Radiation-Grafted Polymers for Biomaterial Applications. II. The Morphology and Structure of 2-Hydroxyethyl Methacrylate and Ethyl Methacrylate Homopolymer Grafts

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Synopsis

The 2-hydroxyethyl methacrylate (HEMA)/ethyl methacrylate (EMA) graft copolymer system has been found to be a useful model for investigating blood/polymer interactions. Studies of the structure of both HEMA and EMA radiation-grafted regions were conducted using extraction methods and transmission electron microscopy of selectively stained HEMA grafts. The effect of the extraction procedure on the surface topography of HEMA- or EMA-grafted films was studied by means of scanning electron microscopy. The existence of internal cells in the bulk of the HEMA network was demonstrated, and their osmotic nature was investigated. As grafting proceeds, the HEMA network becomes increasingly porous. The extraction studies carried out on EMA grafts showed that with this system an increasingly dense structure is obtained as grafting proceeds.

INTRODUCTION

One of the challenges in understanding the performance of blood-contacting biomaterials is elucidating the influence of surface chemistry on blood interactions. During recent years several hypotheses have been formulated which attempt to relate the blood compatibility with the composition of polymeric biomaterials.[1-5] One of these hypotheses suggests that a particular ratio of hydrophilic to hydrophobic sites at a surface may be important for optimum blood compatibility.[4,5] In earlier studies we have proposed a model system designed to test this hypothesis.[6-8] This system is based on grafted hydrogels of 2-hydroxyethyl methacrylate (HEMA) and ethyl methacrylate (EMA) polymers and copolymers.

A previous paper focused on the synthesis and characterization of radiation-grafted homopolymers and copolymers of HEMA and EMA on low-density polyethelene.[9] A detailed radiation dose study was conducted, and the grafted films were also characterized by means of scanning electron microscopy. A number of interesting questions concerning the structure and composition of these radiation grafts and the mechanism of their formation

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were raised by this work. Of particular importance were the following trends: (1) The HEMA grafting process follows a complex kinetic pattern characterized by an induction period, a slight autoacceleration, and a substantial drop in the graft level after a maximum level is reached, (2) EMA exhibits a more usual, diffusion-controlled kinetic behavior in which an early rise in graft level is followed by a plateau value for the graft level, and (3) the two monomers studied have different, inversely varying grafting rates: EMA shows a relatively fast reaction at the beginning of the grafting process, whereas the HEMA graft rate, which starts slowly, increases rapidly as grafting proceeds.

In an attempt to gain further insight into the nature of the HEMA/EMA model system, we have conducted studies complementary to those presented in Ref. 9 on the morphology, structure, and composition of the grafted homopolymers. An enhanced understanding of this system was obtained through extensive extraction studies and electron microscopic examination of selectively stained HEMA grafts. The present paper is a detailed account of this investigation. Complementary studies on the bulk and surface composition of co-grafted HEMA/EMA networks will be reported separately.

EXPERIMENTAL

Preparation of the Polymeric Surfaces

A radiation-induced graft polymerization technique was used to make HEMA and EMA homopolymer surfaces following the method developed in our laboratory and described previously.[6,9] Highly purified 2-hydroxyethyl methacrylate (HEMA) supplied by Hydromed Sciences, Inc., was used as received. Ethyl methacrylate (EMA) was purchased from Polysciences, Inc., and used after distillation at reduced pressure. Low-density polyethylene (LDPE) sheet (20 mil) was obtained from Cadillac Plastics, Seattle, Wa. All solvents were reagent grade.

The following parameters were calculated for each grafted film:

$$\text{Graft level (mg graft/cm}^2\text{)} = \frac{W_d - W_i}{A} \times 1000$$

$$\text{Water content (\%)} = \frac{W_w - W_d}{W_w - W_i} \times 100$$

where

W_w = wet weight of the blotted grafted polyethylene film (grams)
W_d = dry weight of the grafted polyethylene film (grams)
W_i = initial dry weight of the ungrafted polyethylene film (grams)
A = film area (cm^2)

Extraction Procedure

The extraction studies were conducted on dried grafted films. These films had been taken out of the grafting solution upon removal from the radiation source and washed by the following standard procedure: three 30-min rinses in

acetone : methanol (1 : 1) mixtures and three changes of deionized water over a 24-h period with agitation. The samples were then weighed wet after blotting to remove surface water and finally dried for 24 h in a vacuum desiccator at 1 mmHg over anhydrous $Mg(ClO_4)_2$.

To ensure the thorough extraction of ungrafted homopolymer, the extraction of these dry grafted systems was performed with good solvents for poly(HEMA) and poly(EMA): DMF and $CHCl_3$, respectively. The samples were immersed in the solvents at room temperature and taken out periodically for weighing until constant weight was attained. At this point the films were thoroughly washed with three one-hour soaks in an acetone : methanol (1 : 1) mixture, placed in three one-hour changes of deionized water, and then equilibrated in water over a 48-h period with agitation. After weighing the films, they were dried for 48 h in a vacuum desiccator at 1 mmHg over anhydrous $Mg(ClO_4)_2$. Finally, the graft level and the water content of the graft were calculated on the basis of the data obtained for the extracted grafts.

Since the behavior of the polymeric substrate could greatly affect the graft calculations which are based on gravimetric measurements, the extraction behavior of ungrafted LDPE samples in both solvents, DMF and $CHCl_3$, was also studied. The amount of material extracted from the control polyethylene film was subtracted from the total mass extracted from the grafted sample, resulting in the net mass leached out exclusively from the grafted polymer.

The extraction profile is reported in terms of curves showing the percentage graft weight change versus time. ΔW is the weight change of the blotted, swollen graft in the extraction solvent. W_g is the weight of the graft as obtained after the standard grafting, rinsing, and drying procedure.

Selective Staining of HEMA Grafts

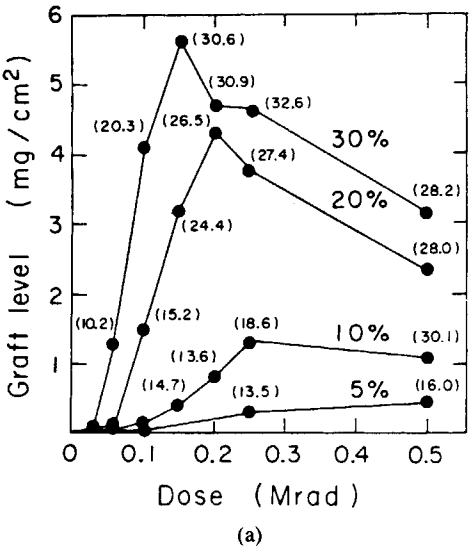
To better visualize the structure of the grafted network in the electron microscope, selective staining of the HEMA graft was performed. A staining technique was developed based upon the reaction of cinnamoyl chloride with the —OH group of HEMA and the subsequent reaction of osmium tetroxide with the C=C double bond present in the cinnamoyl chloride. Thus, an electron-dense agent selectively stained the HEMA regions.

Samples were immersed in 1*M* cinnamoyl chloride in toluene for 20 h at 60°C, rinsed as before, and finally allowed to air-dry overnight before sectioning.

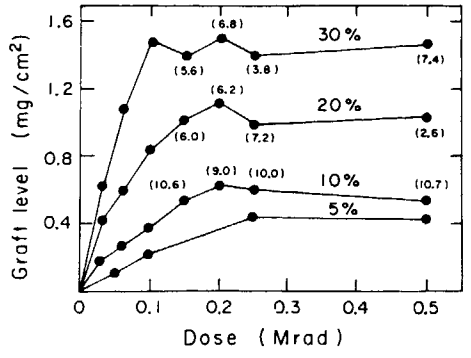
For light microscopy, ~ 10 μm sections were cut perpendicular to the graft surface with a razor blade on the Sorval MT2-B ultramicrotome. Thin sections, ~ 80 nm in thickness, for transmission electron microscopy (TEM) were cut parallel to the graft surface at room temperature using a dry glass knife and examined in a JEOL 100-CX electron microscope.

RESULTS AND DISCUSSION

One of the primary goals of this study was to further investigate the structure of the grafted networks, especially that of the HEMA grafts, and its dependence on important experimental parameters, such as radiation dose and monomer concentration. This investigation was performed to help understand



(a)



(b)

Fig. 1. Effect on the graft level of radiation dose on the grafting of HEMA (a) and EMA (b) onto polyethylene. (The numbers in parentheses report the water content.)

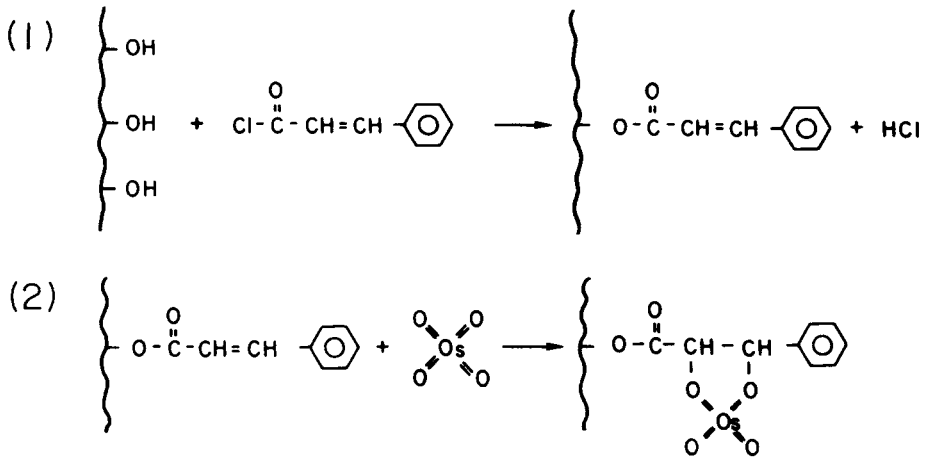
the rather unusual, complex kinetic behavior exhibited during the HEMA grafting process, the main features of which are an induction period, a fast increase in graft level, and a substantial decrease after a maximum graft level is reached [see Fig. 1(a)]. The numbers in parentheses along the graft level versus dose curves report the water content of the graft for that particular specimen. Of special interest is the unexpected substantial drop in HEMA graft level which is especially prominent at higher monomer concentrations.

In a previous study[9] we suggested that this decrease in graft level could be accounted for by considering that entrapped homopoly(HEMA) along with some of the longer grafted chains onto the polyethylene surface would most readily dissolve in the grafting solvent system. This could create pockets under the surface filled with a viscous poly(HEMA) solution. These pockets then could act as "osmotic cells" due to the lower activity of the monomer and solvent molecules in these regions as compared to the surrounding solution. The resulting activity or concentration gradient would accelerate

monomer and solvent diffusion into these pockets. This diffusion, in turn, could lead to expansion of the "osmotic cells" until they eventually burst, allowing the homopolymer to be washed out of the surface and be extracted more efficiently from the bulk of the graft. Thus, the end result would be the observed "apparent" decrease in graft level at higher doses. Clearly, this "osmotic cell" phenomenon should greatly affect the topography of the grafted network and largely determine its structure. In order to understand and illustrate this mechanism better, the HEMA graft structure was systematically investigated by TEM and light microscopy to observe stained graft regions and by conducting extraction studies.

Staining of the HEMA Graft

The staining technique was developed based upon the reaction of cinnamoyl chloride with the —OH groups on HEMA, and the subsequent reaction of the C=C double bond present in the cinnamoyl chloride with osmium tetraoxide. These reactions are shown below:



As a result, an electron-dense staining agent selectively stains the HEMA regions. Since the osmium tetraoxide colors the sample in the visible light region, we also used light microscopy to investigate the grafted films. Figure 2 shows several grafted samples stained by this technique. The EMA graft, lacking the —OH group, clearly contrasts with the various HEMA-containing grafted films. It can be seen that, as a result of increasing HEMA concentration in the grafting solution (upper row) or as a function of increasing radiation dose (lower row), the thickness of the stained layer increases with graft level.

A transmission electron microscopy study of all grafted films was performed. The stained sections presented in Figure 3 clearly reveal an increasingly open, porous structure. It is apparent that at the beginning of the grafting process at low doses, the grafted network is only slightly porous [Fig. 3(a)], but as grafting proceeds, the structure of the graft becomes increasingly porous [Figs. 3(b) and (c)]. This graft level/porosity relationship is even clearer for a higher monomer concentration, as shown in Figure 4 for HEMA monomer concentration of 30%. Such an increase in porosity is consistent with the "osmotic cell" mechanism presented earlier. TEM inspection of the

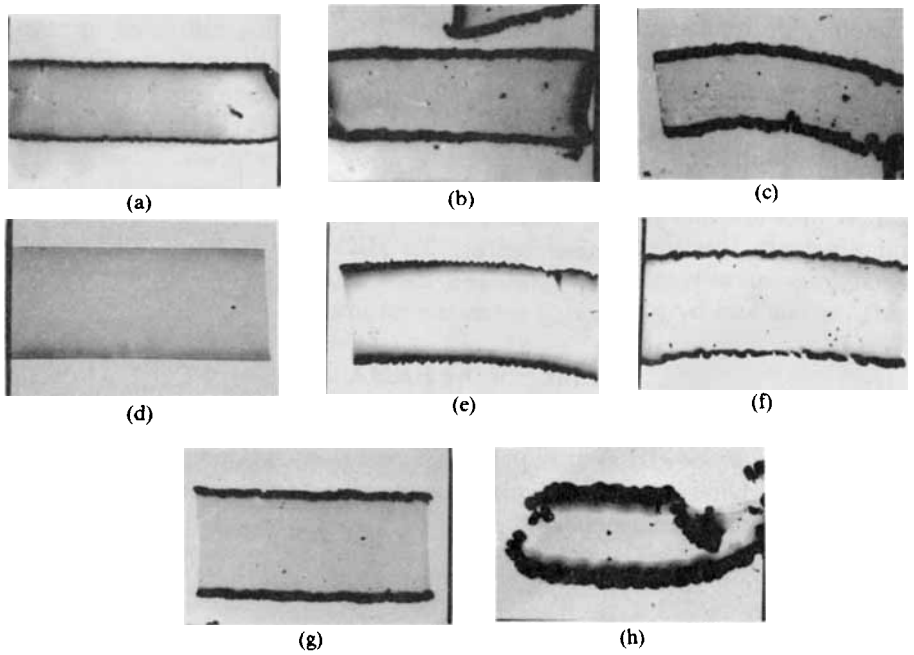


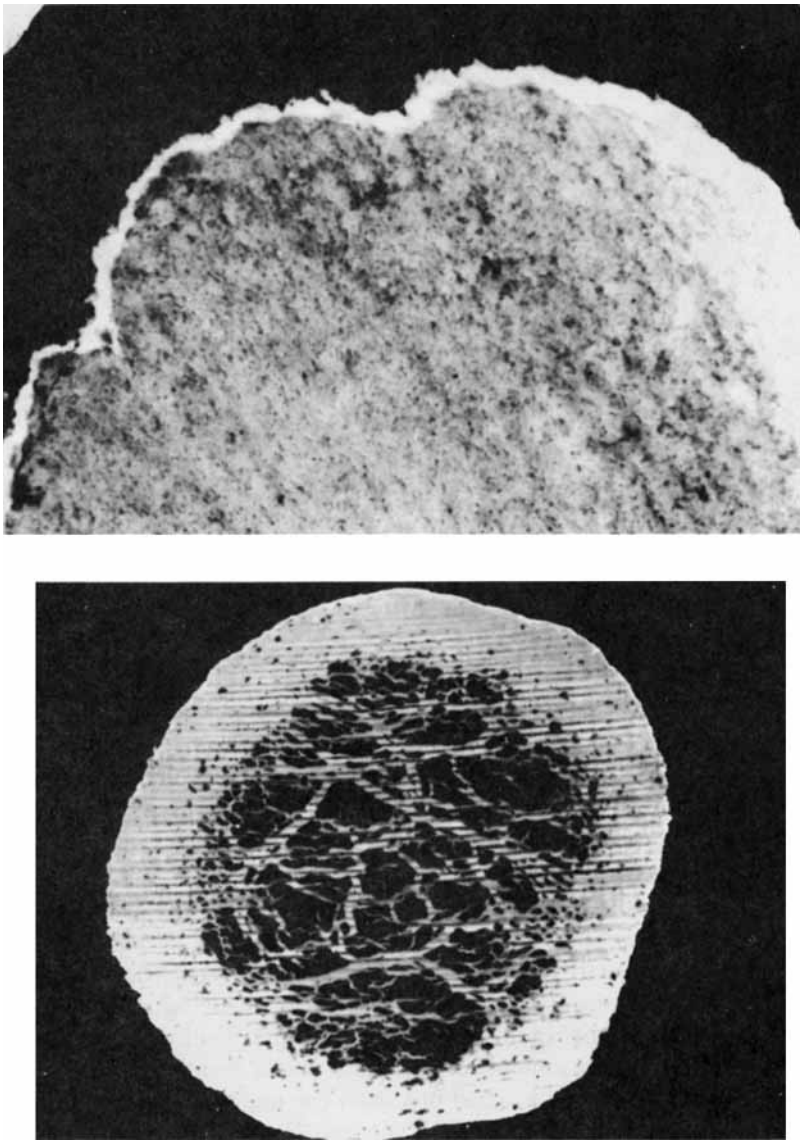
Fig. 2. Cross sections of HEMA/PE and EMA/PE grafts. Light micrographs show graft layers after sequential cinnamoyl chloride and osmium tetraoxide staining. Mag. 50 ×. Upper row: (a) HEMA graft-0.32 mg/cm^2, [5%], 0.25 Mrad; (b) HEMA graft-1.58 mg/cm^2, [10%], 0.25 Mrad; (c) HEMA graft-2.38 mg/cm^2, [20%], 0.25 Mrad; (d) EMA graft-0.59 mg/cm^2, [10%], 0.25 Mrad. Lower row: (e) 0.16 mg/cm^2, [10%], 0.10 Mrad; (f) 0.43 mg/cm^2, [10%], 0.15 Mrad; (g) 1.35 mg/cm^2, [10%], 0.25 Mrad; (h) 4.06 mg/cm^2, [30%], 0.10 Mrad.

stained grafts clearly shows the existence of cells within the grafted networks. These pockets are, in some cases, larger than 15 μm across.

Also, for a given dose, the more concentrated the monomer solution is, the more porous the graft appears to be [compare Fig. 3(b)–4(a) and 3(c)–4(b)]. This finding provides further support for the "osmotic cell" mechanism. For the 30% HEMA concentration system at the relatively low dose of 0.20 Mrad, huge cells, approximately 20 μm in diameter, are barely covered by a thin skin of stained material. These extreme cases provide additional support for the bursting of the cells due to the osmotic nature of the process taking place and are in full agreement with previously reported data.[9] The appearance of burst cells, such as the one shown in Figure 4(b) where the outer skin has been pierced in several places, correlates with the graft level, which reaches a maximum followed by a sharp decrease [see Fig. 1(a)].

HEMA GRAFT EXTRACTION STUDIES

The grafted films in these experiments were thoroughly extracted at room temperature until constant weight was obtained. To ensure that only ungrafted material would be extracted and that the graft itself would not be dissolved or distorted by the extracting agent, extraction of the specimens at both high temperature and ambient temperature was studied. Gravimetric measurements and SEM observations of the sample showed that the structure



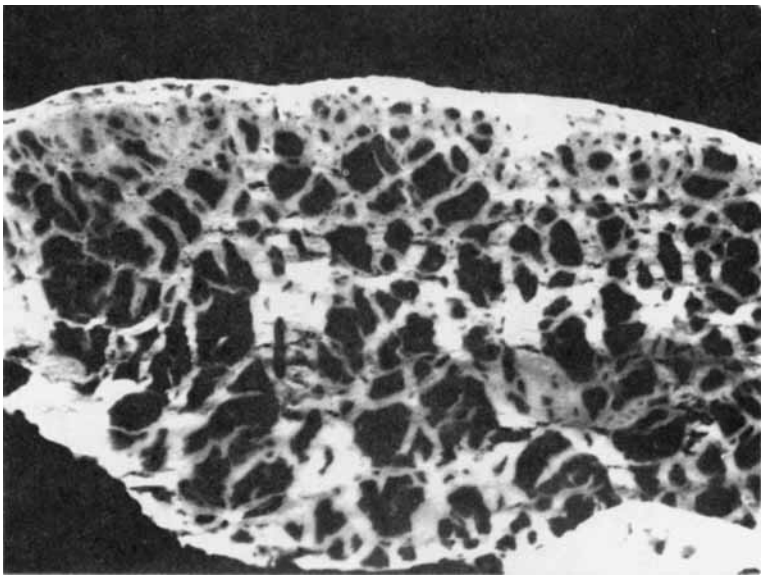
(a)

(b)

Fig. 3. TEM micrographs of HEMA grafts (monomer concentration = 20%), after sequential cinnamoyl chloride and osmium tetraoxide staining. (a) 0.10 Mrad, 1.48 mg/cm^2; (b) 0.15 Mrad, 3.18 mg/cm^2; (c) 0.20 Mrad, 4.30 mg/cm^2.

of the graft is affected to some extent by the DMF treatment only at higher temperatures ($> 50°C$); a minimal effect of solvent on the structure of the graft at low temperatures is expected based upon the resistance of LDPE to this solvent. Therefore, it was concluded that only ungrafted material could be extracted at room temperature.

The effect of the radiation dose and HEMA concentration on the kinetics of the extraction process are graphically illustrated in Figures 5 and 6. The data are plotted as the percentage change in solvent-swollen graft weight versus time curves. The data presented in Figures 5 and 6 represent minimum upper



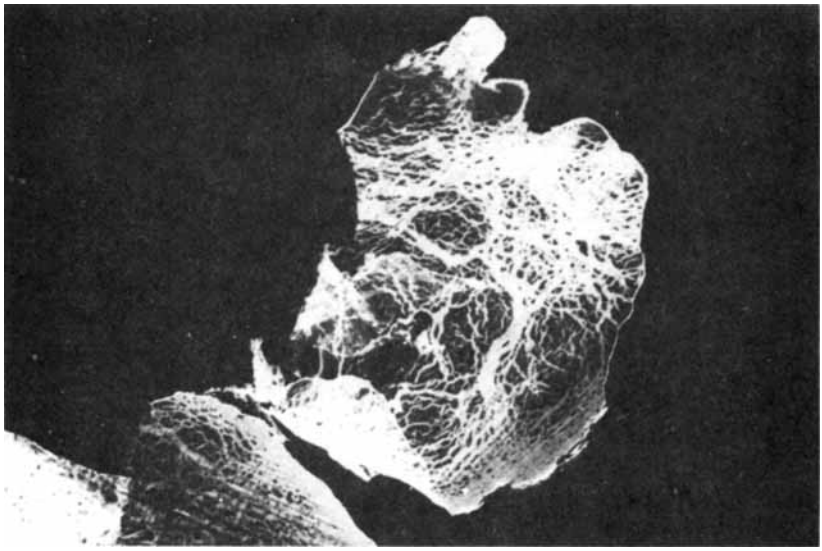
(c)

Fig. 3. *(Continued from previous page.)*

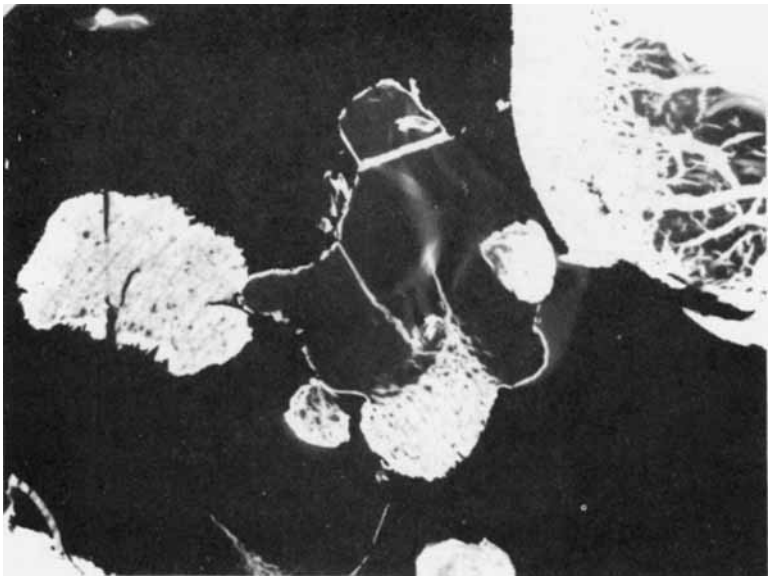
levels of solvent uptake, since the weight increase due to absorption is simultaneously influenced by weight loss due to extracted graft material.

Separate experiments were conducted on ungrafted LPDE films, so that the contribution of the substrate to the gravimetric data obtained for the grafted samples could be accounted for and subtracted. The effect of DMF on the LDPE films was almost negligible. The situation was quite different when the LDPE films were immersed in $CHCl_3$. In this case, the swelling of the film was pronounced, reaching an equilibrium swelling degree of 29 wt%. Chloroform was therefore much more effective as an extraction solvent than DMF and as much as 1.2% of the initial weight of the film was extracted. Although 1.2% is not a high percentage of the initial mass of the LDPE film, it is a very large amount compared to the amount of graft and would greatly affect our results. Correction for the material leached out from the ungrafted substrate and was made as described in the experimental section.

From Figure 5, the basic pattern of the extraction behavior is observed to be essentially similar for systems at different stages of the grafting process, differing only in quantitative terms. Three clearly different stages can be described: an initial, large, fast weight increase, a subsequent weight decrease, and after the extraction process has been completed, an absorption equilibrium swelling level. Two features are worth noting: first, both the maximum and the equilibrium uptake levels are higher for larger radiation doses, and second, excluding the 0.50 Mrad dose plot, the higher the dose, the larger the weight decrease once the maximum absorption level has been attained. This behavior can be attributed to the "osmotic cell" mechanism proposed earlier in which an osmotic pressure difference would accelerate solvent diffusion into the increasingly large pockets created in the increasing grafted network.



(a)



(b)

Fig. 4. TEM micrographs of HEMA grafts (monomer concentration = 30%), after sequential cinnamoyl chloride and osmium tetraoxide staining. (a) 0.15 Mrad, 5.60 mg/cm^2; (b) 0.20 Mrad, 4.69 mg/cm^2.

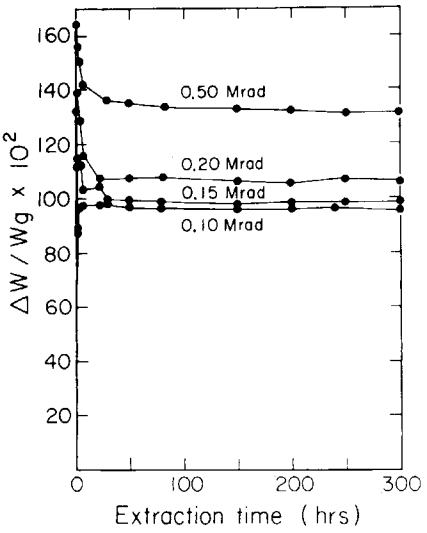


Fig. 5. Extraction profile of HEMA grafts ((monomer concentration = 20%), in DMF.

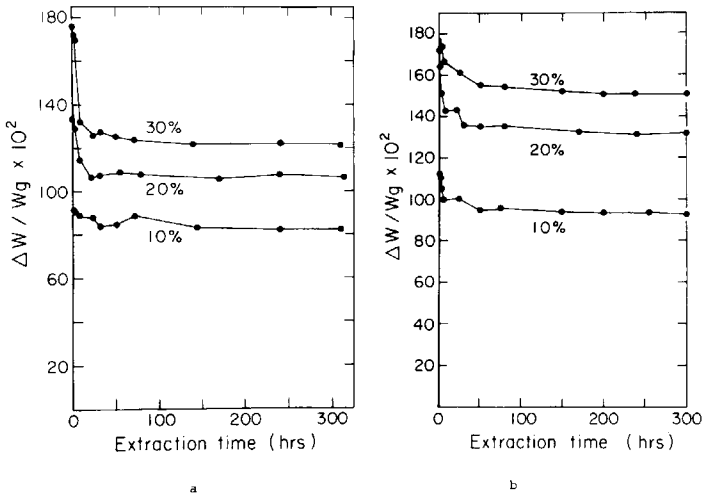


Fig. 6. Extraction profile of HEMA grafts in DMF, for two radiation doses. (a) 0.20 Mrad; (b) 0.50 Mrad.

At the beginning of the grafting process (0.10 Mrad), a relatively low equilibrium swelling level is obtained. The proposed mechanism, in accordance with our TEM findings that showed a relatively dense graft structure [see Fig. 3(a)], would explain the low equilibrium solvent uptake and the absence of measurable amounts of leachable material. As grafting proceeds, the osmotic driving force for the process is evident [see Fig. 3(b) and (c)] with the appearance of open, porous structures. These structures can absorb large amounts of DMF and contain measurable amounts of leachable material. The smaller weight decrease shown by the high-dose (0.50 Mrad) graft when compared to the lower dose curves could be attributed to the fact that many

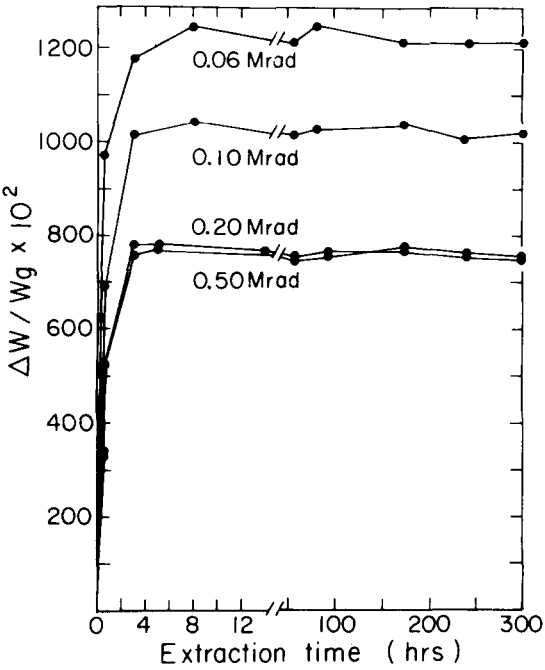


Fig. 7. Extraction profile of EMA grafts (monomer concentration = 20%) in $CHCl_3$.

internal cells have already burst during the radiation period, permitting material expelled from the burst cells to be washed out of the surface and more efficiently extracted from the bulk of the graft by the standard rinsing procedure used. Even though it is not clearly shown by the plotted data (Fig. 5), the gravimetric results also suggest that the larger the dose, the faster the maximum uptake level is attained; this trend indicates that the porosity of the grafted network increases as grafting proceeds.

The extraction trends seen in Figure 6 are similar to the ones discussed already. In this case, the extraction behavior of three grafts having different monomer solution concentrations was studied for two doses: 0.20 Mrad [Fig. 6(a)] and 0.50 Mrad [Fig. 6(b)]. These results support the observations described previously and emphasize the primary importance of osmotic phenomena in determining the structure of the grafted HEMA network. The higher the monomer concentration is in the solution, the larger its concentration gradient between the solution and the cell, and consequently, the larger the driving force causing preferential diffusion of monomer into the graft.

EMA Graft Extraction Studies

The EMA grafts exhibited an extraction behavior substantially different from the one shown by the HEMA systems. Contrary to the trend seen for HEMA samples, Figure 7 shows that the final equilibrium uptake levels are lower for larger radiation doses. Also, the absorption process, as expressed by the first stage of the plots shown in Figure 7, slows down as the grafting reaction proceeds.

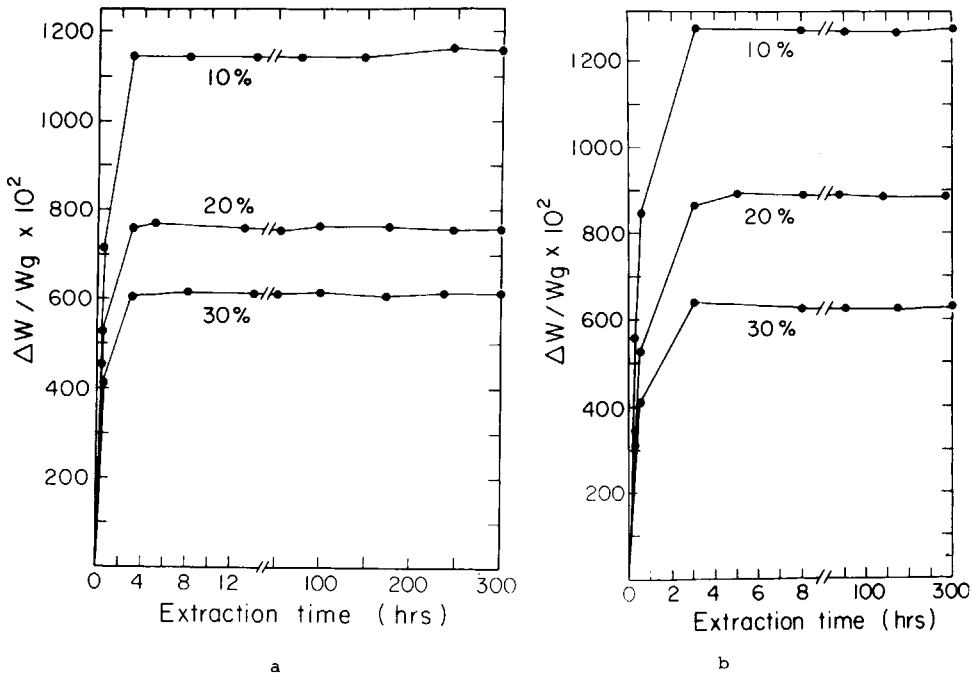


Fig. 8. Extraction profile of EMA grafts in $CHCl_3$, for two radiation doses: (a) 0.20 Mrad; (b) 0.50 Mrad.

A previous study[9] which investigated the kinetics of the grafting process [see Fig. 1(b)] showed that EMA follows a kinetic pattern typical of a reaction which becomes monomer diffusion controlled as the reaction progresses. This behavior was attributed to the polar nature of the grafting solvent (an ethanol–water solvent mixture) which would cause the growing, hydrophobic poly(EMA) graft to be less swollen or even "precipitated" by the grafting solvent system. These considerations are in full agreement with the data shown graphically in Figure 7. The fact that there is no difference in the behavior exhibited by the 0.20 and 0.50 Mrad systems implies that there is no significant structural difference between these two grafts; this similarity also agrees with the kinetic data reported earlier [see Fig. 1(b)].

Further support for this dense EMA graft structure is provided by the data plotted in Figure 8. Once again, contrary to the behavior shown by HEMA, the higher the EMA concentration in the grafting solution, the denser the grafted network and, consequently, the slower the absorption process and the lower the final uptake level.

Characterization of the Extracted Grafts

After the final absorption equilibrium level was attained, the samples were thoroughly rinsed, equilibrated with water, and finally dried. Based on the data obtained from these new wet and dry samples, corrected graft levels and water contents were calculated and compared to those obtained for the same grafts prior to the extraction scheme. The postextraction samples had, as

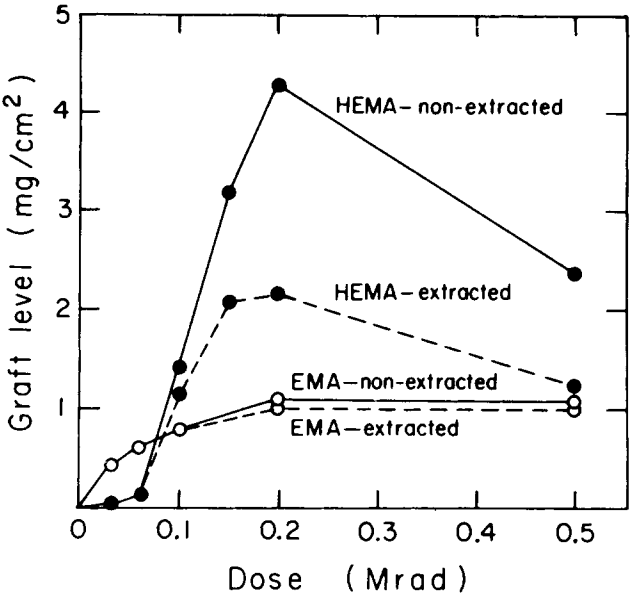


Fig. 9. Effect of the extraction procedure on the graft level versus dose curves, for both monomers (monomer concentration = 20%).

expected, lower graft levels. As illustrated in Figure 9, the graft level versus dose curves for both monomers were affected by the extraction process. It is clear that the extraction lowers the reported HEMA graft levels, while the EMA samples were only slightly affected. Nevertheless, it is apparent that the basic kinetic pattern followed by each monomer (as shown by the curve shape) remains unchanged.

Since, for the EMA system, the lowering of the graft level by extraction was very small, Tables I and II summarize only the data obtained for the markedly affected HEMA grafts. It is apparent from the data reported in Table I that the postextraction graft levels exhibit a significant decrease compared to pre-extraction levels, and that the water content increases after extraction. Because the affinity of the poly(HEMA) for water is a fixed parameter of the system, changes in the equilibrium water uptake are determined primarily by replacement of poly(HEMA) void volume. The increased water content can be attributed to the fact that at least part of the extracted material occupied, prior to the extraction procedure, a portion of

TABLE I
Graft Level and Graft Water Content in HEMA Grafts, Before and After Extraction
(HEMA solution concentration = 20%)

Radiation dose		0.10 Mrad	0.15 Mrad	0.20 Mrad	0.50 Mrad
Graft level (mg/cm^2)	Before extraction	1.41	3.18	4.29	2.37
	After extraction	1.22	2.08	2.15	1.24
Water content (%)	Before extraction	16.0	24.6	26.5	27.7
	After extraction	27.5	34.7	40.0	38.1

TABLE II
Graft Level and Graft Water Content for HEMA Grafts, Before and After Extraction

HEMA solution concentration		Dose = 0.20 Mrad			Dose = 0.50 Mrad		
		10%	20%	30%	10%	20%	30%
Graft level (mg/cm^2)	Before	0.77	4.29	4.65	1.14	2.37	3.13
	After	0.55	2.15	2.40	0.59	1.24	1.70
Water content (%)	Before	12.5	26.5	30.6	28.2	27.7	28.0
	After	24.0	40.0	38.7	49.9	38.1	36.2

the void volume of the graft; after extraction, the newly created voids become available to additional water. Moreover, the calculated graft level and water content prior to extraction were based on systems having, in some cases, an artifactually high graft level due to the weight of the ungrafted material trapped inside or around the grafted regions.

The data presented in Table II establish the effect of the monomer concentration on the behavior of the graft before and after the extraction procedure. As in the data of Table I, the graft level decreases and the water content increases after extraction for each case.

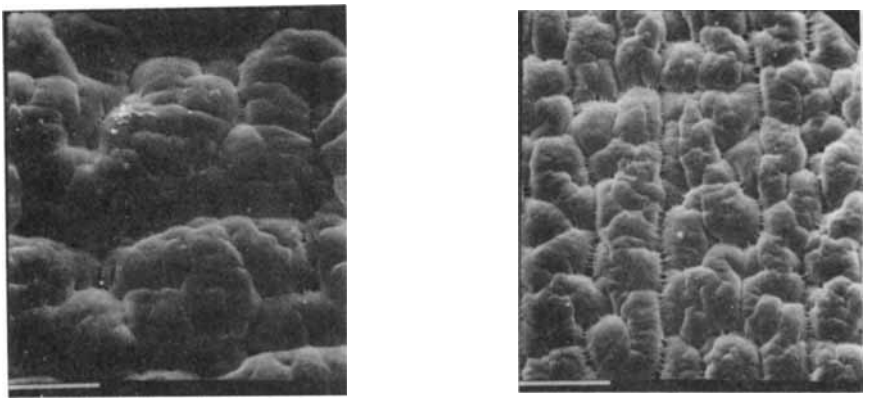
According to the proposed mechanism, the "osmotic cells" created in the graft would grow and expand as grafting proceeds and would eventually burst, allowing the ungrafted material to be washed out of the surface regions and to be more efficiently extracted from the bulk of the graft. Thus, if only the routine rinsing procedure is used, part of the nongrafted material stays inside and around or on top of the unburst cells. When extracted by the DMF procedure, these two components of the ungrafted, extractable material affect the postextraction water content in different ways. Only material extracted from the bulk of the graft will affect the equilibrium water uptake, whereas the material washed out of the surface regions could affect both the appearance of the grafts and the water content.

More detailed calculations were performed in an attempt to verify the source of the extracted material. We present two cases of these calculations for a 20% monomer concentration system, at two different stages of the grafting process: 0.10 and 0.50 Mrad (see Table III).

TABLE III
Graft Weight and Equilibrium Water Absorption Uptake for HEMA Grafts Before and After Extraction
(HEMA solution concentration = 20%)

Radiation dose		0.10 Mrad	0.15 Mrad	0.20 Mrad	0.50 Mrad
Graft weight (mg)	Before	20.76	46.68	62.81	34.79
	After	17.96	30.50	31.45	18.13
Extracted material (mg)	Bulk[a]	2.80	0.88	0	0
	Surface[a]	0	15.30	31.36	16.66
	Total	2.80	16.18	31.36	16.66
Water content (mg)	Before	3.90	15.30	22.60	13.40
	After	6.82	16.17	21.00	11.18

[a] These are calculated values.

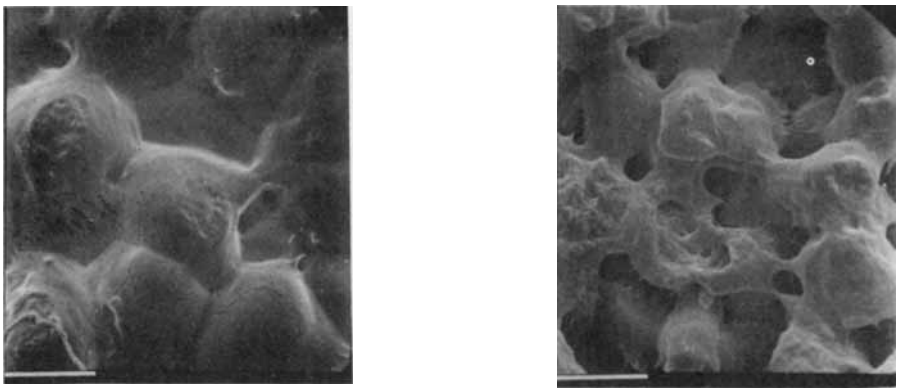


(a) (b)

Fig. 10. SEM micrographs of a HEMA graft, before and after extraction in DMF (monomer concentration = 20%; radiation dose = 0.10 Mrad). Bar = 10 μ. (a) Nonextracted, 2000 ×, 0 tilt; (b) extracted, 2000 ×, 0 tilt.

In the first case, a 0.10 Mrad dose resulted in a graft weighing 20.76 mg which equilibrated with 3.90 mg water. Comparing the postextraction dry weight with the pre-extraction dry weight shows that the DMF treatment caused the extraction of 2.80 mg ungrafted material. The extraction procedure resulted in a 17.96 mg grafted network having 6.82 mg water equilibrium uptake. Neglecting factors such as density differences and more complicated free volume considerations, we hypothesize that the 6.82 mg water absorbed by the graft after extraction occupy (1) the volume of the 3.90 mg water absorbed by the graft prior to the extraction procedure and (2) the internal volume of the graft previously occupied by the 2.80 mg ungrafted, now extracted, material. The excellent agreement existing between the calculated [(3.90 + 2.80 mg) = 6.70 mg] water the graft should absorb and the experimental (6.82 mg) water actually absorbed by the extracted graft indicates that the extracted material was leached out from the bulk of the grafted network. Strong support for this conclusion is provided by the scanning electron micrographs shown in Figure 10. It is apparent that there are no burst pockets in the graft prior to extraction [Fig. 10(a)] and that the surface of the graft is completely clean. Moreover, comparison of Figures 10(a) and 10(b) clearly shows the significant decrease in bump size from ~ 15 μm caused by the extraction procedure.

The 0.50 Mrad dose resulted in a graft different in character from the 0.1 Mrad graft; in this case, the absence of an appreciable effect on the water content by the DMF treatment suggests a washing off of the surface material with little material extracted from the bulk of the grafted network. Based on the same reasoning and following the same steps as above, the calculations for the 0.50 Mrad system (Table III) indicate that the 16.66 mg decrease in the weight of the graft was due to the washing off of poly(HEMA) deposited on the surface of the graft after internal cells burst. This result is consistent with the substantial drop in the weight of the graft, from 34.79 to 18.13 mg, and



(a) (b)

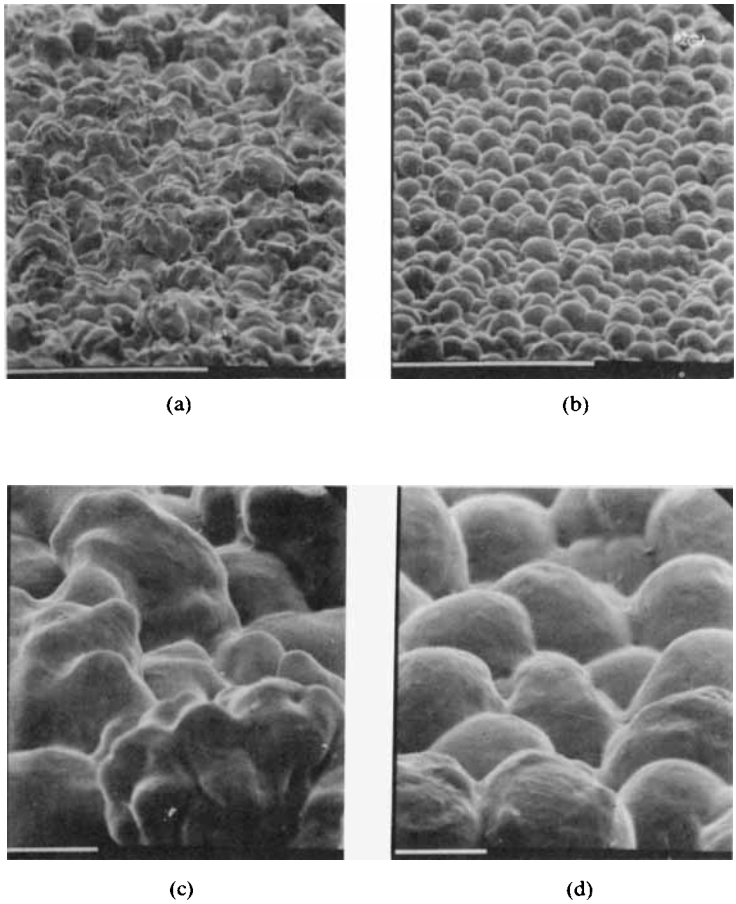
Fig. 11. SEM micrographs of a HEMA graft, before and after extraction in DMF (monomer concentration = 20%; radiation dose = 0.50 Mrad). Mag. 2000×, 0 tilt, Bar = 10 μ. (a) Nonextracted; (b) extracted.

with the fact that there is no net increase in the absolute amount of water the graft is able to absorb.

The electron micrographs presented in Figure 11 illustrate how the DMF treatment affected the HEMA graft surface. The effect of DMF in cleaning the HEMA graft surface is shown clearly in Figure 12 where micrographs of a high monomer concentration (30%), high radiation dose (0.25) Mrad) graft are shown. The graft exhibits, prior to the extraction procedure, an evenly coated surface having a velvet-like appearance; this surface clearly contrasts with the rather clean surface shown by the extracted graft.

The data summarized in Table III show, for a 20% HEMA concentration, that at the early stages of the grafting reaction, the bulk of the graft is the exclusive source of extractable material; however, as grafting proceeds, the ungrafted extractable material exists not only in the bulk of the grafted network but also on its surface, as a result of the bursting phenomenon. For very high graft levels (high dose), the extractability observed for the graft may be restricted to material on the surface of the graft.

Table IV summarizes the data as a function of varying HEMA solution concentrations for two given doses, 0.20 and 0.50 Mrad. Only for quite diluted grafting solutions (10%) there was ungrafted extractable material in the bulk of the graft; for grafts prepared with more concentrated solutions (i.e., 20% and 30%), the calculations suggest that the material was not extracted from the bulk of the network, but rather washed out from its surface. These observations can be understood by realizing that increase in the initial monomer concentration can increase the viscosity of the medium due to the competing homopolymerization reaction. Moreover, the higher the monomer concentration in the solution, the larger the driving force causing preferential diffusion of monomer into the internal cells in the bulk of the graft. The combined action of these factors could cause further polymerization and eventually, cross-linking due to chain transfer reactions of the nongrafted poly(HEMA) trapped in the bulk of the graft; hence, the extractability of the nongrafted poly(HEMA) may be greatly reduced.



(a) (b)

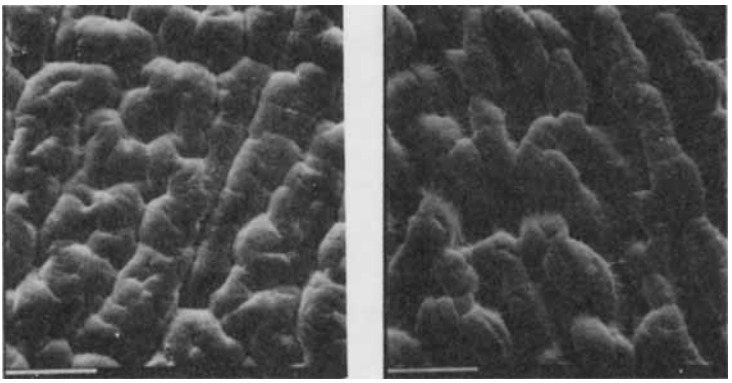
(c) (d)

Fig. 12. SEM micrographs of a HEMA graft, before and after extraction in DMF (monomer concentration = 30%; radiation dose = 0.25 Mrad). (a) and (b) 450 ×, 60 tilt, Bar = 100 μm; (c) and (d) 2000×, 60 tilt, Bar = 10 μ. (a) Nonextracted; (b) extracted; (c) nonextracted; (d) extracted.

TABLE IV
Graft Weight and Equilibrium Water Absorption Uptake for HEMA Grafts Before and After Extraction

HEMA solution concentration		Dose = 0.20 Mrad			Dose = 0.50 Mrad		
		10%	20%	30%	10%	20%	30%
Graft weight	Before	12.29	62.81	68.26	16.74	34.79	45.95
(mg)	After	8.06	31.45	35.16	8.65	18.13	24.98
Extracted	Bulk[a]	0.84	0	0	2.02	0	0
material (mg)	Surface[a]	3.39	31.36	33.10	6.07	16.66	20.97
	Total	4.23	31.36	33.10	8.09	16.66	20.97
Water content	Before	1.70	22.60	30.10	8.09	13.40	17.80
(mg)	After	2.54	21.00	22.20	8.62	11.18	14.16

[a] These are calculated values.



(a) (b)

Fig. 13. SEM micrographs of a HEMA graft, before and after extraction in DMF (monomer concentration = 10%; radiation dose = 0.20 Mrad). Mag. 2000×, 0 tilt, Bar = 10 μm. (a) Nonextracted; (b) extracted.

It is reasonable to assume that washing off ungrafted material from the surface of the graft will not affect the void volume of the grafted network; thus, its water content should remain unchanged. Only when material is extracted from the bulk, the water content of the graft should increase. However, inspection of the data reported in Tables I–IV shows that this water content trend is not always followed. This can be accounted for by recalling that the initial graft level, which is determined gravimetrically, includes not only the weight of the covalently grafted poly(HEMA) but also ungrafted material filling the "osmotic cells"; consequently, an artifactually high graft level is obtained which, in turn, results in the calculation of an artificially low water content.

In Figures 13 and 14, micrographs are presented of grafted films obtained from relatively dilute HEMA grafting solutions (10%) for two radiation doses, 0.20 and 0.50 Mrad, respectively. The graft levels for both systems are approximately 1 mg/cm^2 (Fig. 1). The compact structure exhibiting broad, tightly arranged bumps, as shown in Figure 13(a) for the nonextracted graft, is clearly affected by the extraction process, which causes the formation of smaller, separated globules [Fig. 13(b)]. The change in features is even more pronounced for the 0.50 Mrad radiation dose (Fig. 14). These micrographs distinctly demonstrate the dramatic effect that the extraction procedure has on the grafted network—the collapse of the previously filled osmotic cells in graft copolymers with relatively low graft levels.

CONCLUSIONS

Grafting mechanisms and topographic features of the HEMA/EMA model system have been studied. In particular, the structure of the HEMA-grafted LDPE hydrogel and the factors influencing its behavior have been identified.

We have demonstrated the existence of internal cells by the dissolution of entrapped ungrafted poly(HEMA) chains and investigated their topography by selectively staining the grafted HEMA networks. The staining technique

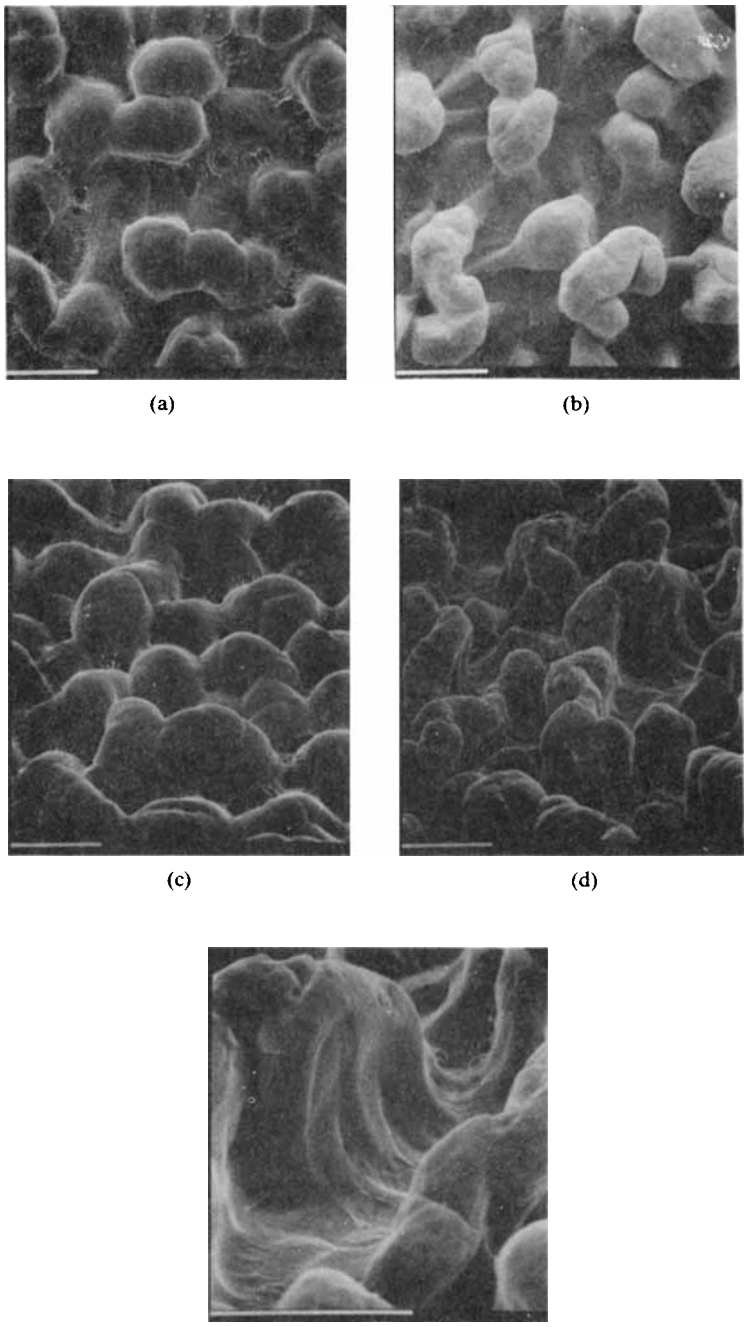


Fig. 14. SEM micrographs of a HEMA graft, before and after extraction in DMF (monomer concentration = 10%; radiation dose = 0.50 Mrad). (a) and (b) 2000×, 0 tilt; (c) and (d) 2000×, 60 tilt; (e) 4500×, 60 tilt. Bar = 10 μm. (a) Nonextracted; (b) extracted; (c) nonextracted; (d) extracted; (e) extracted.

developed proved to be effective for light microscopy and for electron microscopy as well. TEM of stained poly(HEMA) thin sections showed that the structure of the graft becomes increasingly porous as a function of both radiation dose and monomer concentration. Comprehensive extraction studies provided strong support for the proposed "osmotic cell" model.

Similar studies conducted on EMA grafts showed, in contrast to HEMA grafts, that increasingly dense structures were obtained for larger radiation doses as well as for more concentrated grafting solutions.

By providing knowledge of the structure of the grafted networks, the findings reported in this study contribute to the thorough characterization of the HEMA/EMA/polyethylene model system. Furthermore, identifying the nonextractable network structure allows us to work with a clean system free of leachables, an important requirement in biomedical applications. In light of the highly porous structure exhibited by the grafted HEMA network and the possibility of controlling the factors influencing the structure, new controlled drug delivery-related applications are being considered.

The HEMA/EMA graft copolymer system has been found to be a useful model for studying blood/polymer interactions. Complementary studies on the bulk and surface composition of co-grafted HEMA/EMA networks have been conducted; the results will be discussed in a manuscript now in preparation.

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