Methyl Methacrylate and Acrylamide Crosslinked Macroporous Copolymers

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ABSTRACT: In this work the synthesis of a crosslinked macroporous copolymer was effected from methyl methacrylate and acrylamide. The synthesis process began with emulsion prepolymerization, followed by sol–gel copolymerization until a hard block was obtained. Determination of the properties of the obtained material was carried out by FTIR, mercury porosimetry, and SEM microscopy. The material was characterized by a porous structure with open pores. The macroporous copolymer obtained can be used for polymer–analog reactions and the transformation of amide and ester groups into acyl azide groups. It can be used as a hard inert support for the immobilization of enzymes, or other proteins, by condensation of acyl azide group on polymer with the free amino group from the base amino acid of enzyme/protein. For the immobilization of microorganisms it can be used by vacuum diffusion of microorganism suspension into the porous structure, without active group transformation reactions. With microorganisms in the polymer pores, microorganism colonies form within the copolymer by microbial fermentation. © 2003 Wiley Periodicals, Inc. J Appl Polym Sci 91: 387–395, 2004

Key words: macroporous polymers; emulsion polymerization; crosslinking; carrier microorganism; enzyme immobilization

INTRODUCTION

The synthesis of macroporous polymers has always been the subject of great interest because materials with various applications are obtained in the process. Those with open pores can be used as initial substances for different types of ion-exchange resins, adsorbents, inert catalyst particles carriers, enzymes, or complete microorganism cells. If the process is conducted so that the pores remain closed, a foamy polymer material is obtained that can be used as light construction material or a good insulation material.

The immobilization of microorganisms as the producers of pharmaceutical, industrial, and food raw materials on inert carriers is currently very interesting. For such purposes, materials such as various commercial sponges,[1] alginate gel,[2–4] carageenan,[5] sintered glass,[6–8] sugar cane,[9,10] cotton thread,[11] animal bones, wood, porous alumina, and so forth can be used. By means of microorganism immobilization a high concentration of cells in the bioreactor is achieved, the effect of shear forces is decreased on the cells in the porous carrier during mixing of the fermentation medium, and washing out of cells from the bioreactor is hindered.

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Numerous procedures for the synthesis of crosslinked low-density polymer material are described in the patent literature, where the initial emulsion prepolymerization process is carried out with a high water phase content.[12–26] Patents of this type have basically the same procedure:

- The emulsion treatment with different surfactants as emulsion stabilizers, with abundant water content in organic phase monomers
- The prevalent use of water-soluble compounds that break up to make radicals that are the polymerization initiators
- The use of vinyl polymers with one double-bond as the starting polymerization compounds
- The use of monomer compounds with two or more double bonds to make the polymer chains in the polymerization process with one double bond, whereas the other double bond(s) make lateral bonds between the polymer chains, thus obtaining the crosslinked nonsoluble polymer; the density of lateral bonds and the monomers used determine the swelling capacity, elasticity, and the rigidness of the structure of the polymer obtained
- The process of polymer material rinsing, pore opening, and drying

The macroporous polymer based on methyl methacrylate synthesized by suspension polymerization with glycidyl methacrylate[27–29] can be used for selective sorption of metal ions; the synthesis under hypercritical conditions in carbon dioxide by sol–gel polymerization is well known, where methyl methacrylate is one of the comonomers to give the necessary hardness to the finished polymer product.[30,31] In the U.S. patent literature there are data on porous polymethyl methacrylate as human implant material,[32,33] and on porous polymethyl methacrylate with protein content.[34]

The enzyme immobilization by covalent bonding in polyacrylamide gel or on polymethyl methacrylate polymer is also known.[35,36]

The macroporous crosslinked copolymers have a porous structure in the solid state, differing from the common crosslinked copolymers that become porous only after swelling in a solvent. The macroporous crosslinked copolymers can be obtained only by copolymerization of two or more monomers, where at least one of them has more than one double bond, and in some inert media. Opposite to such a traditional approach, the synthesis of a macroporous crosslinked copolymer can be carried out with monomers containing one double bond each. The crosslinking is achieved by means of low molecular compounds having functional groups that can condense with one another, but also with the functional group in one of the monomers (acrylamides) of the copolymer. Thus, lateral bonds are created between the macromolecular chains, resulting in tridimensional crosslinking at the end of the process, with a completely solid, fixed structure of the polymer material that is insoluble and nonswelling.

A macroporous copolymer based on methyl methacrylate and acrylamide is a very interesting material with respect to the immobilization of microorganisms or enzymes within the pores. It is especially adapted for the immobilization of enzymes because the groups on the polymer segments are readily transformed into acyl azide derivatives and so enable covalent bonding of the porous copolymer with the free amino groups from a basic amino acid of any protein. The essential amino acid lysine has an α-amino group that helps create peptide bonds during incorporation into the protein structure. It also has another free amino group that can be used to create a bond with the acyl azide group of the polymer carrier, if the free amino group of the protein has the corresponding orientation toward the outer surface of the protein of which this amino acid is a constituent.

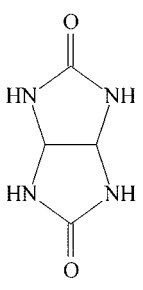
Such a copolymer with the open pores free volume of 50 to 90% can be synthesized from methyl methacrylate and acrylamide with glycoluril (tetrahydro-imidazo[4,5-*d*]imidazo-2,5-dion) based crosslinker and a surface active emulsion stabilizing substance. The emulsion is prepared with copious amounts of water, wherein also one monomer (acrylamide), a water-soluble crosslinker, and an initiator are dissolved. The second monomer (methyl methacrylate) is in the organic phase, where ethyl acetate is added as the pore-opening substance in the termination phase of production of this polymer. In principle, other alkyl esters of the acrylic and methacrylic acids can be used as the second monomer in the organic phase to obtain copolymers for other applications.

EXPERIMENTAL

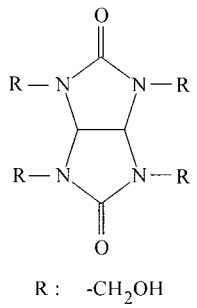
The following chemicals were used in the synthesis: dioctyl sulfosuccinate, sodium salt and acrylamide (Sigma, St. Louis, MO), glycoluril and methyl methacrylate (Aldrich, Milwaukee, WI), potassium persulfate (Riedel-de Haën, Seelze, Germany), formaldehyde 37% (Merck, Darmstadt, Germany), α-amylase (from *Bacillus subtilis;* Serva Feinbiochemica GmbH & Co. KG, Heidelberg, Germany), and locally obtained yeast.

The porous copolymer synthesis began with the synthesis of the crosslinker. The methylol derivative of glycoluril was used as the crosslinker. Then the emulsion containing all the necessary compounds for the copolymer synthesis was prepared. After the prepolymerization and copolymerization phases were finished, the crosslinking phase occurred by chemical condensation of functional groups in the polymer chains, followed by opening of pores and drying.

The synthesis of methylol derivative of glycoluril was carried out in the 0.01 mol/dm^3 (pH = 9.5) concentrated solution of disodium-hydrogene phosphate at 60°C for 1.5 h. To obtain tetramethylol glycoluril (1,3,4,6-tetrakis-hydroximethyl-tetrahydro-imidazo-[4,5-*d*]imidazol-2,5-dion) the necessary glycoluril to formaldehyde ratio is 1 : 4.



Glycoluril



Tetramethylol glycoluril

Emulsion preparation

The corresponding quantities of emulsion stabilizer (sodium dioctyl sulfosuccinate whose concentration in the solution is higher than the critical micellar concen-

tration, 0.2% w/v), initiator (potassium persulfate, 0.83% w/v), acrylamide (2.28% w/v), crosslinker (tetramethylol glycoluril, 0.56% w/v), water (71.65% w/v), and a mixture of ethyl acetate (5.26% w/v) and methyl methacrylate (19.24% w/v) were added to a container and vigorously agitated. Because of the acrylamide present in the water phase, despite the added stabilizer, the emulsion was not completely stable, especially during heating in the prepolymerization phase, and slowly separated into layers.

The container with the stirrer, where the reaction mixture was introduced, was connected to a reflux condenser; the temperature was increased to 60°C with constant mixing and the prepolymerization reaction was carried out for 20 min. During this period, a soft liquid gel was obtained, the separation into layers ceased, and the reaction mixture could then be poured into molds so that particular shapes of particles could be produced.

Polymerization of the reaction mixture by the sol–gel method without mixing at constant temperature of up to a maximum of 60°C proceeded in a reactor of corresponding shape (cylindrical test tube) until the reaction mixture completely hardened. Total prepolymerization and polymerization time amounted to about 2.5 h.

Additional shaping of the particles of the hard polymer (cutting of the cylinder into thin slices to obtain a flat cylindrical shape) was carried out. The finished particles were then further processed by heating in distilled water at 80°C. The heating time at this temperature was 30 min. During that period, ethyl acetate, incorporated mostly between the monomer segments of methyl methacrylate, evaporated within the polymer and the vapor tension opened the pores and escaped from the polymer. At the same time, the remaining initiator terminated the polymerization reaction and the reaction of chemical groups condensation was started to create lateral bonds in the polymer material, thus creating and fixing the tridimensional network of polymer chains. During the process the applied emulgator and the remaining nonpolymerized monomers were washed out.

Further processing of polymer material was carried out by heating in distilled water with continuous stirring. The water to polymer volume ratio was 2 : 1 in each batch, and after the first heating phase, the water was changed. In the second phase, the linear program of temperature increase was applied at 0.5°C increments to boiling, and kept at the boiling temperature for 20 min.

Heating of polymer particles was performed by dry hot air at 130°C, completing the crosslinking reaction; the polymer network became completely fixed, the pores became entirely open, and the polymer material was dried. This phase took 50 min.

Synthesis of the pure polyacryl amide and polymethyl methacrylate homopolymers was effected in water and ethyl acetate solutions, respectively. The polymerization was carried out at 60°C with potassium persulfate as the initiator for 6 h, and then the thin polymer layer was dried at 100°C and used for FTIR spectroscopy for comparison of copolymers and homopolymers.

The crosslinking synthesis product and the polymers were analyzed by FTIR spectra made on a Bomem Hartmann & Braun MB-series IR spectroscope (Quebec, Canada), in the form of a compact disk with 1.2 mg of sample and 160 mg of KBr of spectrophotometric purity (Merck).

A polymer sample was prepared for SEM microscopy by ion-sputtering technique on a JEOL JFC-1100E (JEOL, Peabody, MA). A thin layer of gold (several nm thick) was applied to the polymer sample surface. SEM microscopy was carried out on a JEOL JSM-5300 scanning electron microscope.

Cumulative pore size distribution for the crosslinked macroporous polymer was determined by mercury porosimetry on a Carlo Erba 2000 (Milan, Italy).

For thermal analyses a sample of crosslinked copolymer was ground in a mortar, and the powder sample was used for better heat transfer through the sample mass. The thermogravimetric measurements were carried out using a Perkin Elmer TG S2 (Perkin Elmer Cetus Instruments, Norwalk, CT) in nitrogen atmosphere by dynamic test at 10°C/min from 25 to 600°C. The sample mass was about 10 mg.

The curve of the synthesized copolymer was generated by DSC technique by using a Perkin Elmer DSC 2 differential scanning calorimeter. The sample was heated from room temperature to 250°C in aluminum pans in nitrogen atmosphere. The heating rate was 10°C/min, and an empty aluminum sealed pan was used as a reference.

RESULTS AND DISCUSSION

The porous crosslinked material obtained by the above procedure did not dissolve or swell in any organic solvent. The groups on pore walls could be chemically transformed, oriented from the free copolymer surface toward the liquid phase into which the polymer body was immersed. The density of dry polymer particles was 0.21 g/cm^3.

With respect to the water to organic phase ratio of 2.92 : 1, the initial structure of the emulsion is oil in water, because the oil emulsion is made only when water to organic phase ratio is greater than 4 : 1.[21] The organic phase is made of methyl methacrylate and ethyl acetate mixture. The water phase contains the initiator, acrylamide, and crosslinker. The emulgator is at phase surface contact. This emulsion structure

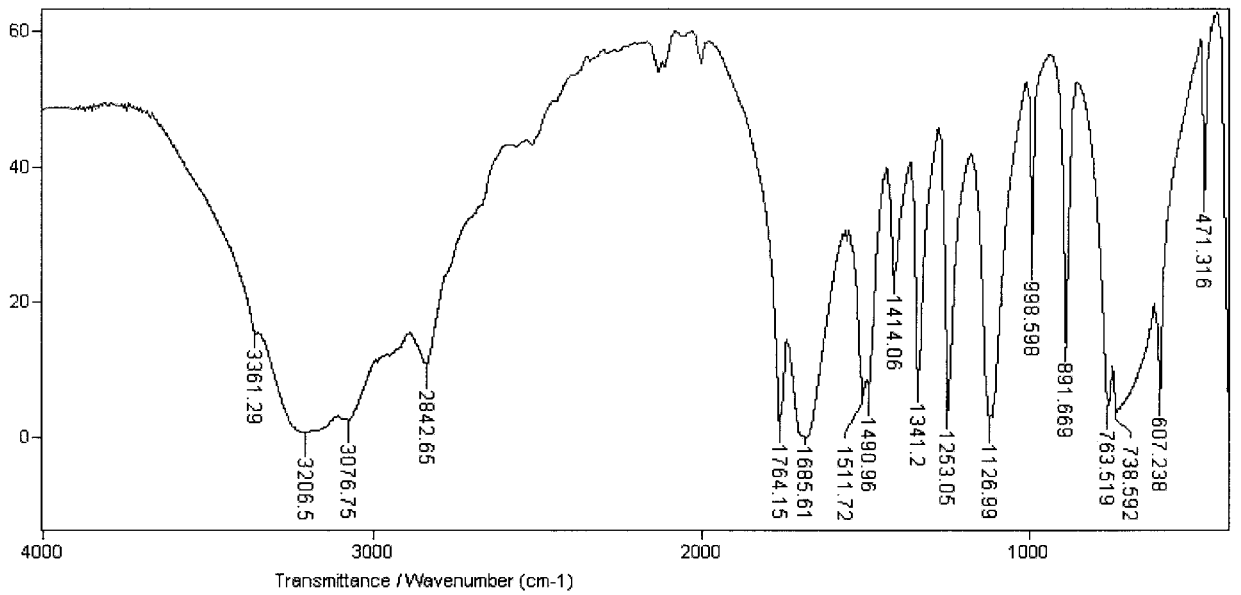


Figure 1 FTIR spectrum of glycoluril in KBr disk.

before gel formation in the prepolymerization is indicative of block copolymer production.

From the IR spectra analysis it can be seen that the valence band of the secondary cyclic amide ν_{NH} at about 3200 cm^{-1} of glycoluril (Fig. 1) was shifting toward higher frequencies because of the C—OH group introduced, ν_{OH}, absorbing at about 3300 to 3470 cm^{-1} (Fig. 2), thus confirming the formation of methylol derivative of glycoluril. The OH bending vibrations band appeared at 1319 cm^{-1}, and valency vibrations ν_{C-O} at 1183 cm^{-1} (Fig. 2). The C—H bond in gycoluril absorbed at 2842 cm^{-1} because the C-atom, bonded with H, is bonded to a heteroatom (N), and as a result there was a shift toward higher frequencies. Three new bands of C—H absorption of CH and CH_2 groups in methylol derivative of glycoluril appeared at 2908, 2957, and 2988 cm^{-1}, respectively (Fig. 2). The valency vibration of the C═O group with ureal structure (glycoluril is acetylene urea) in addition product was at 1718 cm^{-1}, whereas in glycoluril it showed two bands at 1685 and 1764 cm^{-1}, respectively; these were amide bands I and II, where amide band I indicated the C═O group and amide band II was partially attributed to N—H bending vibrations in

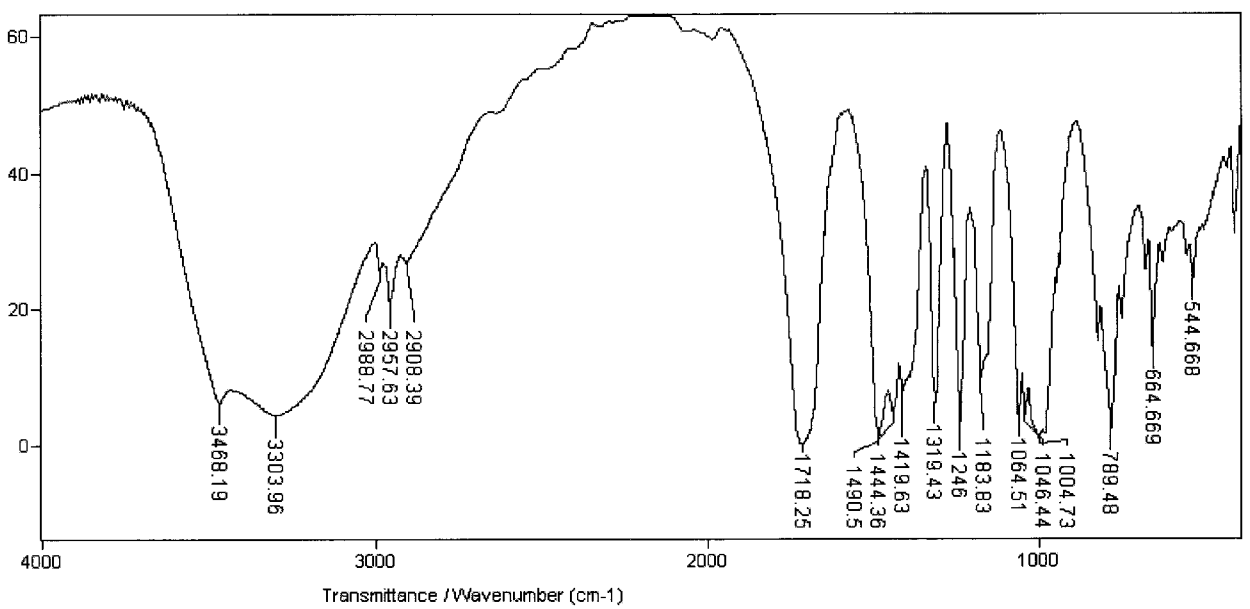


Figure 2 FTIR spectrum of tetramethylol glycoluril in KBr disk.

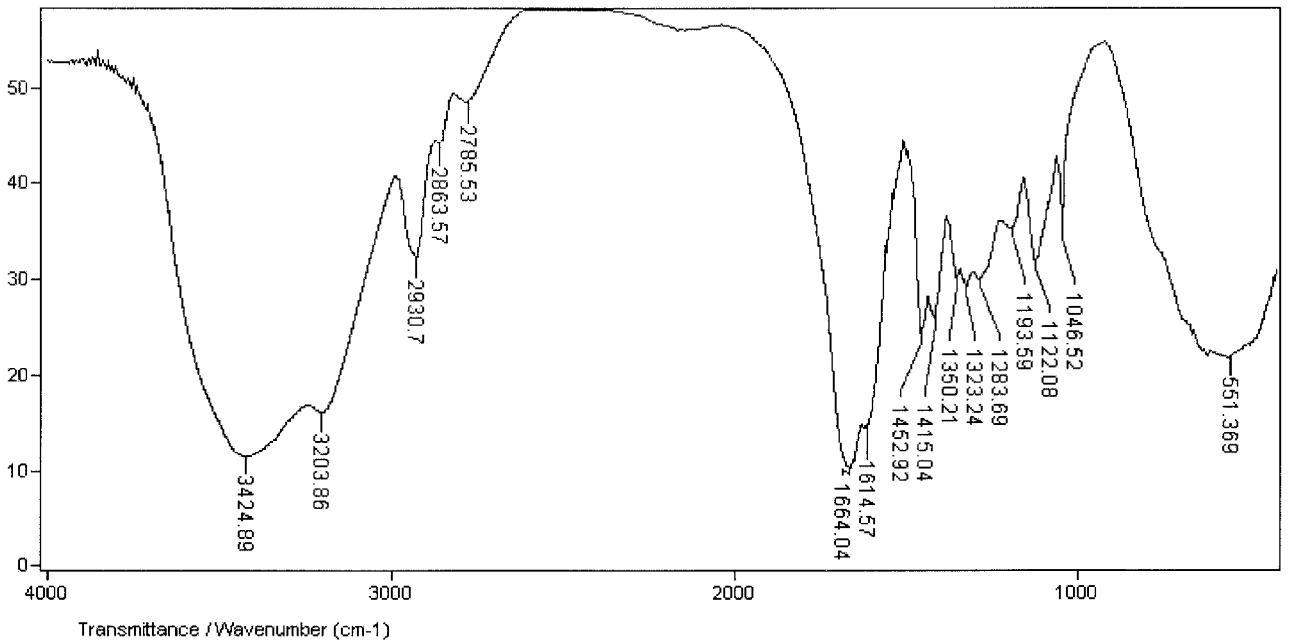


Figure 3 FTIR spectrum of pure polyacrylamide homopolymer in KBr disk.

plane coupled with valency C—N vibrations. After the addition of formaldehyde and the introduction of methylol group, the N-atom was no longer coupled with a free H-atom, there was no more coupling with the C—N group, and amide band II disappeared completely (Fig. 2).

Figures 3, 4, and 5 illustrate by use of IR spectra the synthesis of the porous copolymer with crosslinker. Because the methyl methacrylate content in the copolymer is dominant, up to 90% of the polymer mass (Fig. 5), the similarity of some bands is obvious with the IR spectrum of the pure homopolymer, that of polymethyl methacrylate (Fig. 4). The valency vibration of the C═O group of the polymethyl methacrylate showing absorption at 1728 cm^{-1} (Fig. 4) is also present in the porous copolymer at 1733 cm^{-1}. The amide band I from polyacrylamide with inflection attributed to the vicinity of amide band II of the coupled bending

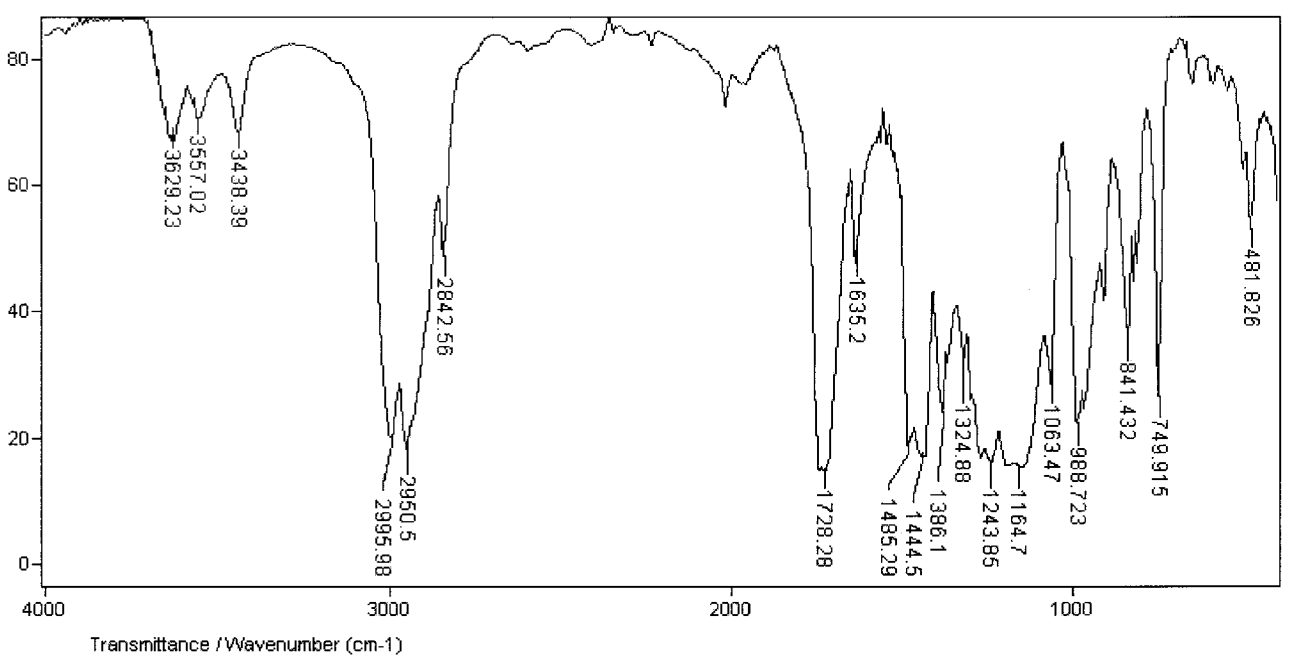


Figure 4 FTIR spectrum of pure polymethyl methacrylate homopolymer in KBr disk.

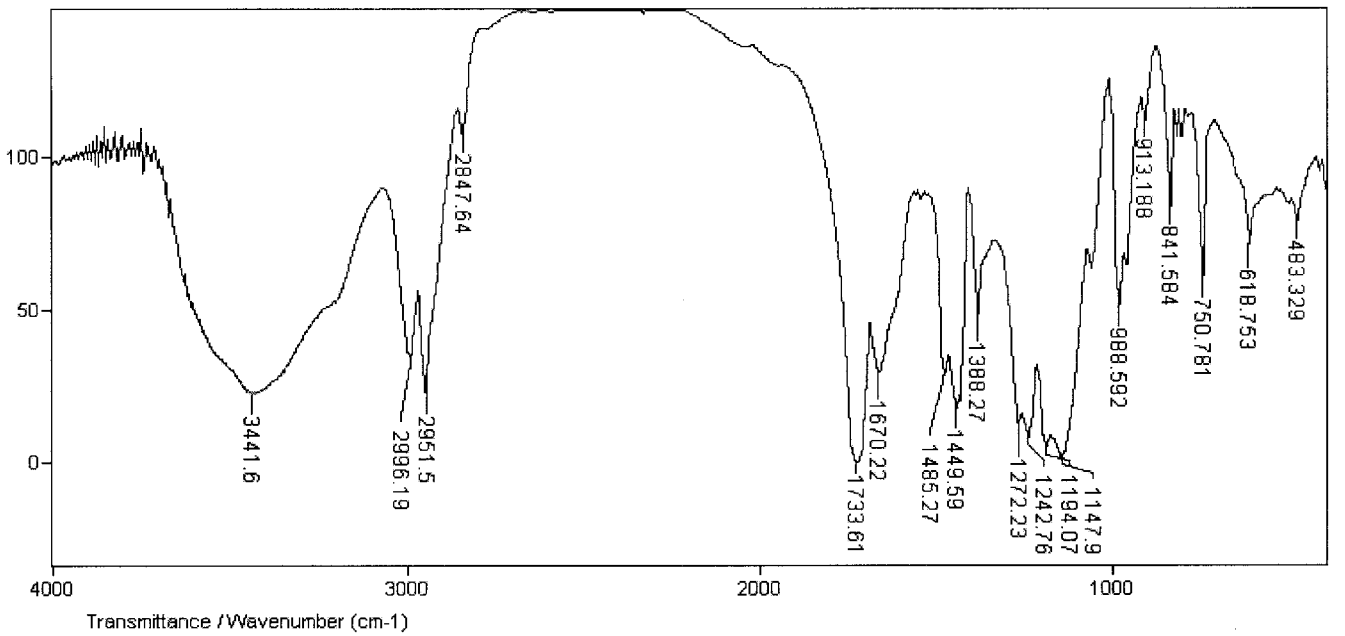


Figure 5 FTIR spectrum of the sample of porous crosslinked methyl methacrylate and acrylamide copolymer with tetramethylol glycoluril as the crosslinker, in KBr disk.

N—H with valency C—N at 1614 cm^{-1} is also present in the copolymer with weaker inflection resulting from partial use of N—H groups for crosslinking; in any case, the whole band has a weaker intensity in the porous copolymer as a result of the smaller percentage of acrylamide and crosslinker content in the copolymer structure. However, at about 3440 cm^{-1} the band also shows the absorption of valency N—H from acrylamide (Fig. 3) and valency O—H from the crosslinker (Fig. 2), which have not been used up for lateral bonds.

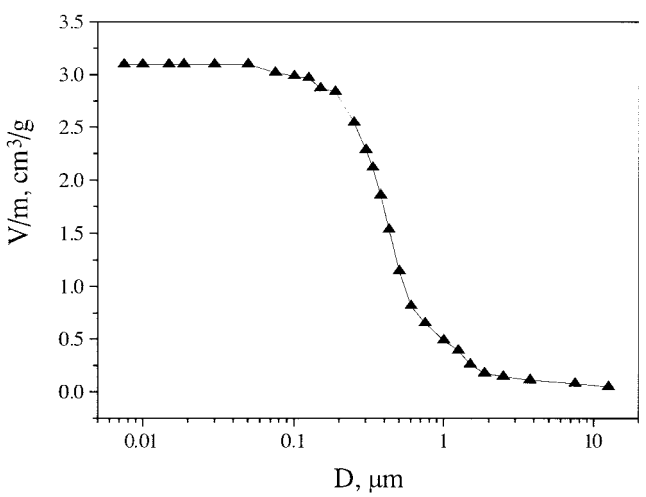


Figure 6 Cumulative pores volume distribution curve with respect to the pore diameter for the crosslinked macroporous copolymer.

From the cumulative curve of pore size distribution (Fig. 6), the total porosity value of copolymer can be seen, about 3.2 cm^3/g, and at the same time one may determine the percentage of $>$ 10-μm pore volume content, which amounts to about 6%, or 0.192 cm^3/g. From the pore size distribution curve, the free surface value $S_{S,Hg}$ in m^2/g can be calculated by use of the following equation:[27,29]

$$S_{S,Hg} = \sum_{i=1}^{n} \Delta S_i \quad \text{where} \quad \Delta S_i = \frac{4000(V_{i+1} - V_i)}{(d_i + d_{i+1})/2}$$

where V_i (in cm^3/g) is the volume of d_i (in nm) diameter pores. For the copolymer sample, the free surface

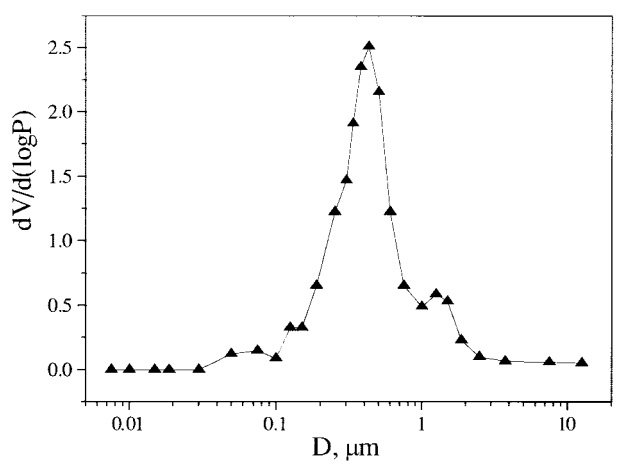


Figure 7 Pore size distribution in the crosslinked macroporous copolymer sample.

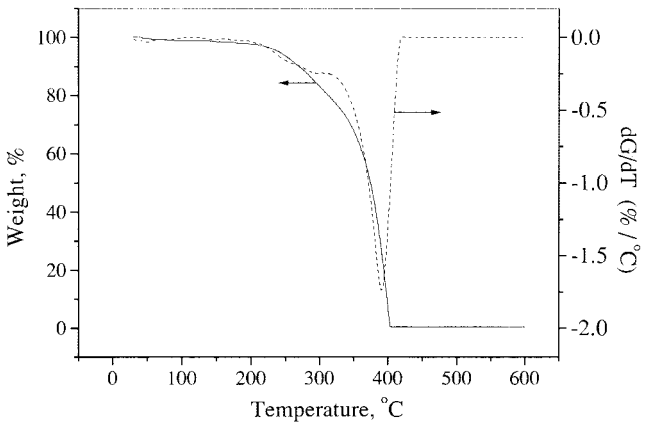


Figure 8 TG and DTG curves of porous crosslinked copolymer.

value obtained was 22.7134 m^2. Cooper[30,31] presents the pore size distribution in porous polymers as a differentiated distribution $dV/(d \log P)$ as a function of D. From the diagram the average diameter of most of the prevalent pores can be seen, ranging from 0.2 to 2 μm (Fig. 7).

In Figure 8, TG and DTG curves are given. Figure 8 shows that the crosslinked porous copolymer was stable up to 250°C, that the maximum mass loss rate was at about 380°C, and that the degradation of the copolymer (pyrolysis) was completed before reaching 400°C. It can be seen that after the complete degradation there were no copolymer residue left, which was expected because the copolymer composition is purely organic.

From the DSC curve shown in Figure 9 the glass-transition temperature (T_g) for the porous copolymer acrylamide and methyl methacrylate was read at 130°C. For the homopolymers, the corresponding glass-transition temperatures were[37] about 200°C for polyacrylamide, 115°C for syndiotactic polymethyl methacrylate, and 45°C for isotactic polymethyl methacrylate. From the above data it can be concluded that the copolymer was mostly in the syndiotactic condition, and that the T_g was greater than that for the pure polymethyl methacrylate homopolymer. This is attributed to the presence of acrylamide units in the polymer chains and the lateral bonds created between them.

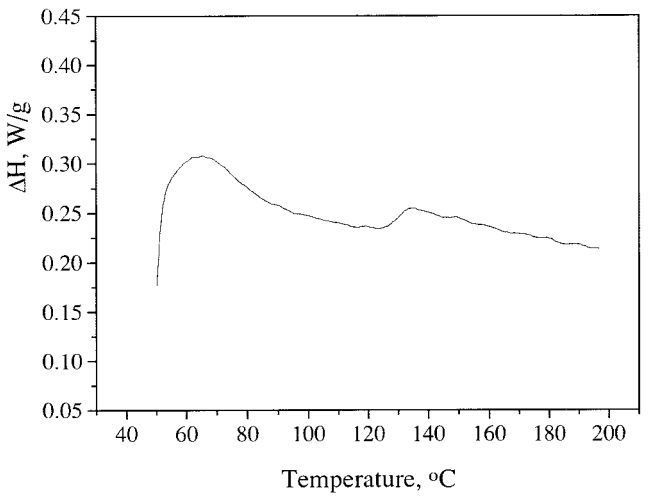


Figure 9 DSC curve of porous crosslinked copolymer.

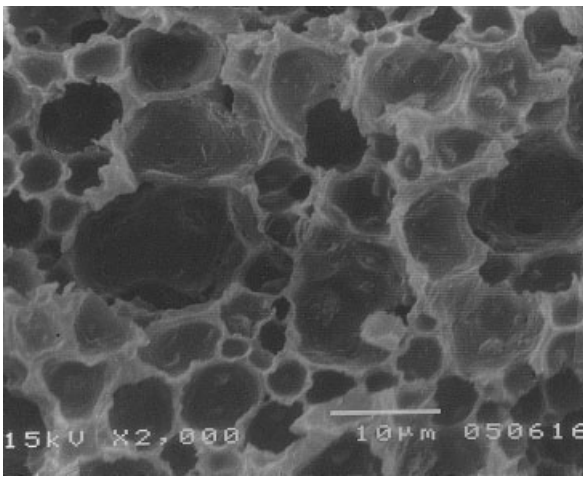


Figure 10 SEM micrograph of porous copolymer; magnified ×2000, bar = 10 μm.

SEM micrographs of the crosslinked porous copolymer are given in Figures 10 and 11. In the photos, the shape and size of the synthesized copolymer pores can be seen. They are mostly oval in shape, between 1 and 10 μm in size, although solitary larger pores are also discernible. The pore size corresponds to the enzyme immobilization, whereas for the immobilization of larger microorganisms greater size pores would be required. An elliptical pore size of 20 × 40 μm is shown in Figure 11.

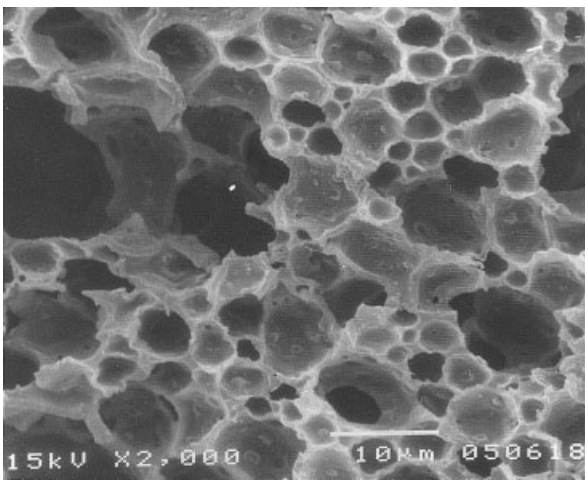


Figure 11 SEM micrograph of porous copolymer; magnified ×2000, bar = 10 μm.

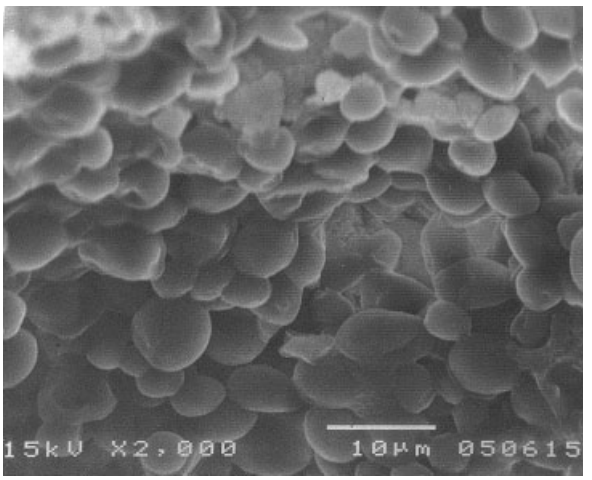
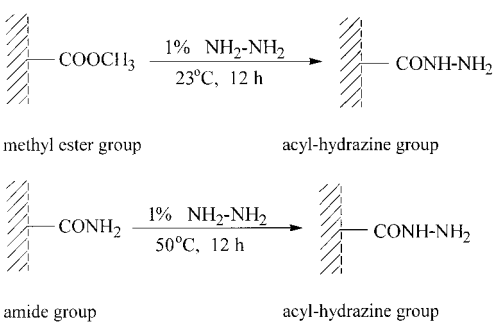


Figure 12 SEM micrograph of crosslinked porous copolymer with immobilized yeast *Saccharomyces cerevisiae* cells, magnified ×2000, bar = 10 μm.

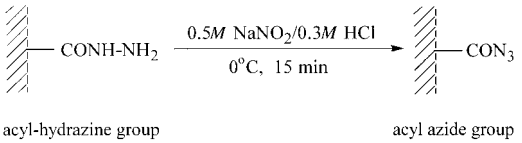
The crosslinked porous polymer material can be used as the carrier in the enzyme or live microorganism cell immobilization, when enzyme or microorganism cells are to be used as biocatalysts in the production of some alimentary, pharmaceutical, or other biochemical substance. The enzyme immobilization is effected by covalent linking of the acyl azide derivative with the amino group of the amino acid in the protein moiety of the enzyme. Thus not only enzymes, but also other proteins can be immobilized.

The simplest way to bring about the immobilization of live microorganism cells is to vacuum the microorganism cell suspension into the porous polymer support. During the fermentation, the cells form their colonies (Fig. 12) and the possibilities for cells being washed out are relatively small because of the pores' depth. Some cells, flowing out from such porous support, may appear freely suspended in the fermentation medium.

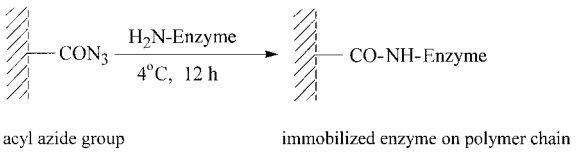
Irrespective of whether the available group on the surface is methyl methacrylate or acrylamide, the polymer–analog reactions on the free copolymer surface are carried out with hydrazine because the end product of the reaction is the same and leads to acyl azide:[35,36]



Rinsing with redistilled water at 0°C:



Rinsing with phosphate buffer solution, pH 7.5, 50 m*M*, and then coupling the reaction with the enzyme:



If the porous copolymer is immersed in hydrazine solution and left long enough for the reaction to terminate, by determination of hydrazine before and after the reaction with the polymer, the quantity of available groups in the liquid phase and in the reaction with hydrazine can be ascertained. In Figure 13 the pH-metric titration of hydrazine with hydrochloric acid is shown on two hydrazine samples, before and after the reaction with the porous copolymer. A typical curve is obtained of neutralization of a weak base with a strong acid. The neutralization points are inflection points of the pH–V curve.

From the difference of the used quantities of HCl of known concentration for the titration of hydrazine before and after the reaction with the synthesized copolymer, the quantity of the hydrazine used per gram of copolymer can be determined, which is equal to the quantity of the available groups of acrylamide or methyl methacrylate in the copolymer. When the

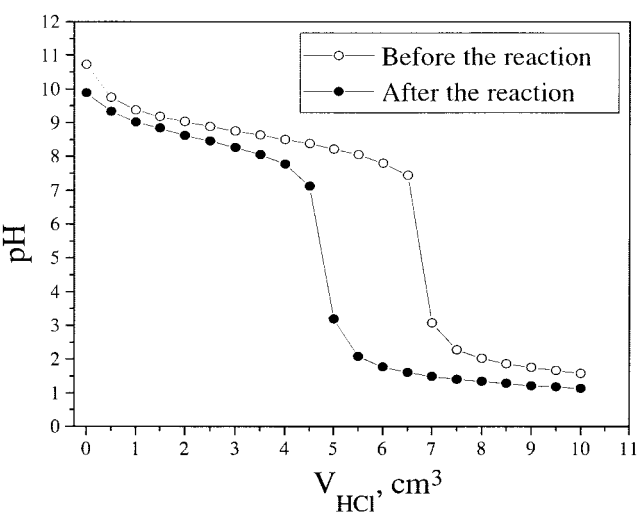


Figure 13 pH-metric titration of hydrazine solution used for copolymer processing before (—○—) and after (—●—) the reaction.

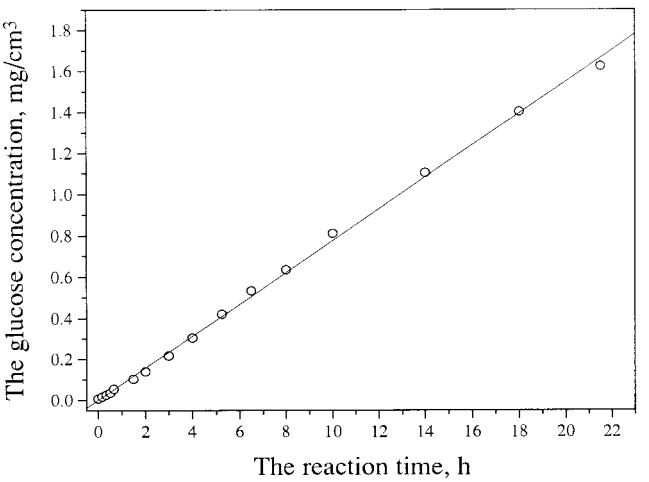


Figure 14 Concentration of glucose from hydrolyzed starch by α-amylase immobilized enzyme.

total quantity of the groups in the copolymer is known, the share of groups available to reaction can be calculated (i.e., the amount of groups on the free polymer surface of phase to liquid contact). For the samples of synthesized copolymers this amounted to 25%. The remaining 75% of groups within the copolymer walls are "blocked" or crosslinked, given that a crosslinker was present and the copolymer synthesis reaction was guided so that the thermosetting crosslinking occurred and thus the structure of the copolymer became stable.

Enzyme α-amylase was immobilized on the prepared porous polymer support and the hydrolysis of the starch solution was carried out by the immobilized enzyme. In Figure 14 the change of concentration of glucose hydrolyzed from starch by immobilized enzyme is shown.

CONCLUSIONS

The synthesis of a macroporous crosslinked copolymer was carried out from methyl methacrylate and acrylamide. The methylol derivative of glucouril was used as the crosslinker, and the crosslinking reactions were of the condensation type, as distinct from the conventional production of crosslinked polymers and copolymers by use of reagents with mostly double bonds or a double bond and a glycidil group. The copolymer obtained can be used as an inert support for the immobilization of live microorganism cells or enzymes.

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