Macroporous poly(2-hydroxyethyl methacrylate-co-ethylene dimethacrylate): Effect of cross-linking density and porogen on pore volume and specific surface area

T. S. Pathak[**], S. Ponrathnam[*], C. R. Rajan[*], G. Ingavle[*] S. V. Deshpande[*], A. M. Kotha[*]

[*]Chemical Engineering Division, National Chemical Laboratory, Pune 411008, India

[**]Seoul National University of Technology, Department of Chemical Engineering, Membrane and Environment Process Lab, 172, Gongneung-dong, Nowon-gu, Seoul, 139-743, Korea

1. Introduction

Macroporous, spherical, beaded, highly cross-linked copolymers [1-8] are commercially pertinent because they possess permanent rigid porous structure that persists in swollen as well as dry states. These are synthesized by suspension copolymerization of a vinyl monomer with a multi-vinyl comonomer, in sufficiently high relative mole ratio. The hydrophilic ones find use in enzyme immobilization, drug delivery devices, and as catalysts, HPLC supports and adsorbents [9-13]. The macroporous structure arises from the phase separation of the inert diluent (porogen) as the polymerization proceeds. This porogen is either a low molar mass compound or a polymer present in the discontinuous monomer phase. During suspension copolymerization, the growing cross-linked copolymer structure precipitates as soon as it becomes insoluble in the reaction medium. The size of the nuclei and hence the porosity formed is dictated by the nature of porogen, its relative volume as well as the cross-linking comonomer type and its volume [14-18]. The porogen, while miscible with the monomers, does not take part in the copolymerization and can be easily removed at the end of the reaction from the copolymer formed [19-23]. During copolymerization the nuclei develop into globules to generate microspheres, which at the same time associate to form particles. The development of microspheres and particles, which constitute clusters, depend on the porogen.

A systematic evaluation of the use of cyclohexanol as porogen was investigated in the preparation of a series of macroporous poly(2-hydroxyethyl methacrylate-co-ethylene dimethacrylate) [poly(HEMA-EGDM)], to be further evaluated as suitable supports for expanded bed chromatography matrices [24,25]. The aim was to establish the relationship between copolymer composition and porogen volume on pore size, pore size distribution as well as pore volume for this application.

2. Experimental

Materials used

2-Hydroxyethyl methacrylate (HEMA) and ethylene dimethacrylate (EGDM) were obtained from Sartomer, USA and used as received. Cyclohexanol used as porogen, was obtained from M/S Aldrich Chemical Co. (USA). Poly(vinyl pyrrolidone) [PVP] was obtained from Polysciences, USA and used as protective colloid. Azobisisobutyronitrile [AIBN], obtained from M/S SISCO, India was used as initiator.

Synthesis of poly(HEMA-EGDM) of differing cross-link densities (CLD).

The synthesis was conducted in double walled cylindrical reactor. The continuous phase comprised of one weight percent aqueous solution of PVP. The discontinuous organic phase consisted of HEMA, cross-linking divinyl monomer (EGDM), polymerization initiator [AIBN] and cyclohexanol. The discontinuous organic phase was introduced into the aqueous phase, stirring (with 6 bladed Ruston turbine) was set at 300 rotations per minute and the temperature was maintained at 70°C by circulating water set at that temperature. The polymerization was continued for 3 hours. The copolymer obtained in beaded form was separated by decantation, washed with water and methanol and dried at room temperature under reduced pressure. The composition of synthesized poly(HEMA-EGDM) are presented in Table 1.

Table 1. Feed ratios of 2-hydroxyethyl methacrylate (HEMA) and ethylene dimethacrylate (EGDM) in the copolymerization

EXPT.No	HEMA (cm^3)	HEMA (mol)	EGDM (cm^3)	EGDM (mol)	CLD %[a]	monomer:porogen[b]
HE1	5.9	0.0486	2.3	0.0122	25	1:2.43
HE2	4.6	0.0379	3.6	0.0191	50	1:2.43
HE3	3.8	0.0313	4.4	0.0233	75	1:2.43
HE4	3.2	0.0264	5.0	0.0265	100	1:2.43
HE5	2.5	0.0206	5.7	0.0302	150	1:2.43
HE6	2.0	0.0165	6.2	0.0329	200	1:2.43

[a]cross-link density (CLD) is defined as the mole percent of cross-linking monomer relative to the moles of reactive functional comonomer.

[b]monomer and porogen ratio were also varied such as 1:1.61 (HE7 to HE12), 1:0.81 (HE13 to HE18), 1:0.405 (HE19 to HE24) and 1:0 (HE25 to HE30), keeping HEMA and EGDM constant.

Porosity measurement

The porous properties were determined by mercury intrusion parameter in the pressure range 0 - 4000 kg/cm^2 with an Auto scan 60 mercury porosimeter from Quantachrome, USA. Evaluation of specific surface area by single point nitrogen adsorption method, also known as BET method, were conducted using monosorb surface area analyzer from Quantachrome Corporation (USA).

Scanning electron microscopy

Pore structure of poly(HEMA-EGDM) beads were studied using scanning electron microscopy (SEM). Specimen preparation was as follows: , dried poly(HEMA-EGDM) beads were mounted on stubs , and sputter-coated with gold. Micrographs were taken on a JEOL JSM-5200 SEM instrument.

IR-Spectra

A Shimadzu 8300-Fourier transform infra-red spectrophotometer (FTIR), with a resolution of 1 cm^{-1} in the transmission mode, was used. The sample poly(HEMA-EGDM) was milled, mixed with potassium bromide, and pressed into a solid disk of 1.2 cm diameter prior to the infra-red measurement.

Particle Size measurement

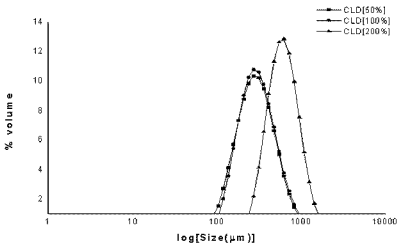
Mastersizer 2000 Malvern instrument was used to obtain size distribution in the synthesized poly(HEMA-EGDM) beads. Mastersizer 2000 uses an integrated optical system to cover the full range from 0.02 to 2000 μm.

3. Results and Discussions

Effect of cross-link density of poly(HEMA-EGDM) beads on particle size

Poly(HEMA-EGDM) beads were produced by suspension polymerization. Figure 1 shows that particle size distribution of poly(HEMA-EGDM) beads shifts to higher values with an increase in relative mole ratio of the cross-linking comonomer, EGDM. This is due to variances in solubility parameter with monomer feed ratios. The solubility parameter of monomer, comonomer and porogen are presented in Table 2 [26] and that of copolymers of differing cross-link density are presented in Table 3.

Figure 1. Particle size distribution of poly[2-hydroxy ethyl methacrylate-co-ethylene



dimethacrylate] beads by varying cross-link density.

Table 2. Solubility parameter δ, of individual component

Component	[a]δ $(cal/cm^3)^{0.5}$
HEMA	11.4
EGDM	8.9
Cyclohexanol	11.4

[a]δ can be calculated using the formula d .∑G/M where G is the molar attraction constant, ∑G is the sum for all the atoms and groupings in the molecules, d is the density and M is the molecular weight.

Table 3. Solubility parameter δ', of poly(HEMA-EGDM) beads having different cross-link density

CLD%	δ' (cal/cm^3)$^{0.5}$
25	10.7
50	10.3
75	10.1
100	9.9
150	9.7
200	9.5

The solubility parameter of copolymer was estimated as: $\delta' = v_1\delta_1 + v_2\delta_2$. (1) where δ_1 and δ_2 are solubility parameters of HEMA and EGDM, while v_1 and v_2 are the volume fraction of the monomer HEMA and EGDM, respectively. It can be seen that the solubility parameter decreases with an increase of the relative mole ratio of EGDM and hence cross-link density. Thus, with decreasing solubility parameter of copolymer, the critical chain length prior to precipitation increases, resulting in larger particles. So at lower cross-link densities, particle size is smaller with a narrower distribution as compared to copolymers with higher cross-link densities.

Suspension polymerization is a process in which monomers are dispersed as liquid droplets, mostly in an aqueous phase, by stirring and then polymerized. Suspension polymerization of a partially water soluble monomer such as HEMA is a challenge and this is overcome by decreasing the solubility of monomer in water using high boiling alcohols to solubilize the monomer and to act as porogen [27].

Infrared Spectroscopy

The typical spectra in Figure 2 shows peaks at 3421.5, 1718.5 and 1072 cm^{-1} due to stretching vibrations of -OH group, C=O of ester group and C-O of -COH group, respectively. The intensity of band at 1637.5 cm^{-1}, a characteristic band of C=C stretching does not disappear but is significantly weakened indicating that it is not completely consumed during polymerization and there are still some residual bonds buried inside the network structure.

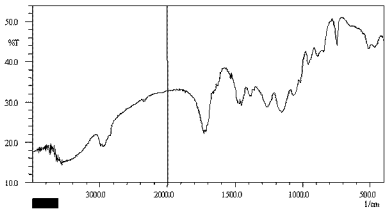


Figure 2. IR spectra of poly[2-hydroxy ethyl methacrylate-co-ethylene dimethacrylate] beads

Porous properties of poly(HEMA-EGDM) beads

Copolymers synthesized by suspension polymerization have specific pore size, pore size distribution, pore volume and surface area. The macroporous morphology and formation of porous texture have been extensively investigated for beaded, cross-linked styrene-divinyl benzene resins [28-29]. The internal pore structure can be controlled by several parameters such as amount of cross-linker, type and volume of diluent/porogen/pore generating solvent, added to the monomer phase. The larger pores, which are responsible for higher pore volume, are located in between agglomerates and arise when larger amount of cross-linker and porogen are used. Macroporous morphology in beaded polymers arises due to formation of gel microspheres, agglomeration of these and binding together of the agglomerates to form the beads. The appearance of gel microsphere, is dependent mainly on the cross-linker and to a smaller extent on the porogen.

During polymerization, the polymer phase separates from the solution due to its limited solubility in the polymerization mixture either due to build of molecular weight beyond that is soluble in the solvent (fractionation) or due to cross-linking. Phase separation generates the microspheres. Porosity in the beads may also be created in a controllable manner by using diluents/porogens, as extensively studied for polystyrene, polyacrylamide, polymethacrylates, poly(hydroxyethyl methacrylate), etc. [30-55]. It has been well established [56-59] that cross-linking and phase separation are responsible for the porous structure development and can be related to the cross-linking agent concentration, type, and concentration of the porogen and temperature. Porosity and surface area of the polymer beads are the most important functional characteristics in many applications [60-65].

Mercury porosimetry provides good estimates of pore size and pore size distribution in the range of importance to the utilization of network polymers as chromatographic materials. Copolymers prepared from monomer feed ratios low in the cross-linking comonomer (EGDM) and porogen (cyclohexanol) have low pore volume and surface area because a large number of nuclei are formed which tend to grow through each other. The data of pore volume and surface area for poly(HEMA-EGDM) are presented in Tables 4 and 5.

Table 4. Change in pore volume of poly(HEMA-EGDM) beads with varying monomer feed ratios and porogen volume

CLD %	m:p= 1:2.43	m:p= 1:1.61	m:p= 1:0.81	m:p= 1:0.405	m:p=1:0
	Pore volume (cm^3/g)	Pore volume (cm^3/g)	Pore volume (cm^3/g)	Pore volume (cm^3/g)	Pore volume (cm^3/g)
25	0.1850 (HE1)	0.0300 (HE7)	0.0080 (HE13)	0.0029 (HE19)	0.0017 (HE25)
50	0.3837 (HE2)	0.0460 (HE8)	0.0110 (HE14)	0.0097 (HE20)	0.0075 (HE26)
75	0.3952 (HE3)	0.2350 (HE9)	0.0540 (HE15)	0.0245 (HE21)	0.0049 (HE27)
100	0.4229 (HE4)	0.2645 (HE10)	0.0630 (HE16)	0.0613 (HE22)	0.0545 (HE28)
150	0.4510 (HE5)	0.4070 (HE11)	0.1380 (HE17)	0.0456 (HE23)	0.0329 (HE29)
200	0.8020 (HE6)	0.6740 (HE12)	0.1780 (HE18)	0.0373 (HE24)	0.0130 (HE30)

Table 5. Effect of copolymer composition and porogen volume on surface area of poly(HEMA-EGDM) beads

CLD %	m:p= 1:2.43	m:p= 1:1.61	m:p= 1:0.81	m:p= 1:0.405	m:p=1:0
	Surface area (m^2/g)	Surface area (m^2/g)	Surface area (m^2/g)	Surface area (m^2/g)	Surface area (m^2/g)
25	6.1501	7.2447	3.1395	0.1094	0.0984
50	77.2704	10.0716	5.2061	0.0987	1.2976
75	48.0534	83.3474	5.9236	8.6472	1.3478
100	65.0785	66.9399	22.2156	16.7574	12.6519
150	93.9843	77.7053	44.4315	12.7658	4.5853
200	76.1570	112.9446	52.7755	14.8769	2.8745

The porogen present during the network (gel phase) formation may remain in the network phase through out the polymerization, resulting in the formation of expanded network (swollen) or may separate out of the network phase, resulting in the formation of porous particle. The distribution of porogen between network and porogen phases (porogen in the pores) at the end of polymerization determines the total porosity of the resulting polymer.

Table 4, shows that pore volume is negligible at lower cross-link density and porogen volume and increases in both cross-link density and porogen. In the absence of porogen, there are no pores. The increase with porogen volume is due to an increase in the number of pores. Phase separation of growing copolymer chain from the porogen present in the monomer phase contributes to the generation of pores rather than phase separation from the monomers yet to be coupled to the copolymer chains. Table 4 shows that at fixed monomer to porogen ratio (1:2.43) an increase in CLD% from 25 (HE1) to 200 (HE6) increases the pore volume from 0.19 cm^3/g to 0.80 cm^3/g. Increase in cross-link density is likely to lead to an early phase separation of the growing copolymer chain. A highly cross-linked nucleus, however, will exhibit less tendency to swell in monomer mixture. The presence of more cross-linked nuclei will also decrease the tendency for the individual spheres to coalesce during polymerisation and thereby prevent additional enlargement of microglobules.

The inner pore volume is negligible at lower cross-link density and lower volume of porogen also because most porogen molecules are embedded in the network (gel) phase through out the copolymerization. At higher volume the porogen phase separates out of the network (gel) phase resulting in high pore volume. Table 4 shows that a decrease in monomer to porogen ratio from 1:2.43 to 1:0.405 at 100% CLD (HE4 and HE22), results in a decrease in the total pore volume from 0.42 cm^3/g to 0.06 cm^3/g.

It is observed in Table 5 that surface area is low at low cross-link density and lower amount of porogen, and that it increases with cross-link density and volume of porogen for copolymers of similar particle size distribution. In the absence of porogen, copolymer particles formed are devoid of inner pores and surface area is entirely due to the surface of the particles. While surface area increases with cross-link density, this trend is not very exact due to a concomitant decrease in the size of the microsphere.

Different applications of macroporous polymers require tailored pore size distributions. Macroporous copolymers that have the same chemistry but different pore size distributions can be prepared by varying the mole ratio of monomers as well as relative volume of porogen. Tables 6-10 shows pore size distribution profiles obtained for macroporous poly(HEMA-EGDM) beads.

Table 6: Effect of cross-link density on pore volume distribution in poly(HEMA-EGDM) beads without porogen

Expt. No	CLD %	Distribution in pore radii (vol%), radius in nm								
		<5	5 -10	10 -15	15 -20	20 -30	30 -50	50 -100	100 -300	>300
HE26	50	48.2	31.53	0.01	0.01	0.02	0.03	0.31	0.95	18.93
HE28	100	20.19	34.86	2.75	7.34	3.67	2.75	25.69	0.00	0.00
HE30	200	18.46	26.92	18.46	13.08	10.77	7.69	2.31	0.00	0.00

Porogen=cyclohexanol; CLD=cross-link density

Table 7: Effect of cross-link density on pore volume distribution in poly(HEMA-EGDM) beads at constant monomer:porogen ratio of 1:1.61

Expt. No	CLD %	Distribution in pore radii (vol%), radius in nm								
		<5	5 -10	10 -15	15 -20	20 -30	30 -50	50 -100	100 -300	>300
HE7	25	44.99	42.49	2.50	0.00	0.00	0.00	0.00	0.00	10.00
HE10	100	15.69	40.45	17.58	8.13	8.51	3.97	2.65	0.94	0.19
HE12	200	7.64	21.29	15.65	13.51	19.43	12.24	6.53	1.93	0.00

Porogen=cyclohexanol; CLD=cross-link density;

Table 8: Effect of cross-link density on pore volume distribution in poly(HEMA-EGDM) beads at constant monomer:porogen ratio of 1:2.43

Expt. No	CLD %	Distribution in pore radii (vol%), radius in nm								
		<5	5 -10	10 -15	15 -20	20 -30	30 -50	50 -100	100 -300	>300
HE1	25	0.00	0.00	0.00	0.00	0.00	50.00	50.00	0.00	0.00
HE4	100	24.83	42.81	3.60	2.29	0.00	0.98	0.00	23.20	0.00
HE6	200	2.88	3.59	2.69	2.33	3.66	6.73	18.20	52.60	3.21

Table 9: Effect of porogen volume on pore volume distribution in poly (HEMA-EGDM) beads at constant composition (200% CLD)

Expt. No	m:p	Distribution in pore radii (vol%), radius in nm								
		<5	5 -10	10 -15	15 -20	20 -30	30 -50	50 -100	100 -300	>300
HE30	1:0.00	18.46	26.92	18.46	13.08	10.77	7.69	2.31	0.00	0.00
HE18	1:0.81	23.31	48.6	15.17	6.46	5.62	0.28	0.00	0.00	0.00
HE12	1:1.61	7.64	21.29	15.65	13.51	19.43	12.24	6.53	1.93	0.00
HE6	1:2.43	2.88	3.59	2.69	2.33	3.66	6.73	18.20	52.60	3.21

Table 10: Effect of porogen volume on pore volume distribution in poly (HEMA-EGDM) beads at constant composition (50% CLD)

Expt. No	m:p	Distribution in pore radii (vol%), radius in nm								
		<5	5 -10	10 -15	15 -20	20 -30	30 -50	50 -100	100 -300	>300
HE26	1:0.00	45.95	33.78	0.00	0.00	0.00	0.00	0.00	0.00	0.00
HE14	1:0.81	72.72	18.18	0.00	0.00	0.00	0.00	0.00	0.00	0.00
HE8	1:1.61	17.39	29.35	6.52	5.43	1.09	0.60	3.26	2.18	5.43
HE2	1:2.43	13.84	30.68	19.45	5.35	0.00	0.00	27.16	3.26	0.00

Figure 3 represents the surface morphology of poly(HEMA-EGDM) beads.

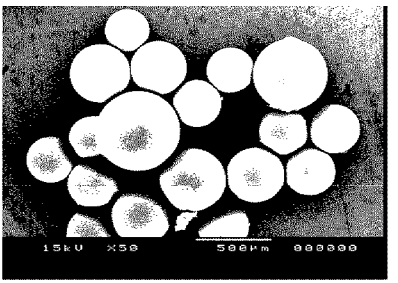


Figure 3. Surface morphology of poly[2-hydroxy ethyl methacrylate-co-ethylene dimethacrylate] beads

Table 6 indicates that at low CLD (50%) micropores are formed and the distribution spreads with increase in CLD, to the macropore range of 100 nm, though a large percent are still micropores.

The pore size distribution presented in Table 7 indicates that the median consist of micro and mesopores. Thus, cyclohexanol is a weak porogen for the poly(HEMA-EGDM) series.

Table 4 indicates that the pore volume increases to 0.19 to 0.80 cm^3/g at higher relative volume of porogen (1:2.43). As compared to copolymers synthesised at monomer: porogen ratio of 1:1.61, the pore size distribution (Table 8) shifts to meso and macroporous range. This shift decreases the surface area of copolymers (Table 5), indicating that the number of pores probably decreases with the relative increase in porogen volume.

Table 9 shows that pore size distribution shifted to smaller pores as the porogen volume decreases. The changes in these characteristics, which are obtained upon decreasing the percentage of porogen in the polymerisation mixture, are mainly due to a decrease in the volume of large pores (> 50 nm) and a parallel increase in volume of pores with size less than 50 nm.

Similarly, Table 10 shows that pore size distribution shifted to smaller pores as the porogen volume decreases. The changes in these characteristics, which are obtained up on decreasing the percentage of porogen in the polymerisation mixture, are mainly due to a decrease in the volume of large pores (> 50 nm) and a parallel increase in volume of pores with size less than 50 nm. The volume percentage of pore > 50 nm in HE2 (monomer:porogen = 1:2.43) is 30.42 which is 2.80 times greater than HE8 (monomer:porogen = 1:1.61) at fixed 50% cross-link density.

Figure 3 shows that the procedure yields spherical beads.

These copolymers will be evaluated for their suitability as base supports for anchoring chiral ligands.

Conclusion

Pore size and its distribution, as well as pore volume, in poly(HEMA-EGDM) are dictated by a combination of cross-link density and hence mole fraction of cross-linking comonomer in the feed as well as porogen type and its volume. In the 30 copolymers investigated surface area was in the range 0.1 - 98.0 m^2/g while pore volume was in the range 0.002 to 0.802 cm^3/g, depending on copolymer composition and porogen volume. Cyclohexanol, used as an

effective porogen for other methacrylate net-works, is a weak porogen for poly(HEMA-EGDM) system.

References

1. Guyot A, Bartholin M, (1992) Prog Polym Sci. 8: 277

2. Hodge P In, Hodge P, Sherrington DC, Eds, (1989) Syntheses and Separations Using Functional Polymers, Wiley, New York

3. Wijnhoven JEGJ, Vos WL (1998) Science 281:802

4.Tanev P T, Chibwe M, Pinnavaia TJ, (1994) Nature 368: 321

5. Maquet V, Jerome R, (1997) Mater Sci Forum 250: 15

6. Peters M.C, Mooney D J, (1997) Mater Sci Forum 250:43

7. Bancel S, Hu WS, (1996) Biotechnol Prog 12: 398

8. Viklund C, Svec F, Frechet JMJ, (1996) Chem. Mater 8:744

9. Glass E, Hydrophilic Polymers, (1996) Adv Chem Series, American Chemical Society, Washington, 248

10. Fishman M, Friedman R, Huang S. (1994) Polymers from Agricultural Coproducts, ACS Symposium Series, American Chemical Society, Washington DC

11. Jhonson P, Lloyd-Jones G, (1987) Drug Delivery Systems. Fundamentals and Techniques. (Ellis Horwood Serires in Biomedicine), VCH Verlagsgesellschaft:Weinheim, Germany

12. Kenawy E, Sherrington DC, (1992) Eur Polym J 28:841.

13. Galia M, Svec F, Frechet JMJ, (1994) J Polym Sci Polym Chem Ed 32: 2169.

14. Sederal WI, DeJong GJ, (1973) J Appl Polym Sci 17:2385.

15. Horak D, Svec F, Ilavsky M., Bleha M, Baldrian J, Kalal J, (1992) Angew Makromol Chem. 192: 113

16. Rosenberg JE, Flodin P, (1987) Macromolecules 20:1518

17. Okay O, Gurun G,(1992) J Polym Sci Polym Chem Ed 46: 401

18. Rabelo D, Coutinho FMB, (1994) Eur Polym J 30:675

19. Hamid MA, Naheed R, Fuzail M, Rehman E, (1999) Eur Polym J 35:1799

20. Lewandowski K, Svec F, Frechet JMJ, (1998) J Appl Polym Sci 67: 597

21. Horak D, Svec F, Ilavsky M, Bleha M, Baldrian J, Kalal J, (1981) Angew Makromol Chem, 95: 117

22. Horak D, Svec F, Ilavsky M, Bleha M, Baldrian J, Kalal J, (1981) Angew Makromol Chem, 95: 109

23. Liang YC, Svec F, Frechet JMJ, (1997) J Polym Sci Polym Chem, 35: 2631
24. Horak D, Pelzbauer M, Bleha M, Ilavsky M, Svec F, Kalal J, (1981) J Appl Polym Sci, 26: 411
25. Nayak DP, Ponrathnam S, Rajan CR, (2001) J Chromat A, 922: 63
26. Okay O, Gurun C, (1992) J Appl Poly Sci, 46: 401
27. Lowell S, Shields JE, Powder Surface Area and Porosity, Chapman and Hall Ltd., London, New York, pp97-120.
28. Hodge P, Sherrington DC, (1980) Polymer Supported Reactions in Organic Synthesis, Wiley, New York, London.
29. Jacobelli H, Bartholin M, Guyot A, (1979) J Appl Poly Sci, 23: 927
30. K.F. Mueller, S. Heiber, W. Flanká U.S. Patent, 4,224,472, 1978.
31. J. Seidel, J. Malinsky, K. Dusek and W. Heitz. (1967), Adv. Polym. Sci. 5: 113.
32. J.C. Moore. (1969), J. Polym. Sci., Part A 2 p. 835.
33. J. Coupek, M. Krivakova and S. Pokorny. (1973), In: J. Polym. Sci. Polym. Symp. 42: 185.
34. W. Heitz. (1977), Adv. Polym. Sci. 23: 1.
35. J. Kalal. (1978),In: J. Polym. Sci., Polym. Symp. Ed. 62: 251.
36. D. Horak, Z. Pelzbauer, M. Bleha, M. Havsky, F. Svec and J. Kalal. (1980), J. Appl. Polym. Sci. 26: 411.
37. P. P. Wieczorek, M. Ilavsky, B.N. Kolarz and K. Dusek. (1982), J. Appl. Polym. Sci. 27: 277.
38. A. Guyot and M. Bartholin. (1982), Prog. Polym. Sci. 81: 277.
39. Y. Ohtsuka, H. Kawaguchi and Y. Yamamoto. (1982), J. Appl. Polym. Sci. 27: 3279.
40. H. Galina, N.B. Colaz, P.P. Wiezorek and M. Woiszynska. (1985), Br. Polym. J. 17: 215.
41. M. Dimonie, H.D. Schell, G. Hubca, M.A. Mateescu, C.G. Oprescu, S. Todireanu, O. Maior, J. Languri and M. Iosif. (1985), J. Macromol. Sci.- Chem. A22: 729.
42. C. C. R. Robert, P.A. Buri and N.A. Peppas. (1985), J. Appl. Polym. Sci. 30: 301.
43. D. Horak, F. Svec, J. Kalal, K. Gumargalieve, A. Adamyan, N. Skuba, M. Titova and N. Trostenyuk. (1986), Biomaterials 7: 188.
44. O. Okay. (1986), J. Appl. Polym. Sci. 32: 5533
45. O. Okay. (1986), Angew. Macromol. Chem. 143: 209.
46. O. Okay. (1987), Angew. Macromol. Chem. 153: 125.
47. B. D. Barr-Howell and N.A. Peppas. (1987), Eur. Polym. J. 8: 591.
48. O. Okay. (1988), Angew. Macromol. Chem. 157: 1.

49. T. G. Park and A. S. Hoffman. (1990), J. Biomed. Mater. Res. 24: 21.

50. T.G. Park and A.S. Hoffman. (1990) Biotech. Bioeng. 35: 152.

51. B.N. Lolarz, M. Wojaczyriska and A.W. Trochimczuk. (1990), Macromol. Chem. 194: 1299.

52. O. Okay and W. Funke. (1990), Makromol. Chem. Commun. 11: 583.

53. T. G. Park and A S. Hoffman. (1992), J. Polym. Sci., Polym. Chem. Ed. 30: 505.

54. C. G. Gomez, C. I. Alvarez, M. C. Strumia, B. L. Rivas and P. Reyes, (2001), J. Appl. Polym. Sci., 79: 920.

55. Kemal Kesenci and Erhan Piskin, (1998), Macrmol. Chem. Phy., 199: 385.

56. Kucuk, Y.; Kuyulu, A.; Okay, O. (1995), Polym Bull. 35: 511.

57. Svec, F., Frechet, J. (1995) Macromolecules 28: 7580.

58. Horak, D.; Lendnickly, F.; Bleha, M. (1996) Polymer, 37: 4243.

59. Kuroda, H.(1995) Eur Polym J 31: 57.

60. G. Subramanium, B. A. Rhods, J. F. Cooper, V. S. Sodd, "Radiopharmaceuticals", Eds. Soc. of nuclear acad. New York, Ny, 1975.

61. J. Woodward, Ed. "Immobilized cells and Enzymes: A practical approach" IRL Press, Oxford. 1985.

62. A. Rembaum and Z. A. Tokl, "beads medical and biological applications" CRC Press, Bocaraton, FL, USA, 1976.

63. K. Mosbach. "Immobilized enzymes" in methods in Enzymology, Vol. 44, Eds., Academic press, New York, Ny, USA, 1976.

64. " Cell Separation Science and Technology" D. S. Kompale, P. Todd, Eds. ACS Symp.ser., 464, ACS Washington 1991.

65. R. Arshady, (1993), Biomaterial, 14: 5.