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Jiří Štamberg[ab]
[a] Institute of Macromolecular Chemistry, Czechoslovak Academy of Sciences, Prague, Czechoslovakia [b] U Petřin 1859/5, Prague, Czechoslovakia

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BEAD CELLULOSE

Jiří Štamberg
Institute of Macromolecular Chemistry, Czechoslovak
Academy of Sciences, CS 162 06 Prague 6, Czechoslovakia*

ABSTRACT

A new polymeric structure has been developed which fills a blank in available hydrophilic supports for separation processes.

Bead cellulose is a pure regenerated cellulose which is prepared by a modified viscose procedure. It is characterized by a regular spherical shape of individual particles, controlled porosity, accessibility for high molecular weight substances, high deformation stability and adequate chemical reactivity.

Diverse uses of this new material are described, viz., physical supports, chromatographic materials, dried preparations and various derivatives with different functions like ion exchangers, metal chelating adsorbents, chemisorbents, affinity adsorbents, immobilized enzymes. Bead cellulose can also be coupled with various active substances giving composite systems.

INTRODUCTION

Various polymeric structures are recommended as carriers in various separation techniques. Most of them are synthetic polymers - which are more or less hydrophobic - but biopolymeric matrices are preferred in some cases, due to an explicitly hydrophilic nature.

* Present address: U Petřín 1859/5, CS 162 00 Prague 6, Czechoslovakia

These promote fast kinetics of interaction in aqueous media and show a better tolerance to the biochemical systems generally. Cellulose belongs to carriers of this type but, unfortunately, its physical structure is much inferior by comparison with synthetic polymeric materials.

A significant step ahead in the implementation of cellulose in the branch of effective polymeric matrices was made by the development of porous spherical cellulosic materials. Beaded cellulose was first prepared before 1951 by O'Neil, R.Reichardt, E.P.Reichardt[1] through dropping a cellulose solution into a coagulation bath. In this way rather big spherical particles resulted (ca 2.2 mm), which were not intended for the use as polymeric matrices for separations. At the end of the sixties more experiments were carried out by Determann, Wieland, Rehner, Meyer[2,3], and spherical cellulosic chromatographic materials were obtained. In mid-seventies a new original method for the preparation of spherical porous cellulose was discovered by Peška, Štamberg and Blače[4,5], based on the thermal solidification of viscose - an aqueous solution of cellulose xanthogenate. This method was developed soon to technical maturity and was called TSGT (Thermal-Sol-Gel-Transition) process. The product obtained - bead cellulose - is reprecipitated wood cellulose, i.e., regenerated cellulose of high purity. It is partly crystalline and therefore mechanically stable, insoluble in water and most solvents, so that it can be successfully used for applied interactions in aqueous and non-aqueous media. The regular spherical shape allows an easy handling, a regular arrangement of beds and optimal column flow characteristics. It is reasonably porous even for high molecular weight substances to permit their access to the inner surface of the matrix and to facilitate their diffusion through the mass. This bead cellulose was introduced to the public by several reviews[6-9] and has been described in numerous papers and patents loc.cit.

PREPARATION

Two different methods are used for the preparation of spherical cellulose[6,7]: (a) The ejection method gives the shape to the individual particles through dropping or extrusion. (b) The suspen-

sion process consists in mixing a cellulosic liquid phase with an immiscible inert liquid medium. In both cases the scheme of preparation includes the same steps.

1) Providing a solution of cellulose or cellulose derivative. Cellulose can be dissolved in an ammoniacal copper solution (Schweizer's solution), in Fe tartrate, cadoxen, or calcium thiocyanate aqueous solution.[10-13] Also, labile chemical derivatives can be used, from which cellulose can be easily regenerated, e.g. an aqueous solution of cellulose xanthate (viscose),[1,5.10,11,14-16] melt of cellulose acetate,[17] or a solution of cellulose acetate in organic solvents.[18,19] Solutions of stable cellulose derivatives are also used and corresponding spherical products are prepared, e.g. hydroxyethylcellulose or cellulose with ionic groups.[22-25]

2) Shaping the liquid phase into spherical objects. - Use of a correct dispersion medium with respect to the dispersed phase is of great importance. It has to be an inert and immiscible liquid, or a liquid which precipitates cellulose from the dispersed phase.[13] For aqueous cellulosic solutions, mostly aprotic organic solvents are used, like hydrocarbons or halogenated hydrocarbons;[10-12,16,20,22-24] exceptionally, methanol is recommended, due to its precipitating ability.[13] Sporadically, water or aqueous solutions are used as suspension media for cellulose derivatives, which can be dissolved in water immiscible organic solvents.[18] The size of liquid spherical objects is controlled by mixing parameters and by the addition of surface active compounds.[2,5]

3) Solidification of liquid droplets is the next step. It is mostly a sol/gel transition - i.e. a changeover of the cellulose phase from a liquid to a swollen solid. This has to be carried out so as to avoid deformation and sticking together to agglomerates. So far the precipitation of cellulose by salts and acids has been mostly used, similarly as it is practised in the production of fibers and foils.[1,10-12,14] Besides the chemically induced precipitation, an addition of poor solvent for cellulose is used (e.g. of methanol).[13] The melt of cellulose acetate is solidified by a decrease in temperature. Often the sol/gel transition in aqueous

cellulosic solutions is achieved by chemical cross-linking,[16,20-25] esp. by reacting cellulose hydroxyls with epichlorohydrin or similar compounds in an alkaline medium.

4) Regeneration of cellulose in solid beads - if need be - has to be completed without impairing the spherical shape and porous structure. It consists in a perfect decomposition of the cellulose complexes or in the splitting-off of all the residual labile side groups. Also, removal of all by-products and non-cellulosics by thorough washing is needed. Perfection of the regeneration process decides about the purity and hydrophilicity of the final structure.

5) Useful aftertreatments (drying, partial drying, solvent-exchange and drying, adjustment of porosity, introduction of special active component et al.) are occasionally used in the final step.[26-30]

Using a similar preparation scheme, diverse reaction conditions may be selected, and thus various spherical products are obtained. Though numerous procedures have been described, only few were brought to ripeness. The greatest attention has been paid to bead cellulose prepared from technical viscose by the TSGT-process (Thermal-Sol-Gel-Transition-process).[5] The product is regenerated cellulose of high purity, higly hydrophilic and therefore well tolerated through biosystems. It is partly crystalline (not cross-linked), which grants mechanical stability and perfect insolubility. Its physical microstructure recalls a macroreticular structure of synthetic polymers with free accessibility for high molecular weight substances. It has a regular spherical shape of individual particles. TSGT-bead cellulose is prepared by the dispersion method in a stirred reactor. The drops are solidified solely by an increase in temperature and the regeneration is completed by hydrolysis in an aqueous alkaline medium. The porous structure can be altered through various aftertreatments,[26] and most diverse active groups can be introduced into the structure by polymer-analogous transformations without impairing sphericity.[31]

A special feature of the TSGT-process consists mainly in the type of the sol/gel transition corresponding to requirements of the

suspension method: It occurs under stable hydrodynamic conditions, quickly, in the total volume of the particle. Owing to the presence of ionic side groups, cellulose xanthate is soluble in a dilute sodium hydroxide solution. This solution decomposes slowly even at room temperature under gel formation. But it was found that as the temperature rose from 25 to 95°C, the time of gelation was reduced from several days to few minutes.[32] Under these conditions, the spherical shape and the individuality of the drops can be easily preserved during solidification. If the gelation is induced through composition changes in the dispersion medium - e.g. if a precipitant is added - the hydrodynamic conditions are disturbed, the sol/gel transition starts at the interphase, and thus, an undesirable "skin effect" can develop.

The TSGT-method was already optimized with respect to the chemical composition of the dispersion medium, useful additives, and regeneration procedures. Now, it is in the stage of an industrial plant operation.

PROPERTIES

The freshly prepared bead cellulose (i.e. by TSGT-process) consists of pure regenerated cellulose which is swollen in water. It posses a high porous structure with macropores filled with water. The polymeric chains have been oriented through spontaneous processes and cross-linked by hydrogen bonds; the product is therefore insoluble in most solvents. It is heterogeneous consisting of crystallined and amorphous region. The physical microstructure of the product depends on its history, a first approximate picture is indicated by water regain values: Never dried bead cellulose contains c. 90 vol.% (c. 85 weight %) water.[26]

Chemical composition

The dry substance of bead cellulose consists of rather pure poly-1,4-beta D-glucopyranose. Non-cellulosics - like hemicelluloses, lipids, mineral substances etc. - are mostly removed by re-

precipitation of the cellulose polymer and by thorough washing of the spherical product. The purity of the so called regenerated cellulose is given by the content of residual sulphur (xanthate groups or their decomposition products), which is usually <0.03% S. The ash content of normal beads is represented by <0.15%; after this the product is rinsed with diluted mineral acids (e.g., 0.1 Mol HCl/l) and the ash value falls down to <0.01%. It is well known that cellulose undergoes oxidation, e.g. during treatments in strong alkaline media. As a result, carboxylic groups also appear in bead cellulose. Their content fluctuates at <0.02 mMol/g. The chemical characterization of bead cellulose includes the determination of molecular weight. The starting raw material (cellulose pulp) shows polymerization degree values 600-700, the regenerated spherical product, 300-500; similar relations are known for viscose rayon, silk, and foils.

Particle geometry and size

Bead cellulose consists of perfectly spherical particles. In examining the microscopical pictures, a standard deviation of c.1% from the circular projection was found, the beads were transparent or slightly turbid, and, infrequently, very few undesirable aggregates were observed.

A random distribution of particle sizes was found like that in other suspension processes; the position and width of the distribution curve depended on mixing conditions. Typical curves are shown in Fig. 1. Screen analysis is used for technical grain (0.2-1.2 mm), which is assigned to macro scale or industrial applications. The particle size of fine grain is evaluated by image analysis or by multichannel Coulter counter apparatus.[33]

Porous structure

The physical microstructure of spherical cellulose is characterized by the total volume of pores (the porosity value) and by the accessibility for interacting substances.

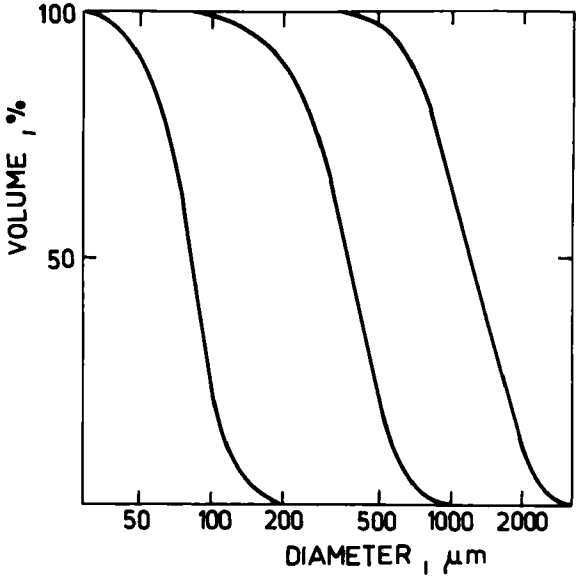


FIGURE 1

Particle size volume distribution curves of bead cellulose samples

The porosity values are expressed for swollen centrifuged samples in volume % or in the volume fraction of water. The initial porosity value of bead cellulose (c. 90 vol.%) can be altered by drying or by partical drying and reswelling. Values between 50 and 90% can be obtained if water is exchanged for an organic solvent with different polarity before drying.[26] (Table I)
Bed volume values (corresponding to 1 g of dry substance) are also used to indicate porosity; usually, values of 8-10 ml/g are found for undried cellulose. If much higher values (e.g. 11-13 ml/g) are found, an undesirable presence of agglomerates has to be assumed.

The accessibility for interacting substances was characterized by gel chromatography using linear dextranes or globular proteins as test substances; the results were expressed as a dependence of distribution coefficients on the molecular weight of test substances.

TABLE I

Modification of Porous Structure of Bead Cellulose by Solvent Exchange [26]

Solvents	Porosity, vol.%	
	Dry State	After Reswelling
$H_2O/DMSO/Et_2O$	71	77
$H_2O/Me_2CO/Et_2O$	61	74
$H_2O/MeOH/Me_2CO$	28	65
$H_2O/MeOH$	19	57
H_2O	0	56

DMSO - dimethylsulphoxide, Et_2O - diethyl ether, Me_2CO - acetone, MeOH - methanol

Bead cellulose (TSGT-method product) was found to contain accessible pores for substances with molecular weights of 10^3-0.5×10^6.[7-9] Spherical celluloses of another origin reached an even broader range of accessibility up to several million[34].

APPLICATIONS

Spherical cellulose does not yet belong to the materials with well known and specified uses. Still, even the first announcement about its availability[8] raised an extraordinary response in the field of separation systems and techniques. Up to now, more than 100 papers and more than 80 patents have reported the uses of spherical cellulose; they deal mostly with bead cellulose which is prepared by the TSGT-process.[6,7]

In most cases, bead cellulose can successfully substitute former cellulosic supports, but it can do much more, if its spherical shape, controlled porosity, mechanical stability, chemical reactivity are properly utilized; it is especially useful in large scale applications. Bead cellulose also inspired new discoveries:

beaded active carbon was prepared for special laboratory applications by the carbonization of bead cellulose.[35]

Physically active supports

Bead cellulose has proved to be a very effective means for simple phase separation; it can support liquids, so that they can be used as solids. The swollen undried bead cellulose is in fact "solidified water", as it contains 90 vol.% H_2O.

Bead cellulose as an efficient physical support for aqueous media has been successfully utilized in cryotheraphy, e.g. in the treatment of rheumatic disorders.[36] Bead cellulose was saturated with antifreeze liquids (concentrated salt solutions), placed in sealed plastic bags, cooled down to -17°C, and applied to the affected joint for 10-20 min. A very good shaping of the bag according to the uneven surface of the joint occurred due to an easy regrouping of the spherical filling, and heat was withdrawn slowly in agreement with the therapeutical need. The cooling of the treated joint had both analgetic and anti-inflammatory effects. This new technique has also been used in sport medicine, traumatology, neurology, orthopedics and other medicine branches.

Similarly, bead cellulose can also be used as a physical support for other liquids. Water may be substituted by any organic solvent using the solvent exchange method, e.g., in the series: water - ethanol - a non-polar solvent.[26] The final solvent can be an extraction agent which transforms the inert bead cellulose to an selective adsorbent.

Bead cellulose was also used as a physical support for high molecular weight substances, closing them in a polymeric cage. This was based on a discovery that a contraction of the polymeric skeleton of initially high porous bead cellulose occurred not only due to the removal of water, but also by decreasing the temperature. Urease in aqueous solution was filled in to swollen undried bead cellulose and then physically immobilized by deep colling (e.g. down to -20°C).[37]

Column packings

Water swollen bead cellulose has been used as an effective filtration medium for the mechanical separation of fine particles from drinking water or aqueous solutions. It has been successfully used as a scavenger filter before an ion-exchange or reverse osmosis installation. Similarly, fine particles were separated from the sodium chloride solution, which served as a medium in a Coulter counter apparatus for granulometric analysis.

Several papers have appeared, [38-41] referring to the application of spherical celluloses in liquid chromatography, in gel permeation chromatography or in the desalination of aqueous solutions of high molecular weight substances by the gel filtration method. The efficacy of cellulosic supports in these and similar applications depends on the type of porous structure. Different results have to be expected with spherical cellulose products of different origin. Thus, bead cellulose (prepared by the TSGT-process) is a macroporous support with a broad distribution of pore sizes, which is of advantage in many applications. But it cannot substitute quite different materials like cross-linked polysaccharides - cellulosics or dextrans - which possess a soft gel structure with a narrow distribution of pores. All structural features of various spherical celluloses have to be kept in mind, and right uses are to be found.

Dried preparations

Dry insoluble, but highly hydrophilic beaded polymers can be used as adsorbents for water. Also, some applications of this kind were suggested for bead cellulose, e.g., in medicine and cosmetics.

Polysaccharidic powders were used for treatments of suppurating and non-healing wounds. This application consists in covering the surface wound (e.g. ulcer) with hydrophilic powder, washing it after c. 12 hours and repeating this simple treatment during several weeks. The first preparation of this kind was made from cross-linked dextran ,[42] but properly dried bead cellulose showed the same effect though more economically.[27] The action of these preparations con-

sists in the separation of the pus, cleansing the wound, supporting the formation of new granulation at the base, and epithelialization of the scar. The defined particle dimensions and their spherical shape determine the properties of the preparations significantly. The powder particles must be >0.1 mm so as not to penetrate into the blood stream; their action is limited to the interphase boundary only. The beaded powders can be easily handled and simply separated from the wound - more effectively than powders of irregular particles. The treatment is much more efficient than the application of old methods. Cleansing of the wound was achieved after 2-4 weeks, even in those cases, which did not improve after months or years, if the old treatments were applied.[42,43]

Recently, an addition of spherical cellulose powders in cosmetic creams has been suggested. The reason was to improve the spreadability and adhesion to the skin.[44]

The dry bead cellulose powders are still waiting for more applications, esp. in cases which are not hampered by the decrease of porosity through drying. Peroral medical applications are expected, e.g. using bead cellulose as a support for minute quantities of medicaments.

Adaptability is one of the characteristic features of biopolymeric structures; their physical microstructure is mostly fixed by hydrogen bonds which can be cancelled or newly formed, according to external conditions. Also, bead cellulose is not bound through covalent crosslinking, and its porous structure can be altered by the abolition or formation of hydrogen bridges, i.e. by partial dissolution, swelling in good solvent systems, or by drying under proper conditions (Table 1). If it is dried from non-polar solvents in the absence of water, a true porous product results with 80% free accessible pores in the dry state (c. 300 m^2/g specific inner surface area by mercury porosimetry or by the BET adsorption method).[7]

Mechanical stability

Bead cellulose shows a relatively high mechanical strength and different behaviour in comparison with synthetic polymeric supports.

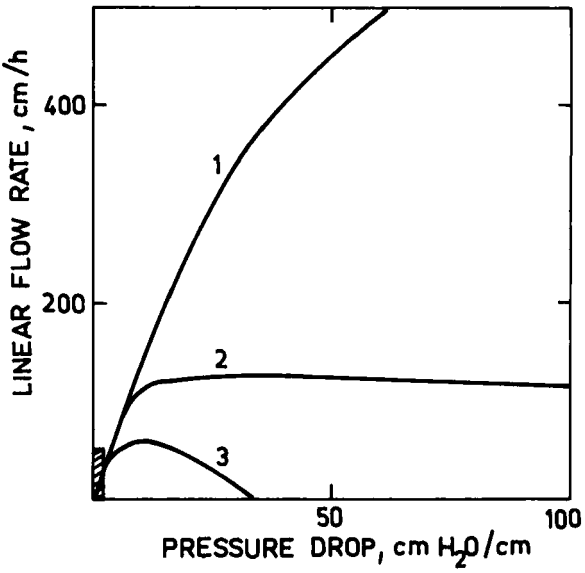


FIGURE 2

Flow characteristics, 1 bead cellulose, 40-100 µm, bed vol. 8 ml/g; 2 dextran gel, 40-130 µm, bed vol. 9 ml/g; 3 dextran gel, 40-130 µm, bed vol. 16 ml/g

It is not brittle, and therefore perfectly stable against attrition, in contrast to swollen ion-exchange resins and many other synthetic supports. This was demonstrated in a usual test by stirring a sample in an aqueous suspension with foreign hard objects like glass beads. Osmotic shocks - which destroy synthetic ion exchangers - do not impair bead cellulose and its derivatives, so that the latter withstand variations of ionic strength or pH in treated media very well.

The deformation stability appears to be more critical for polysaccharide supports. The penetration moduli for the individual particles of bead cellulose are much higher than for dextrane gels and also higher than for acrylic carboxyl cation exchangers in the sodium form.[8]

High mechanical stability enables bead cellulose be used in applications in big columns and filters, in contrast to other bio-

polymeric supports, like spherical dextrans. Pressure losses in the filter bed were measured, and it was found that flow through bead cellulose remained unrestrained even under conditions where comparable dextran beds were blocked (Fig. 2)

Beaded ion-exchange celluloses

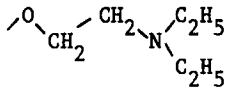
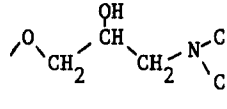
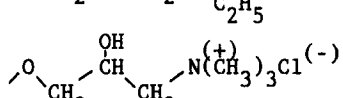
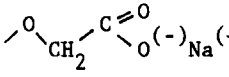
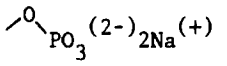
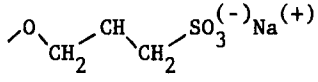
The old ion-exchange celluloses were introduced by the pioneer work of Peterson and Sober.[45] They prepared the most ion-exchange derivatives from fibrous native cellulose and demonstrated their effective use in biochemistry. Their hydrophilic structure - which implied a low non-specific adsorption in biochemical systems - was the main reason for the substitution of synthetic ion-exchange resins with a more or less hydrophobic skeleton. But beaded ion-exchange celluloses offer more advantages. They can have a controlled physical macro- and microstructure, they can be used equally in laboratory columns and industrial filters, i.e. not only for analysis and micropreparation, but also for macropreparation and a large-scale production of biochemical products. They are useful in biochemistry and biotechnology, primarily, but they can be used in technical ion-exchange processes, generally, if a high ion-exchange capacity is not needed, while fast kinetics are much more appreciated.

Spherical ion-exchange celluloses were described by Determann, Meyer, Wieland as the first.[2,3] Bead cellulose (prepared by the TSGT process) was derivatized to ion-exchangers later.[31] All main types of ion-exchange bead cellulose are now available: materials with diethylaminoethyl (DEAE) or diethylaminohydroxypropyl (DEAHP) weak base groups, trimethylammoniohydroxypropyl (TMAHP) strong base groups, carboxymethyl (CM) or phosphate (P) weak acid groups and sulphohydroxypropyl (SHP) strong acid groups. Basic data characterizing these products are given in Table II.

Weak base and strong base ion exchange celluloses can be used in the isolation of proteins from blood plasma.[46] Another applications of ion exchange bead cellulose were also studied: isolation of endodeoxyribonuclease on the P derivative,[47] immobilization of

TABLE II

Ion Exchange Derivatives of Bead Cellulose

Type	Signature	Exchange Group	Water Regain,[a] gH_2O/g	Exchange[b] Capacity, mmol/g
weak base	DEAE		2.6-3.5	1.1
weak base	DEAHP		3.5-4.8	0.7
strong base	TMAHP		3.5-4.8	0.7
weak acid	CM		2.6-3.3	1.6
weak acid	P		2.7-4.8	0.2
strong acid	SHP		4.0-5.7	0.5

a - related to the centrifuged state, b - titration capacity (dry weight)

invertase on a DEAHP derivative,[48,49] and immobilization of growing cells on ion exchange derivatives of various type.[50] The main applications of these materials are in biochemistry, but they were also examined in other branches. Weak base and strong acid derivatives stirred up interest in water treatment because of the fast exchange kinetics and a reversible uptake of organic substances.[51,52] Certain possibilities also appeared in the separation of uranium isotopes by DEAHP and P derivatives.[53,54]

Several authors have described beaded cellulose ion exchangers of another origin.[55-57] Sephacel was used in the clinical analysis,[58] other DEAE derivatives were applied in the immobilization of enzymes.[59] Properties of Japanese products were also reported.[60]

The applications published up to now are the first examples of a much broader possible use. An inspiration for further exploitation can be aspired to - e.g., large-scale operations instead of analytical or micropreparative procedures.

Sorbents of heavy metals

A great number of organic reagents have been known which form complexes with ions of heavy metals selectively; through their immobilization on polymeric supports, selective and also specific adsorbents were obtained.[62] But at the same time serious problems arose. The ligands for binding metals are usually only weakly dissociated and so they do not expand sufficiently the synthetic polymeric structure at swelling, opening it to interactions with metal ions. There are two possible solutions of this task: The ligands have to be located on an external or inner surface of the polymeric support, or a definitely hydrophilic matrix has to be used, which alone causes swelling regardless of the presence of hydrophilizing dissociated groups. Porous bead cellulose is a support like this.

A number of adsorbents have been described,[63] based on bead cellulose, able to bind heavy metals, swelling sufficiently and possessing fast kinetics of sorption and desorption (Table III). As examples of possible applications, concentration of trace elements from surface waters[64] and separation of metals which inhibit the enzymatic activity of urease[65] can be mentioned.

Chemisorbents

Various groups with different binding abilities were fixed to bead cellulose. Some of them can be classified among chemisorbents and used mostly in biochemistry. Many of these structures were described by Gemeiner and his coworkers. (loc.cit.)

TABLE III

Sorbents Complexing Heavy Metals

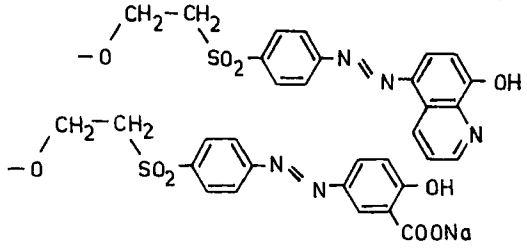
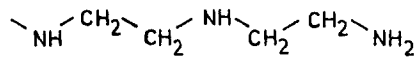
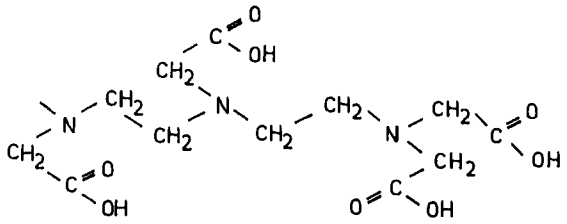
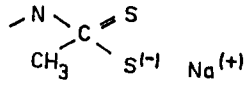
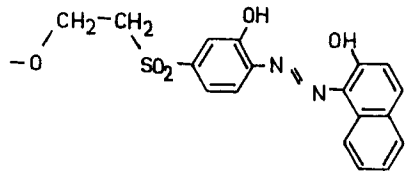
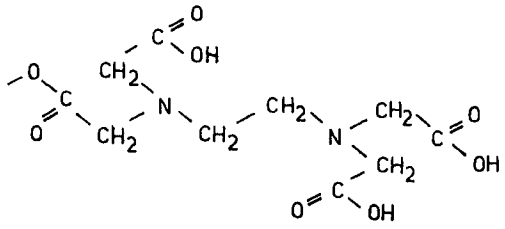
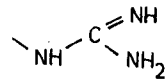
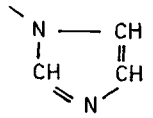
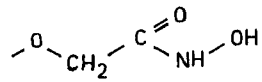
Active group	References
	66-70
	71
	72
	73
	67,74
	75

Table III - continuation

Active group	References
	76
	76
$-SH$	76-80
	81

Defined hydrophobic interactions can be utilized effectively for sorption. Cellulose allows the localization of defined hydrophobic binding sites on an inert and fully hydrophilic support. Thus, the absence of uncontrollable non-specific interactions is guaranteed and the necessary swelling is preserved. Attention was paid to phenyl derivatives of bead cellulose. Their preparations were described[82] and an isolation of calmoduline published.[83] An experimental method for the evaluation of hydrophobic sorbents was suggested.[84]

Adsorbents containing thiol groups were also prepared. At first chlorohydroxypropyl groups were introduced into bead cellulose,[85] chlorine was substituted by iodine during the second step,[86] and at last, the product was reacted with sodium thiosulfate.[87] Effectiveness of this chemisorbent was verified through the uptake of

cysteine and papain ; regeneration was possible with low molecular weight thiol compounds.[87] The use of bead cellulose thiol derivatives has been continued.[88]

Another example of bead cellulose chemisorbents is a derivative with benzaldehyde groups.[89] Attention was also paid to formyl derivatives of bead cellulose.[90,91]

The study of chemisorbents based on spherical cellulosics has been continued and in connection with it a mathematical treatment of sorption processes was suggested.[92,93]

Affinity sorbents

This type of sorbents can be considered as a special case of chemisorbents. Their binding functions are based on biospecific interactions, most of which are operative in bioaffinity chromatography - e.g. on interactions of enzymes with their inhibitors, of antigenes with antibodies, and the like. High specificity and easy loosening of the bonds ranks among the priorities of affinity sorbents.[94,95]

High porous bead cellulose is an effective support for affinity functions. Papers have been published where interactions between hemoproteins and imidazol derivatives of bead cellulose have been mentioned.[96,97] Other subjects of interest were interactions between immobilized DNA and enzym DNAse,[98] bead cellulose bound trypsin inhibitor and chymotrypsin,[99] immobilized aprotinine and kallikreins,[100,101] bead cellulose bound glucose and lectins.[102]

Dye ligand chromatography is one of the special affinity techniques.[103-105] Reactive dyes can be easily fixed on bead cellulose[106,107] and reasonably utilized. Remazol derivatives of bead cellulose have been prepared and used in the chromatography of lactate dehydrogenase,[108,109] for its isolation and purification,[110] and in the chromatography of yeast dehydrogenases.[111] Bead cellulose with dye ligands has also been used in the isolation of human serum albumin.[112]

Covalently immobilized enzymes

Polymer bound enzymes and the respective immobilization techniques have been described in numerous papers and reviewed in monographs.[113,114] The old type cellulose often appeared among popular polymeric matrices,[115] and beaded celluloses of different origin have also been described to this purpose;[55,56,116-119] the immobilization of whole cells with active enzymes to a beaded cellulose has been reported as well.[120-123]

Bead cellulose (i.e. the product which was prepared by TSGT process) joined to well-established matrices and evoked the interest of several research groups.[124,125] Various activation procedures were examined and many enzymes immobilized (Table IV). A special method was developed, comprising the glycosylation of proteins.[138] Bead cellulose is one of the few polymeric supports which are not covalently cross-linked, and therefore it can be dissolved in proper solvent systems. This fact was exploited for the determination of bound enzyme by direct photometry of cadoxen solutions of bead cellulose [139] with covalently bound lysozyme or trypsin. Close attention was paid to the immobilization of chymotrypsin, and the kinetics of this process were studied.[140]

An effective application of bead cellulose bound enzymes has been developed in medicine. It was found that the proteolytic action of an immobilized proteinase can be utilized for the separation of purulent matter, i.e. for cleansing non-healing suppurating wounds. A new healing powder was prepared through the immobilization of chymotrypsin on bead cellulose. It combines effectively an absorption effect with proteolyse.[27,141]

Composites

Bead cellulose is a hydrophilic high porous polymeric support that is rather inert and inactive. It can be used as a host matrix for various active components and increase their effectiveness. There are many insoluble inorganic substances which possess interesting sorption properties, but unfortunately also insufficient porosity and poor mechanical strength of individual particles, esp.

TABLE IV
Immobilized Enzymes

Activation	Enzyme	References
cyanogen bromide	hemoglobin	126
	trypsin	127
	hydroxysteroid dehydrogenase + diaphorase	139
periodate oxidation	trypsin	129,130
	chymotrypsin	27,130
	glucose oxidase	131,132
	invertase	133
benzoquinone	galactosidase	134-136
	chymotrypsin	137
4-nitrophenyl chloroformate	trypsin	127
N-hydroxysuccinimidyl chloroformate	trypsin	127
epichlorhydrin + diamine + glutaraldehyde	glucose oxidase	131
AE-cellulose + glutaraldehyde	glucose oxidase	131
CM-cellulose + carbodiimide	glucose oxidase	131

due to instability against attrition. A method for its precipitation in macropores of bead cellulose has been described, and similar technique can be used for other systems.[142]

Also, synthetic reactive polymers were introduced into macropores of bead cellulose. E.g., glycidylmethacrylate was fixed by grafting and reacted with sodium sulfite to a strong acid cation exchanger.[28] Through polymerization in situ poly(ethylene methycrylate toluenesulfonate) was filled in the macropores[29] and transformed to adsorbents chelating heavy metals by reactions with amines.[143] Urea or guanidine were polycondensated with formaldehyde inside bead cellulose, yielding sorbents for the separation of uranium isotopes.[144] Bead cellulose was also coupled with

mercurials, namely with mercurated phenol-formaldehyde resins that are effective selective adsorbents for thiols, halogenides, and other anions which form mercaptides.[30]

Composite systems can also be prepared through the addition of a second component to a cellulose xanthate solution before dispersion, i.e. during the preparation of beads. E.g., carboxymethylcellulose was added to hydrophilize the structure and to promote formation of large pores; this method was used in the production of healing powder with increased absorbability for the treatment of suppurating wounds.[43,145] Similarly, other substances were added before dispersion like novolac (as support for mercuration)[146] or sorbent powders, e.g. inorganic selective adsorbents,[142] synthetic ion exchangers,[147] or biosorbents.[148]

CONCLUSIONS

There are many separation systems based on interactions between solid macroparticles and a surrounding liquid. They are subject of an intensive development continuously. Close attention is paid to the perfection of the interacting solid phase, to its chemical and physical structure. Spherical celluloses with controlled porosity are objectives of these efforts.

Cellulose is a traditional material which is accessible from renewable sources in a relatively high chemical purity. It is highly hydrophilic and possesses sufficient chemical reactivity for the introduction of active groups. Now the physical structure has also been essentially improved according to the needs of separation systems. The conventional fibrous form was substituted by regular spheres with controlled porosity and accessibility for high molecular weight substances. Beaded celluloses of gel and macroreticular type have been prepared.

Bead cellulose ranks among supports with a broad application range. It can successfully substitute fibrous forms and inspire to new uses. Due to the excellent flow characteristics, bead cellulose is an etraordinary interesting material for large-scale applications. Numerous examples of diverse used have been cited above, namely, as

a physical support for active liquids or solids and as the starting material for the preparation of ion-exchange substances, metal chelating sorbents, chemisorbents, affinity sorbents, immobilized enzymes. The new support can be coupled with various active substances combining hydrophilicity, inertness and mechanical stability of the cellulose matrix, on the one hand, and useful activities of materials with poor practical qualities, on the other.

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