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Effect of inert components on the porous structure of 2-hydroxyethyl methacrylate–ethylene dimethacrylate copolymers

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The products obtained by suspension copolymerization of 2-hydroxyethyl methacrylate with ethylene dimethacrylate in water in the presence of 1-dodecanol and cyclohexanol as inert diluents were characterized by scanning electron microscopy, mercury porosimetry, water and cyclohexane regain and volume swelling experiments. The porosity of the resulting poly(2-hydroxyethyl methacrylate) beads was readily adjusted by a change in the ratio of 1-dodecanol (non-solvating diluent) to cyclohexanol (solvating diluent). The morphological structure of the porous samples was also influenced by the choice of drying technique. Freeze-drying of samples swollen in water increased porosity compared to samples air-dried from ether.

(Keywords: porosity; HEMA–EDMA copolymers; inert diluents)

INTRODUCTION

Macroporous hydrophilic matrices, such as copolymers of 2-hydroxyethyl methacrylate (HEMA) with ethylene dimethacrylate (EDMA), have potential as support carriers for entrapment of drugs that are slowly released or for immobilization of enzymes and cells for biocatalysis in biomedical engineering[1]. If implanted in living tissue, their structure supports cellular ingrowth and proliferation[2]. Macroporous matrices are obtained only if copolymerization of the monomers is performed in the presence of an inert diluent. The inert diluent is a low-molecular-weight or polymeric substance that is miscible with the monomers but does not react during the copolymerization and at the end of the reaction can be easily removed from the resulting product. Cyclohexanol (CYOL) and 1-dodecanol (DOL), used in the production of highly crosslinked HEMA–EDMA sorbents marketed under the tradename Spheron or Separon[3], are examples of inert diluents, the former being a thermodynamically good solvent whereas the latter is a thermodynamically poor solvent for the poly(HEMA) matrix.

The objective of this study is to investigate the relationship between the synthesis conditions and the porosity and morphology of HEMA networks crosslinked with low amounts of EDMA. Unlike some of the previous investigators[4], we made no attempts to influence the properties by varying the degree of crosslinking or by changing the overall amount of diluents in the suspension polymerization system. CYOL and DOL were used as the diluents and the effect of their ratio on the network properties was studied.

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EXPERIMENTAL

Materials

The following materials were obtained from commercial sources: 2-hydroxyethyl methacrylate (HEMA; Léčiva Prague), ethylene dimethacrylate (EDMA; Ugilor), 1-dodecanol (DOL; Fluka), cyclohexanol (CYOL; Lachema Brno), magnesium chloride (Lachema Brno). The monomers and solvents were freed from stabilizers and impurities by distillation; their purity was higher than 98% (g.c.). Azobisisobutyronitrile (AIBN; Ferak) was recrystallized twice from ethanol.

Preparation of the beads

Suspension polymerization was conducted in a 250 ml Büchi reactor fitted with an anchor-type stirrer, according to the Mueller procedure[5]. The suspension stabilizer based on magnesium hydroxide was prepared *in situ* from magnesium chloride and sodium hydroxide. A mixture of 90 g 20% aqueous NaCl solution containing $MgCl_2 \cdot 6H_2O$ (5.75 g) was introduced into the reactor, heated to 75°C and stirred at 700 rpm for 10 min. Then 30.75 ml 1 N NaOH was added dropwise to this solution, the stirring speed was reduced to 310 rpm and a mixture of HEMA (13.54 ml, i.e. 108 mmol), EDMA (0.3 ml, i.e. 1.6 mmol) and a mixture of CYOL and DOL (23.3 ml) containing AIBN as an initiator (0.15 g, i.e. 0.9 mmol) was added. In all experiments the stirring speed was kept constant. The polymerization was allowed to proceed for 8 h at 75°C. After polymerization, 2.5 ml of concentrated HCl was added and the beads were successively washed with water, methanol, acetone and ether. In this way, the gel was transformed from the rubbery to the glassy state. Finally, the beads were dried *in vacuo* at room temperature.

Table 1 Properties of poly(HEMA) beads

Sample	DOL[a] (vol%)	D_0[b] (μm)	D' (μm)	V_{Hg}[d] (ml g^{-1})	V_{WR}[e] (ml g^{-1})	V'_{WR}[f] (ml g^{-1})	V_{CX}[g] (ml g^{-1})	q_V[h]
A	0	648	722	0.30	0.61	0.77	0.00	1.11
B	20	583	662	0.68	1.09	1.33	0.45	1.14
C	40	519	623	0.73 (1.94[i])	1.24	1.50	0.68	1.20
D	60	500	570	0.99	1.18	1.58	0.84	1.14
E	80	384	596	0.30	1.25	1.62	0.03	1.55
F	100	356	526	0.23	1.41	1.80	0.05	1.48
G[j]	–	494	541	–	0.54	–	0.03	1.10

[a] 1-Dodecanol
[b] Dry bead diameter
[c] Wet bead diameter
[d] Pore volume by mercury porosimetry
[e] Pore volume by water regain
[f] Pore volume by water regain of freeze-dried samples
[g] Pore volume by cyclohexane regain
[h] Equilibrium swelling ratio
[i] Value for freeze-dried sample
[j] Standard copolymer prepared without any diluent

Freeze-drying

Dry beads were swollen in water to equilibrium and the swollen particles were freeze-dried in liquid nitrogen or propane in GT2 Leybold-Heraeus (Germany) lyophilizer.

Methods

The pore volume was determined from water and cyclohexane regain[6] and mercury porosimetry (Carlo Erba Strumentazione porosimeter series 200). The pore-size distribution was also determined by mercury porosimetry. Micrographs of the surface and of the internal structure of the beads were obtained using a JEOL JSM 6400 (Japan) scanning electron microscope. Particles cut with a razor blade were sputter-coated with a 10 mm thick gold film.

The equilibrium swelling ratio of copolymers, q_V, was calculated[4] as $q_V = D/D_0$, where D and D_0 are the mean diameters of the water-swollen and initial beads, respectively. These values were obtained by measuring on photographs the diameters of 500–600 beads in each run.

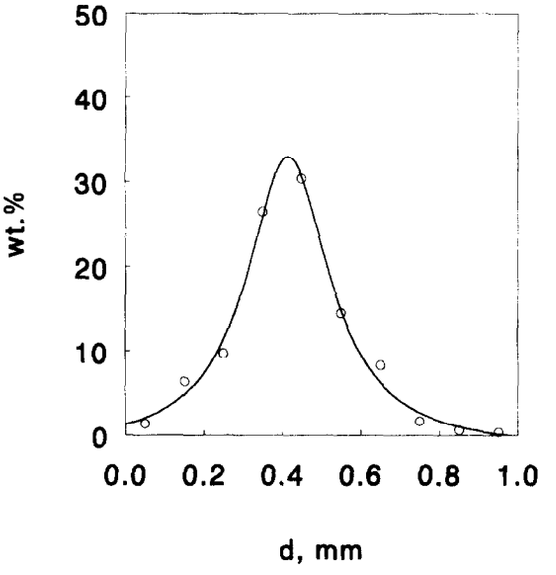

Figure 1 Bead-size distribution of poly(HEMA) sample D

RESULTS AND DISCUSSION

Formation of porous poly(HEMA) beads

Poly(HEMA) beads were produced by suspension polymerization under the conditions listed in *Table 1*. This technique resulted in beads 0.1–1 mm in size with the majority in the range 200–600 μm. *Figure 1* shows a typical integral bead-size distribution determined by counting the beads on photographs. An increase in the concentration of DOL in the diluent phase caused a shift in the bead-size distribution towards lower values: the average bead diameter in the dry state decreased from ca. 650 μm to ca. 360 μm (samples A and F in *Table 1*).

The presence of DOL in the diluent phase is necessary if porous poly(HEMA) beads are to be obtained. In its absence, only non-porous, transparent beads are formed (sample A, *Table 1*). However, some non-porous beads appeared also among the opaque, porous particles prepared at 80 vol% and more DOL in the diluent phase (samples E and F). The appearance of non-porous beads may be explained in terms of phase separation occurring before the gel point when the thermodynamically poor diluent partly separates from the polymerization mixture. The polymerization then results in non-porous beads.

At the same time, the effect of water, a poor solvent for poly(HEMA), on the phase separation, which forms a porous structure, cannot be excluded. However, the concentration of water dissolved in the polymerization mixture was found to be low (2–2.8 wt%)[7]. It can be assumed that this amount cannot substantially influence the thermodynamic quality of the diluent phase.

Morphology

Particle morphology both in the bulk and on the surface was examined by SEM. The morphology is strongly influenced by the type of a diluent. If neat CYOL was used as a diluent (sample A), particles were non-porous but transparent.

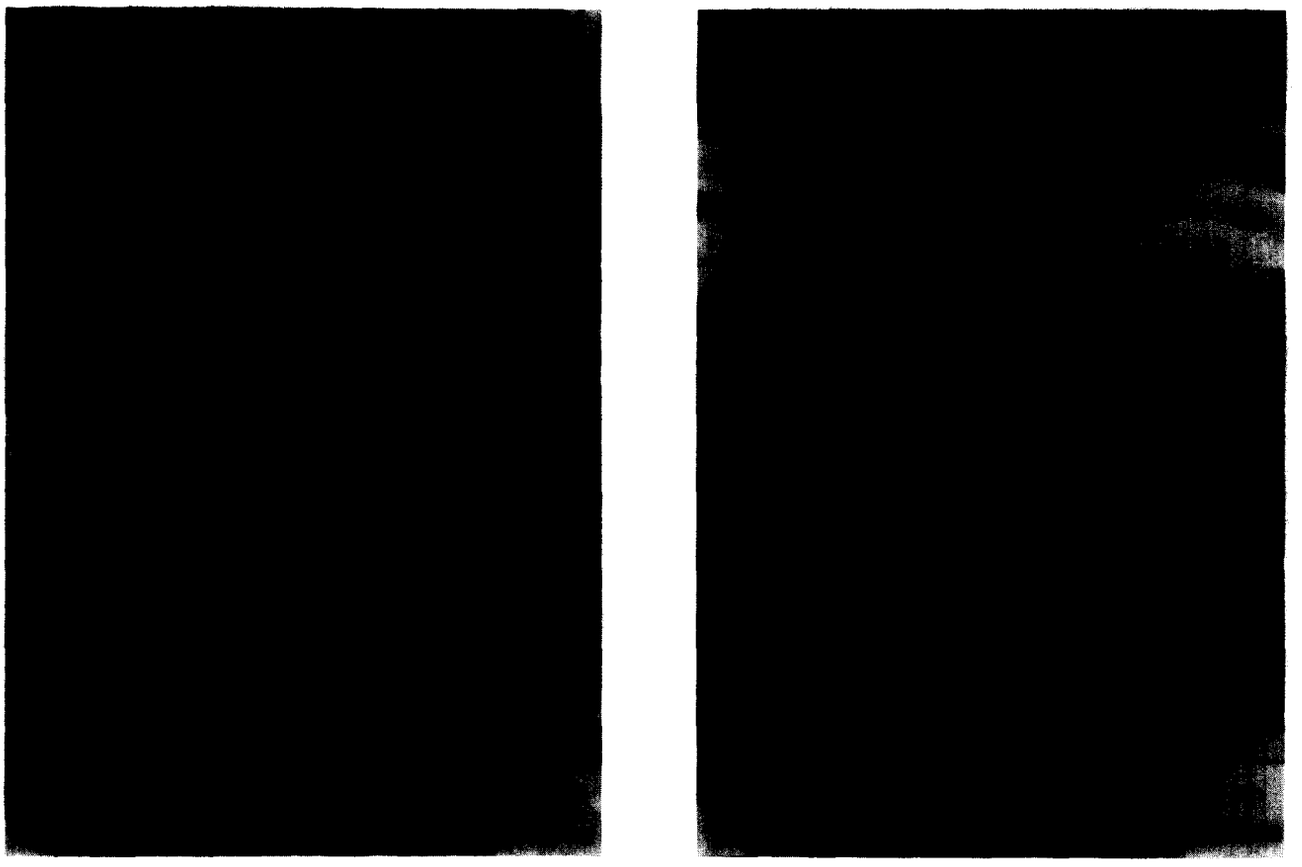

Figure 2 Scanning electron micrograph of the interior (a) and periphery (b) of poly(HEMA) sample B

The other samples, B–F, reveal a different situation. Their interior is composed of many voids (interconnected pores) between microspheres and/or their agglomerates (*Figure 2a*), formed by phase separation, which already occurs at an early stage of polymerization[8]. The porous core is surrounded by a thin shell of compactly fused microspheres with a smooth surface (*Figure 2b*).

In sample C (40% DOL), the size of microspheres is rather smaller than in sample B (20 vol% DOL) (cf. *Figures 2a* and *3*). The aggregates of microspheres in sample C, as regards the size, are approximately the same as in sample B; they consist, however, of larger numbers of distinctly separated microspheres than in sample B.

In sample D (60% DOL, *Figure 4a*) the findings are similar to those in sample C, with the difference that the wrinkled surface of the bead is visibly cracked (*Figure 4b*). From cyclohexane regain and mercury porosimetry, it appears that sample D contains a larger void volume (its porosity is higher) than samples C and B. This was also confirmed by SEM analysis.

Both samples E (80% DOL) and F (100% DOL) have a similar structure. Some non-porous particles with wrinkled surface are present among white opaque particles which are porous and brittle. Clusters of partly fused microspheres are observed inside the porous bead (*Figure 5a*). The sizes of the microspheres are the same as in samples C or D. The surface shells are similar to those in previous samples, but the cracks are absent (*Figure 5b*).

Samples discussed so far were air-dried from ether. If their dehydration was performed by freeze-drying

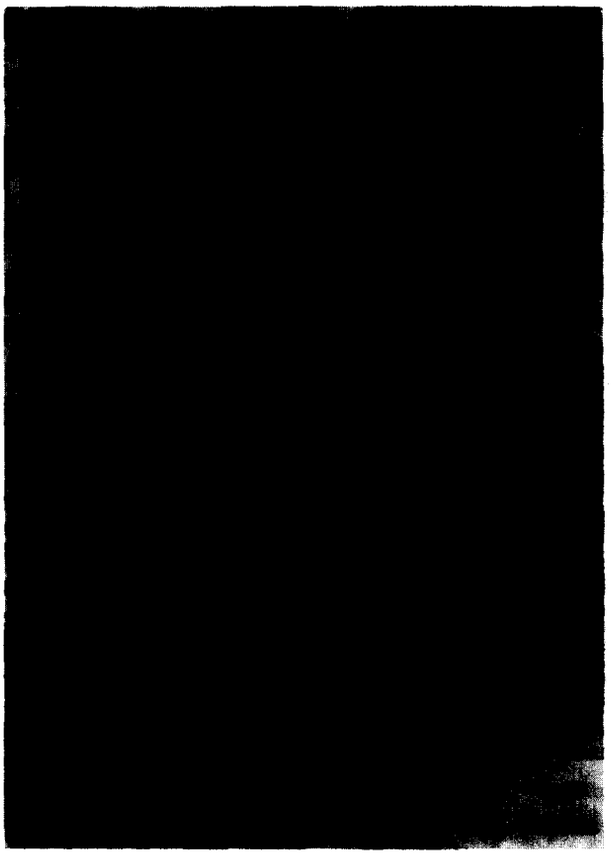

Figure 3 Scanning electron micrograph of the interior of poly(HEMA) sample C

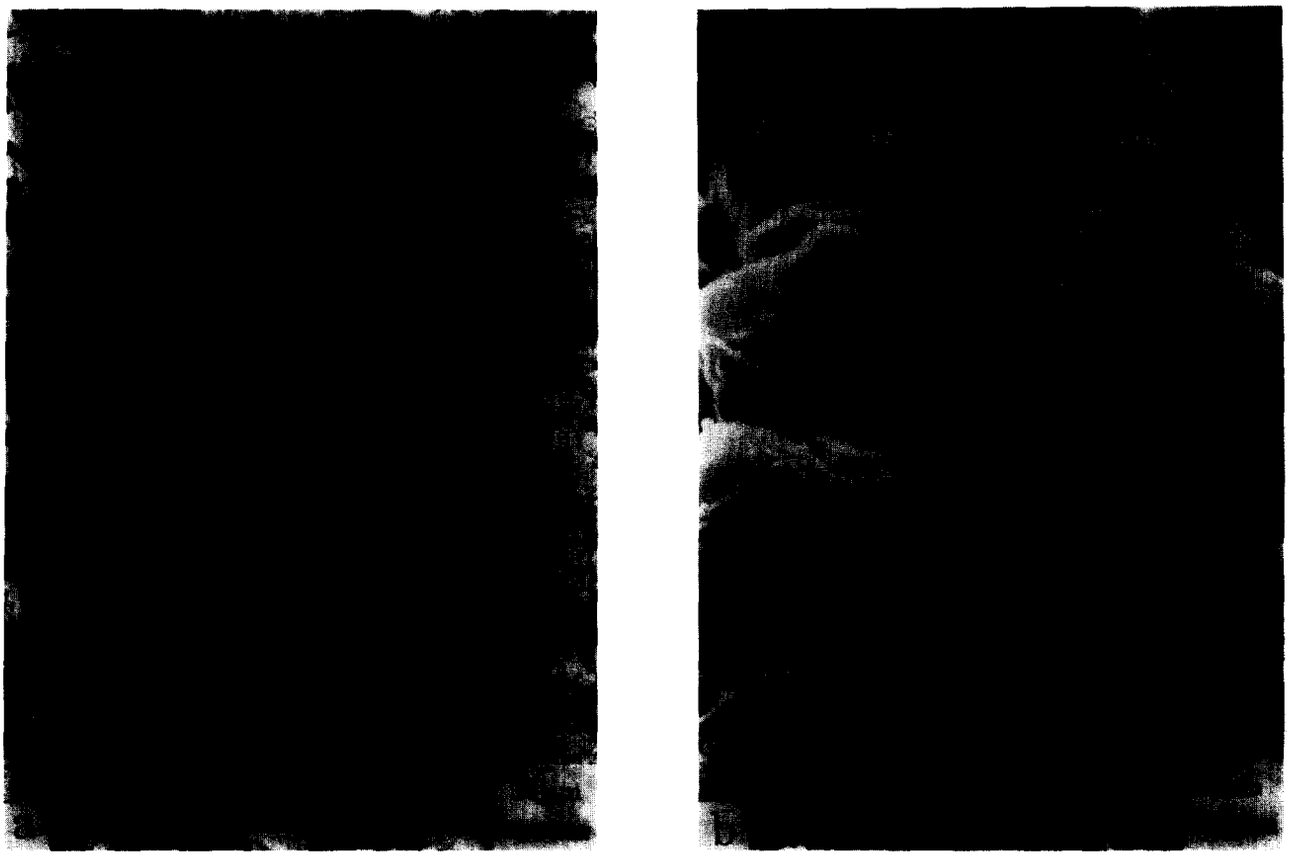

Figure 4 Scanning electron micrograph of the interior (a) and surface (b) of poly(HEMA) sample D

Figure 5 Scanning electron micrograph of the interior (a) and periphery (b) of poly(HEMA) sample F

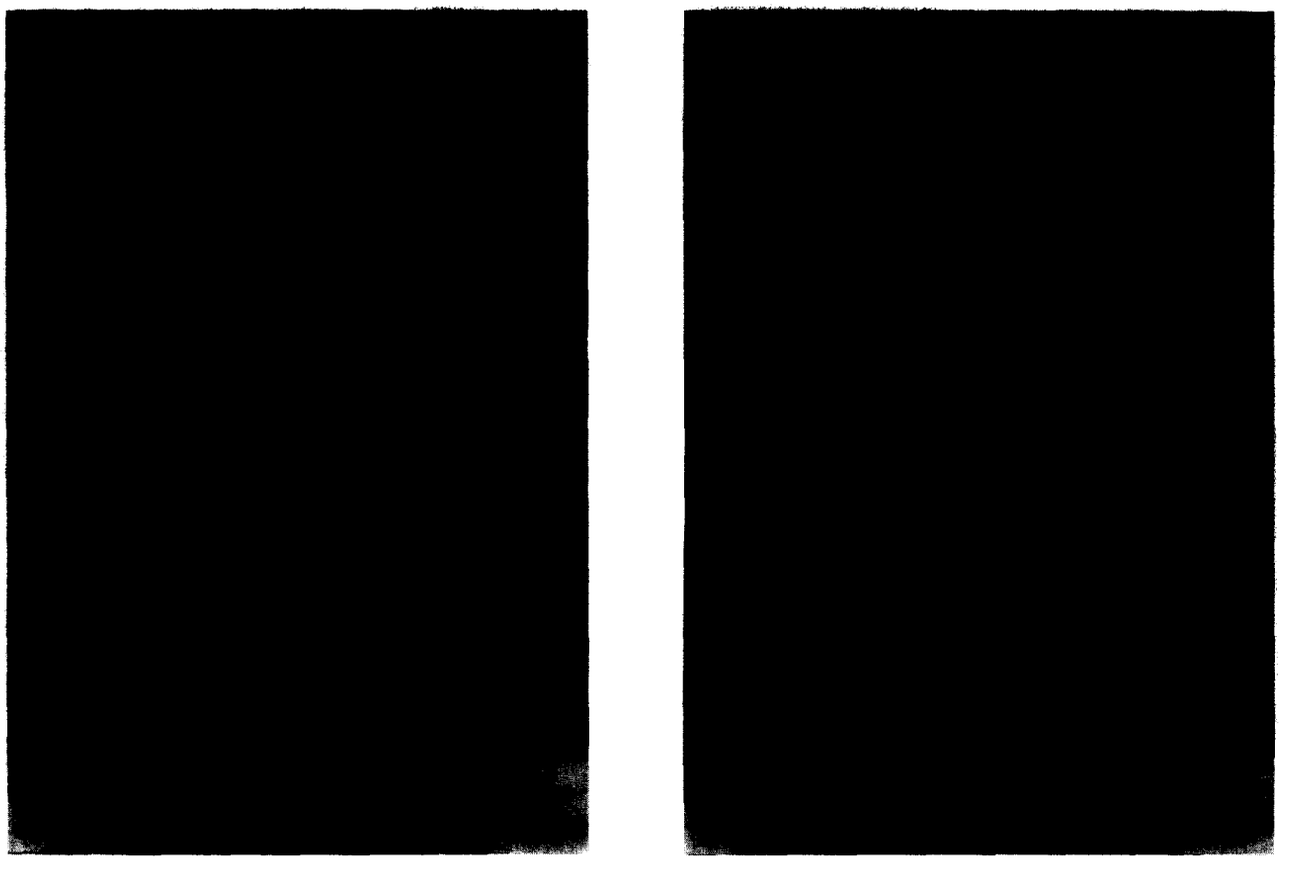

Figure 6 Scanning electron micrograph of the interior (a) and surface (b) of poly(HEMA) sample B after freeze-drying

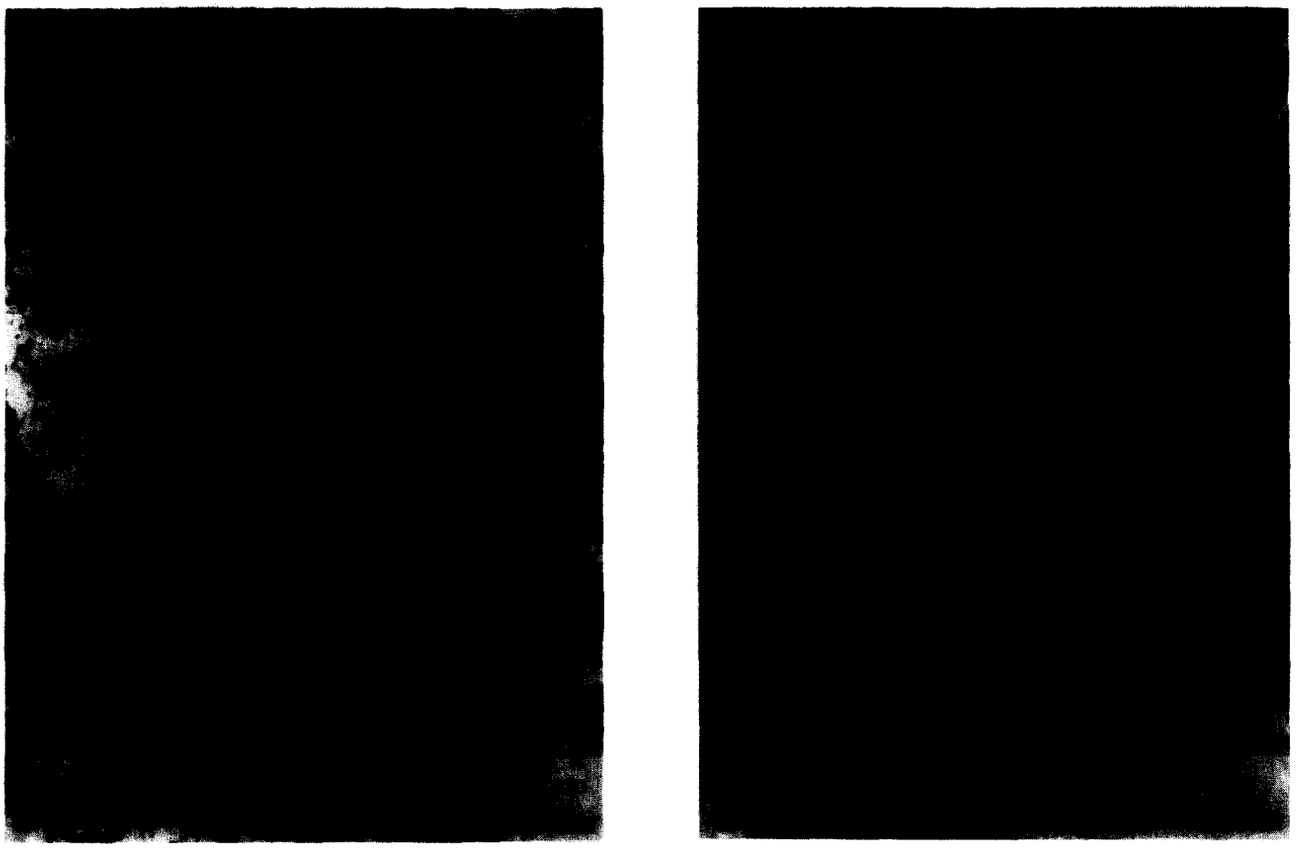

Figure 7 Scanning electron micrograph of the interior (a) and surface (b) of poly(HEMA) sample F after freeze-drying

poly(HEMA) beads swollen in water, the surface shells of the beads changed from smooth (*Figures 2b* and *5b*) to rough, coarse ones containing large holes (*Figures 6b* and *7b*). The second noticeable difference is apparent in the interior of the beads. Although the sizes of the microspheres do not change, the interstices (pores) between the aggregates in freeze-dried samples are larger than in the samples dried from ether (compare *Figures 2a* and *5a* with *Figures 6a* and *7a*). At the same time, in freeze-dried samples the water regain is increased as compared to those dried from ether.

In order to prove that the breaking of the shell is not an artefact caused by crystalline ice, we freeze-dried sample F in liquid propane. Compared to samples freeze-dried in liquid nitrogen, no differences were observed, either in the bulk or on the surface.

It can be concluded that the structure of samples is preserved in the swollen state if ordinary air-drying from ether is avoided and freeze-drying of the hydrated gels was used. The structure of freeze-dried samples as obtained by SEM is probably the best estimation of the real pore size. On the other hand, structural collapse occurred during drying from ether.

Porosity

The pore volume of poly(HEMA) samples was determined by three independent methods: water and cyclohexane regain and mercury porosimetry. The results of measurements are summarized in *Table 1*.

Mercury porosimetry measures samples in the dry state. During the removal of the diluent and drying, the expanded network collapses, though reversibly, so that it re-expands to its original size[9] on addition of water. As a consequence, the pore volume determined by mercury porosimetry is lower than the pore volume obtained from water regain. The pore volume from mercury porosimetry is virtually in accordance with pore volume from cyclohexane regain. The latter two methods give lower values of pore volume than water regain due to the swelling of the networks in water.

As the ratio of DOL to CYOL increases (samples B, C and D in *Table 1*), water regain also increases and the dependence of pore volume in the dry state (determined by both mercury porosimetry and cyclohexane regain) on the concentration of DOL in the diluent phase has a maximum at about 60 vol% DOL. The same behaviour was observed with styrene–divinylbenzene copolymers[10] and was explained as a result of the deteriorating solvating power of the diluent–monomer mixture with increasing concentration of non-solvating diluent. First, as the content of the non-solvating diluent in the diluent phase increases, pore volume increases as well. However, at a high concentration of the non-solvating diluent (80 vol% and more), it partly separates from the polymerization mixture before reaching the gel point and non-porous beads result. The presence of some non-porous beads in samples E and F thus reduces the pore volume, according to mercury porosimetry or cyclohexane regain, compared to samples C and D. However, this is not the case for water regain. As non-porous beads swell in water (not in cyclohexane), water regain of both samples E and F is not reduced.

The pore size and pore-size distribution were determined by mercury porosimetry. Pore-size differential distribution curves (*Figures 8* and *9*) show that the proportion of large pores (1 μm in diameter and larger) increases with increasing concentration of DOL in the diluent phase up to 60 vol%. Such size of pores in poly(HEMA) sponges implanted subcutaneously in rabbits supports cellular ingrowth and neovascularization[2]. However, the fraction of large pores is low again at a concentration of DOL in the diluent phase of 80 vol% and more (*Figure 9*).

Changes in the pore volume were observed depending on the drying method. The pore volume from mercury porosimetry of a freeze-dried sample is higher than that of a sample dried from ether (*Table 1*). The fraction of large pores in a freeze-dried sample (*Figure 7a*) also increased compared to that in the air-dried sample (*Figure 5a*). This fact is also confirmed in *Figure 10*. Water regain values of freeze-dried samples are higher than those of air-dried ones as well. This may be explained by the fact that in freeze-dried samples the swollen structure remains fixed even after the removal of water, resulting in a higher pore volume compared to

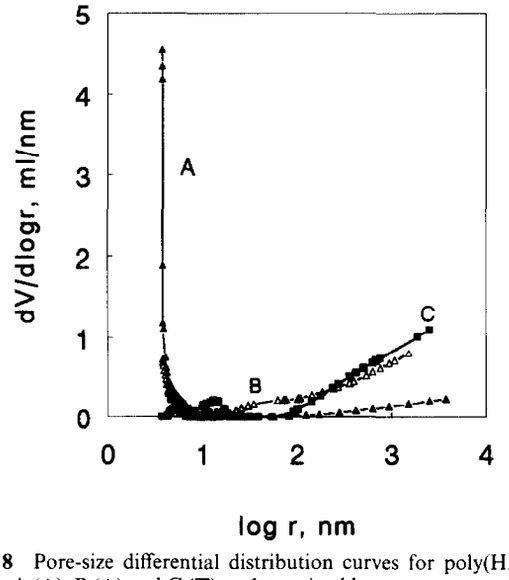

Figure 8 Pore-size differential distribution curves for poly(HEMA) samples A (▲), B (△) and C (■) as determined by mercury porosimetry

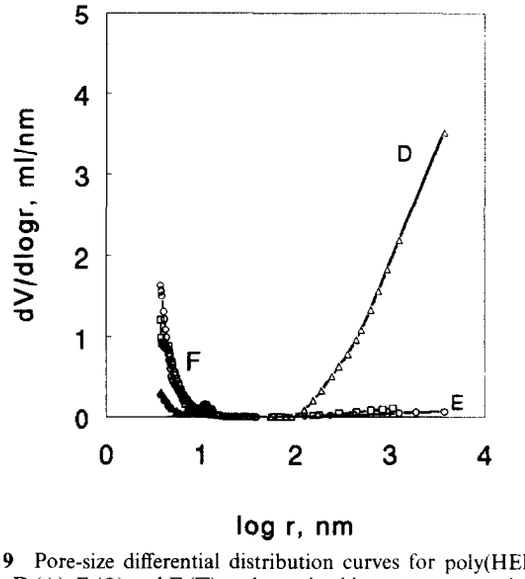

Figure 9 Pore-size differential distribution curves for poly(HEMA) samples D (△), E (○) and F (□) as determined by mercury porosimetry

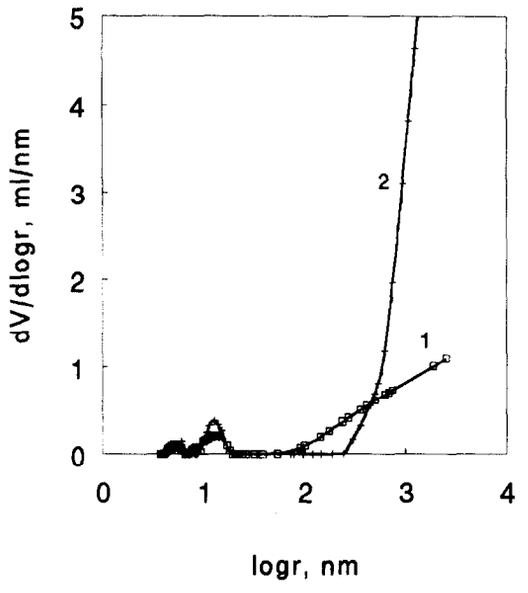

Figure 10 Pore-size differential distribution curves for poly(HEMA) sample C air-dried from ether (1) and freeze-dried (2)

that of air-dried samples from ether, where collapse of the structure occurs.

Swelling

The swelling of poly(HEMA) networks in an aqueous medium is especially important from the point of view of biocompatibility of the material. As a consequence of swelling, the dry porosity of poly(HEMA) networks does not correspond to the swollen-state porosity, i.e. to the situation after network formation[11]. Water regain of poly(HEMA) networks is thus always significantly higher than cyclohexane regain or pore volume from mercury porosimetry. The swelling is governed by two separate processes: (i) water filling of pores determined by the volume of diluent separated from the network phase during the polymerization; and (ii) solvation of network chains determined by the volume of diluent remaining in the network structure during the polymerization. The solvation depends on the crosslink density and on the interaction between water molecules and the network chains. This process is characterized by the equilibrium swelling ratio, q_V, of the network in water, if isotropic swelling is assumed (i.e. the volume of pores remains constant on swelling). Thus, q_V only includes the amount of water taken up by the gel portion of the network. Accordingly, the q_V values of samples E and F (prepared from polymerization mixtures containing 80 vol% DOL and neat DOL in the diluent phase, respectively) are relatively high compared to that of the corresponding standard copolymer G, thus indicating that most of the diluent remains in the network (gel) phase at the end of the network formation. Samples E and F thus show a high degree of swelling. As the ratio of CYOL to DOL in the diluent phase increases, q_V decreases rapidly, showing the separation of the diluent from the network phase. Sample D with the maximum pore volume exhibits a similarly low value of equilibrium swelling ratio in water as the standard copolymer G (*Table 1*) since most of the diluents separate from the network phase during the polymerization. A low value of swelling ratio was also observed with copolymer A formed in the presence of neat CYOL as a diluent. It can thus be concluded that, during network formation, a higher amount of DOL than CYOL remains in the gel throughout the polymerization.

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