5	6.0–72.0	65
3	63 Serikose[a]	63.8% acetone in water	triethanolamine	20.0:80.0	28	70	1.1	12.0–82.0	90
4	44 Serikose[a]	81.7% acetone in water	25% diethylamine in water	37.5:62.5	36	55	9.0	7.0–540.0	38
5	44 Serikose[a]	acetic acid	8% hydrochloric acid	37.5:62.5	30	67	1.0	13.0–70.0	100
6	44 Serikose[a]	81.7% acetone in water	8% hydrochloric acid	37.5:62.5	210	9	33.0	1.4–260.0	32
7	44 Serikose[a]	DMF	1,3% sodium hydroxide	37.5:62.5	42	47	1.2	7.2–67.0	75
8	123 DAC[b]	DMF	25% ammonia	32.1:67.9	33	60	1.5	8.4–100.0	66

[a] Serikose is acetylcellulose that contains 44.2% of bound acetic acid.
[b] DAC=55.2% of bound acetic acid.

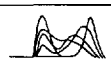
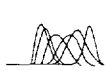
change and affinity chromatography. Even products with pores of diameter that can be considered to be perfusive can be synthesized (Table 3, sample N4). The pores are accessible to analytes of a size up to 540 nm. However, further investigations have to be made, whether these pores are throughpores (pores which transect particles).

3.3. The controlled morphology cellulose granules

As was shown in a previous section, the morphological structure of the adsorbent granule affects the thermodynamics of the chromatographic retention process in the SEC mode (entropy-controlled process) and in the ion-exchange mode (enthalpy-controlled process). In order to predict the results of the chromatographic separation, the morphology of the adsorbent granule should be evaluated. Usually, the qualitative definition of the morphology (homogeneous/heterogeneous) is sufficient to predict the chromatographic behaviour of the adsorbent. Controlled morphology granules can be obtained by the regulation of the process of the cellulose gel formation or by the evaluation of the morphology of the produced granules. The regulation of granule morphology by proposed methods of cellulose gel formation from the acetylcellulose solution is summarized in Table 4. A homogeneous formation process always produces granules of homogeneous morphology. A heterogeneous formation process, depending on the affinity of the dispersion medium to the gelating polymer of the cellulose, can produce both heterogeneous and homogeneous morphology granules.

Several cellulose matrices of defined porous structure and controlled morphology have been used for synthesis of bioadsorbents by Fermentas (Vilnius, Lithuania). The flexibility of the developed methods

Table 4
The influence of the formation process on the particle morphology

Formation process		Product	
Mode	Conditions resulting in polymer solidification	Characteristic chromatographic behavior	Microstructure
Heterogeneous	Two side mass transfer between the two phases of the emulsion.		Heterogeneous beads 
	Elimination of the solvent transfer into another phase by the presaturation of this phase.		Heterogeneous beads with higher porosity and better permeability of the outer layer (so called membranous adsorbents)
	Increase of the chemical agent concentration, which causes faster gelation of polymer.		
	Addition of a solvent to the dispersion medium in order to obtain good affinity to the polymeric material.		Homogeneous beads 
	Collapse of the emulsion by an increase of the solvent concentration in dispersion medium. Disintegration of solidified polymer block by grinding.		Homogeneous granules 
Homogeneous	The formation of granules in an emulsion. The two phases are inert with respect to mass transfer processes.		Homogeneous beads 
	The formation of a gel as a block and grinding of the product		Homogeneous granules

for the regulation of the porous and the morphological structure permits the optimization of adsorbents. Those materials have been used for the chromatographic separation and purification of biopolymers on a preparative scale [58].

4. Conclusions

The adsorbents based on cellulose fibres or on cellulose powder have a native morphology that is not or is slightly affected by adsorbent manufacturing procedure.

The morphology of granules, formed from a cellulose polymer solution, depends on the mass-transfer processes in the formation system. The morphology can be regulated by affecting the interphase mass-transfer or by excluding it.

Cellulose adsorbents of controlled morphology were obtained by regulating parameters of the formation process. Cellulose granules of defined morphology have a predictable chromatographic behaviour.

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