Macromolecules 1991, *24*, 6065–6072 **6065**

Immobilization of Poly(ethylene glycol) onto a Poly(vinyl alcohol) Hydrogel. 1. Synthesis and Characterization

Gerard R. Llanos and Michael V. Sefton*

Department of Chemical Engineering and Applied Chemistry and Centre for Biomaterials, University of Toronto, Toronto, Ontario, Canada M5S 1A4

Received February 6, 1991; Revised Manuscript Received June 5, 1991

ABSTRACT: Poly(ethylene glycols) (PEG) were immobilized onto a poly(vinyl alcohol) (PVA) hydrogel using either acetal or urethane linkages. The hydrogel was cross-linked with glutaraldehyde using $MgCl_2$ (water based) or toluenesulfonic acid (DMSO based) as the acid catalyst. The water-based gel was stiffer (compression modulus of 465 kPa) than the DMSO gel (compression modulus of 48.6 ± 2.4 kPa). The terminal hydroxyl group of PEG (M_n 2000 and 5000, with or without a monomethoxy end cap) was oxidized to an aldehyde by using an acetic anhydride–DMSO mixture or modified with a diisocyanatohexane to produce a terminal isocyanate. Optimum conversion to an aldehyde was realized at 8 h at room temperature, independent of molecular weight. Maximum conversion to the isocyanate occurred at 6 and 10 h/90 °C for M_n 2000 and 5000, respectively. Using the aldehyde-terminated PEG5000 in the presence of toluenesulfonic acid, XPS analysis of the immobilized product determined that an average maximum oxyethylene content of 71% was obtained after 4 h at 70 °C. Both molecular weight species of poly(ethylene glycol isocyanate) were bound to PVA on the order of 74% oxyethylene content as measured by XPS. Covalent binding of PEG M_n 5000 via an aldehyde had no effect on the advancing contact angle whereas immobilization through a urethane moiety resulted in a significant decrease, indicating that PEG dominated this surface in air. Since receding contact angles indicated total wetting of both modified and unmodified PVA surfaces, it was not possible to distinguish the presence of PEG from that of PVA on the surface of the gel in water. These surfaces are of potential interest as a means of reducing the thrombogenicity of cardiovascular biomaterials.

Introduction

Hydrogels, a group of cross-linked polymeric materials that have the ability to imbibe large quantities of water, have numerous biomedical applications.[1] A hydrogel prepared by cross-linking poly(vinyl alcohol) (PVA) with glutaraldehyde has been studied extensively in our laboratory for potential use in cardiovascular devices.[2,3]

Observations[4–6] that protein adsorption and cell deposition were greatly reduced on surfaces preexposed to poly(ethylene glycol) (PEG) solutions have led to the development of numerous techniques to prepare PEG-rich surfaces. These include block or graft copolymerization[7–19] and direct covalent attachment to a surface.[20–25] Direct attachment provides the advantage of having pendant PEG molecules which presumably retain most of the properties of the free counterparts in solution. With reduced protein and cell adsorption, immobilized PEG has the potential to improve the biocompatibility of polymeric substrates as well as enhancing the protein resistant properties of synthetic membranes and affinity chromatography supports.

Here we report the immobilization of PEG onto a PVA hydrogel to lower its thrombogenicity. To achieve this, the hydroxyl terminus of the PEG was oxidized to an aldehyde or modified with a diisocyanate to produce an isocyanate end group. This was followed by reaction with the hydroxyl groups of the PVA substrate (Figure 1). The resulting PEG-grafted PVA hydrogels were characterized by using X-ray photoelectron spectroscopy (XPS), Fourier transform infrared spectroscopy (FTIR), and water contact angle measurements. The effect on thrombogenicity is discussed separately.[26]

Materials and Methods

PVA Hydrogels. PVA hydrogel was prepared by using either water or dimethyl sulfoxide (DMSO) as solvents. The formulations for each solvent are given in Table I. The water-based gels were similar to that prepared before,[27] but with formaldehyde and glycerol being omitted from the formulation. For the DMSO-based gel, PVA was dissolved in the solvent with heating. After cooling to room temperature glutaraldehyde and toluenesulfonic acid (100 mg/mL of DMSO) were added. Entrapped air was removed from the solution by allowing it to stand for 2–3 min.

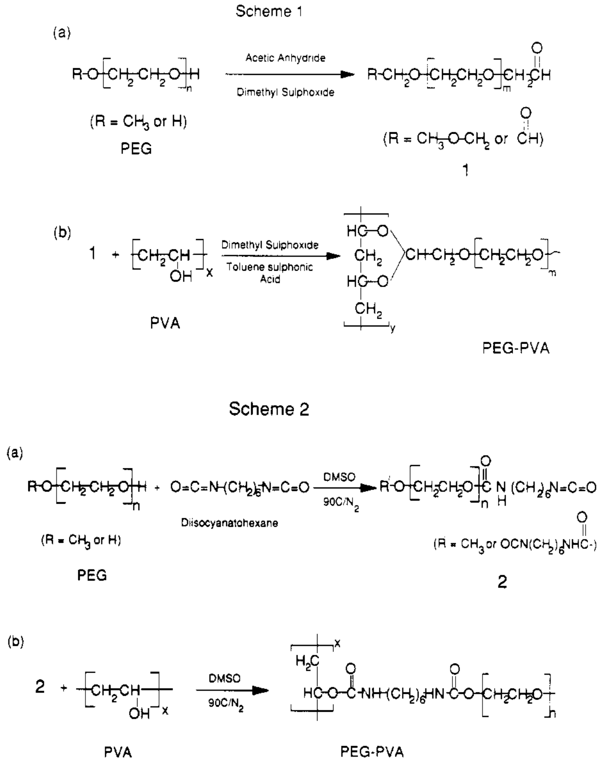

Figure 1. Reaction schemes for the immobilization of PEG onto PVA hydrogel.

Chromic acid etched polyethylene sheets (2 cm × 1 cm × 1 mm; Warehouse Plastics, Toronto, Ontario, Canada) or tubings

---

* To whom correspondence should be addressed.

0024-9297/91/2224-6065$02.50/0

Table I
Poly(vinyl alcohol) Hydrogel Formulations

	DMSO based		water based	
	wt %	mol %	wt %	mol %
PVA[a]	7.3	12.32	7.0	3.27
glutaraldehyde[b]	0.19	0.19	2.0	0.41
TSOH[c]	0.33	0.13		
$MgCl_2$[d]			5.0	0.5
DMSO[b]	92.18	87.35		
water			86.0	95.82

[a] MW 86 000; 99–100% hydrolyzed; Aldrich, Milwaukee, WI. [b] BDH, Toronto, Ontario, Canada. [c] TSOH = toluenesulfonic acid (Aldrich) in DMSO at 100 mg/mL. [d] Hexahydrate salt; Fisher Scientific, Toronto, Ontario.

(2.69 mm i.d. × 35 cm; Intramedic, Clay Adams, NJ) were coated with PVA hydrogel by dipping (sheets) or by filling the tubes with gel solution and allowing them to drain. This was repeated twice. In the case of aqueous gel solution, but not for DMSO-based gels, both sheets and tubing were allowed to "hang-dry" for 30 min between coats followed by overnight drying. The chromic acid etching and glow discharge cleaning of the PE surfaces have been described before.[27]

The hydrogels, either coated on PE or cast into glass Petri dishes, were cured at 70 °C for 1 h for the DMSO-based gel or 2 h for the water-based gel. During curing the coated tubing and sheets were hung vertically, with the sheets being placed in a covered glass vessel to prevent rapid evaporation of solvent. The hydrogels were then washed extensively with deionized water and dried in vacuo.

Aldehyde-Terminated Poly(ethylene glycol). The terminal hydroxy group of PEG was oxidized to an aldehyde (PEG–CHO) following a modification of a method reported by Harris et al.[28] Monomethoxypoly(ethylene glycol) (10.0 g; MW 5000; Aldrich) was dissolved in freshly distilled, dry, dimethyl sulfoxide (40 mL; DMSO; BDH, Toronto, Ontario). Acetic anhydride (20:1 mole ratio of anhydride to hydroxyl; Aldrich) was added and the mixture stirred for a given time. A 5.0-mL aliquot of the reaction mixture was then withdrawn, and the polymer, precipitated in 20 volumes of diethyl ether (ether; BDH, Toronto, Ontario). The polymer was redissolved in minimum methylene chloride and precipitated again in 20 volumes of ether. This was repeated twice. The polymer was dried overnight under vacuum over Drierite ($CaSO_4$; BDH, Toronto, Ontario). Each sample was analyzed by FTIR and subjected to a Schiff test for aldehydes.[29] The above procedure was repeated for PEG2000, with the polymer being dissolved in 17 mL of DMSO.

Isocyanate-Terminated Poly(ethylene glycol). Monomethoxypoly(ethylene glycol) (5.0 g; MW 5000; dried in vacuo overnight) was dissolved in 20 mL of DMSO (freshly distilled, dry). This solution was added slowly, using a gas-tight glass syringe (Hamilton Syringe fitted with a Teflon-coated plunger; Chromatographic Specialities, Inc., Brockville, Ontario) and a syringe pump (Razel Scientific Instruments, Stamford, CT), to a solution of 2 mL of diisocyanatohexane (12.6 mmol; Aldrich) in 10 mL of DMSO and heated to 90 °C under a continuous N_2 purge. These conditions were maintained for the entire length of the reaction. The reaction mixture was then cooled to room temperature and the solution added to 400 mL of diethyl ether (stored over 4-Å molecular sieves). After stirring the mixture for 30 min, it was filtered under a N_2 atmosphere and the resulting precipitate was dissolved in a minimum amount of methylene chloride. This solution was added to 300 mL of ether, stirred for 30 min, and filtered again under a N_2 atmosphere. The precipitation procedure was repeated again. The polymer was stored desiccated at -15 °C and always used within 48 h of being synthesized.

This procedure was repeated for 2000 MW PEG except as follows: R-PEG2000 (5.0 g; R = OH or OCH_3) was dissolved in 10 mL of DMSO. At the end of the reaction the mixture was added to 200 mL of ether and the mixture stirred for 30 min. At this point the polymer did not separate out of solution as a solid but as a viscous liquid. This was allowed to settle, and the excess ether was decanted. A small amount of methylene chloride was added to the remaining solution. Ether (200 mL) was then added to this solution while stirring, and the resulting precipitate was treated in the same manner as that for MW 5000.

Immobilization of PEG. Dry PVA hydrogel films (10 fragments each ~1.0 cm × 0.5 cm × 1 mm; approximate weight 400 mg) were added to a solution of PEG–CHO (2 g) and toluenesulfonic acid (50 mg) in DMSO (10 mL; freshly distilled). The reaction mixture was heated at a given temperature. At a prescribed time a sample of gel (two fragments) was then removed, washed extensively with deionized water, and dried in vacuo. Similarly, dry PVA hydrogel films were added to a solution of R–PEG–NCO (2.0 g; either MW 2000 or 5000, R = NCO or OCH_3) dissolved in 10 mL of DMSO, and the reaction was carried out at a given temperature under a continuous N_2 purge.

To immobilize the modified PEG onto PVA-coated PE, sheets were suspended in or tubing was filled with the PEG solution. The solution of PEG was incubated in the tubing (PEG–CHO) or circulated through PVA-coated PE tubing (PEG–NCO) using a roller pump fitted with Viton tubing (Cole-Parmer, Chicago, IL). The reaction temperature was maintained by placing the tubing in an oven (Lab-line Instruments, Inc., Melrose Park, IL).

Chemical Analysis. Infrared spectroscopic examination of all compounds was performed by using a Nicolet 60sx FTIR spectrometer (Nicolet Analytical Instruments, Madison, WI) at a spectral resolution of 4 cm^{-1}. The instrument was supplied with dedicated software, and this was used for all spectral manipulations.

The transmission mode was used to analyze all modified PEG prior to immobilization onto PVA hydrogel. The polymer was dissolved to the desired concentration (125 mg/mL for PEG–NCO and 50 mg/mL for PEG–CHO) in chloroform. The modified PEG–PVA hydrogel (or coated PE) was analyzed in the ATR (attenuated total reflectance) mode. Two samples (1.0 × 0.5 cm), cut from the dried gel fragments or coated PE, were placed in contact with the KRS-5 crystal (45° bevel; Harrick Scientific Corp., Ossining, NY) provided with the ATR cell.

XPS analysis was performed on both the hydrogel films and the hydrogel-coated PE surfaces to quantify the extent of chemical modification of PVA under various reaction conditions. The analyses were performed on a Surface Science Instruments (SSI) X-probe ESCA instrument (NESAC-BIO Center, University of Washington). This instrument, with its dedicated software, provided the capability of probing the outermost 50–100 Å of each sample over an elliptical area ranging from 150 to 1000 μm. Photoemission was stimulated by using an aluminum $K_{\alpha 1,2}$ monochromatized X-ray source, and the energy of the emitted electrons was measured by using a hemispherical energy analyzer at pass energies ranging from 25 to 150 eV. An electron flood gun set at approximately 5 eV was used to minimize surface charging of the sample. To offset shifts in binding energies that occur during the analysis, the binding energy scale was referenced by setting the CH_x in the C 1s spectrum to 285.0 eV. Both elemental survey scans (0–1000 eV) and high-resolution scans at the C and O binding energy regions (285 and 539 eV, respectively) were performed.

Physical Characterization. Dynamic contact angles were measured by a Wilhelmy balance (courtesy of Dr. A. W. Neumann, Department of Mechanical Engineering, University of Toronto) at a temperature of 25 °C and relative humidity of ~45%. The sample, preswollen in water, was immersed into and withdrawn again from the bath at a rate of 1–1.5 mm/min. The force (*g*) and the depth of immersion (*h*, mm) were recorded at 20-s intervals. The advancing and receding contact angles were then derived from the force versus immersion curves in the standard fashion.

The compression modulus and cross-link density of the fully hydrated hydrogels were determined by using the method of Cluff et al.[30] Swollen gel disks (10.0 mm × 1.0 mm) were placed, submerged in water, beneath the plunger of a micrometer gauge (Testing Machines, Inc., Amityville, NY). Weights in the range of 50–775 g were applied, and each corresponding displacement was recorded. The dry dimensions of each disk were obtained after drying them at 70 °C for 24 h. The compression modulus was derived from the linear portion of the stress–strain curve as before.

For water uptake, gel films, equilibrated in deionized water overnight, were sandwiched between two double layers of Kim-

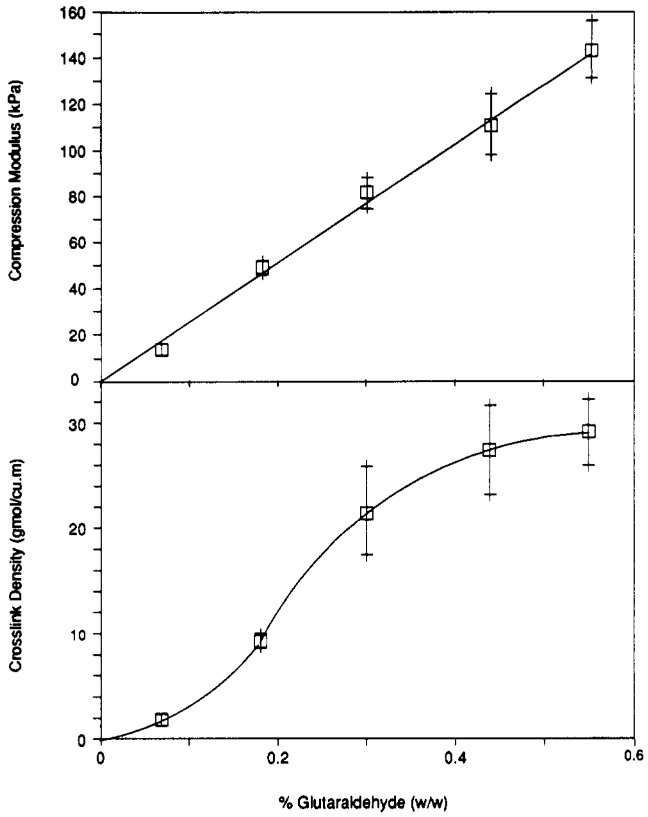

Figure 2. Effect of the glutaraldehyde content on the compression modulus (upper) and corresponding cross-link density (lower) of DMSO-based PVA hydrogel (n = 3-5; ±SD).

wipe tissue (Kimberly Clark Ltd., Toronto, Ontario) draped over Teflon slabs, for 30 s. The gel films were then weighed, freeze dried, and weighed again. The water content was expressed as a percentage of the weight of the swollen gel.

Results

DMSO-Based Hydrogel. The compression modulus was determined from the slope of the linear portion of the compression stress–strain curve. Figure 2 shows the effect of glutaraldehyde concentration on the compression modulus and cross-link density of DMSO-based gels. The compression modulus varied linearly with glutaraldehyde concentration in the range studied. This implied that the cross-links in the DMSO-based gel were primarily chemical in nature, with little or no contribution from physical cross-links such as those that could be attributed to PVA crystallites. This was confirmed by the water content of the gel, which decreased linearly with glutaraldehyde concentration (Figure 3).

The DMSO-based formulation used for the preparation of PVA-coated PE surfaces, contained 0.19 wt % glutaraldehyde and 7 wt % PVA. This produced a gel network with a mean compression modulus of 48.6 ± 2.4 kPa and a corresponding cross-link density of 9.26 ± 0.6 g·mol/m^3. This translated into a molecular weight between cross-links (M_c) of approximately 100 000. The water content of the hydrogel was approximately 90%. The hydrogel formulation of 7 wt % PVA and 2 wt % glutaraldehyde used in the water-based system produced a gel network with a compression modulus of 465 kPa and a cross-link density of 91 g·mol/m^3; its water content was approximately 70%.

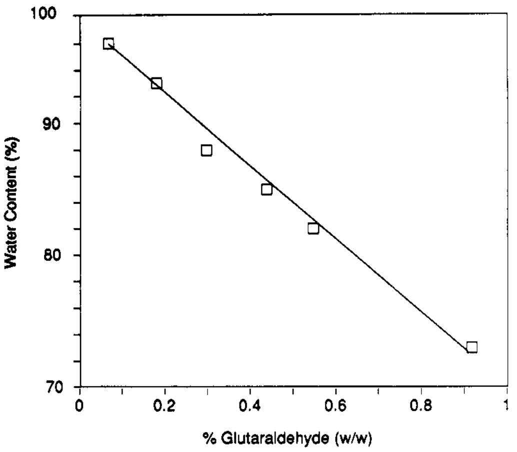

Figure 3. Effect of the glutaraldehyde concentration on the water content (based on wet weight) of a DMSO-based hydrogel.

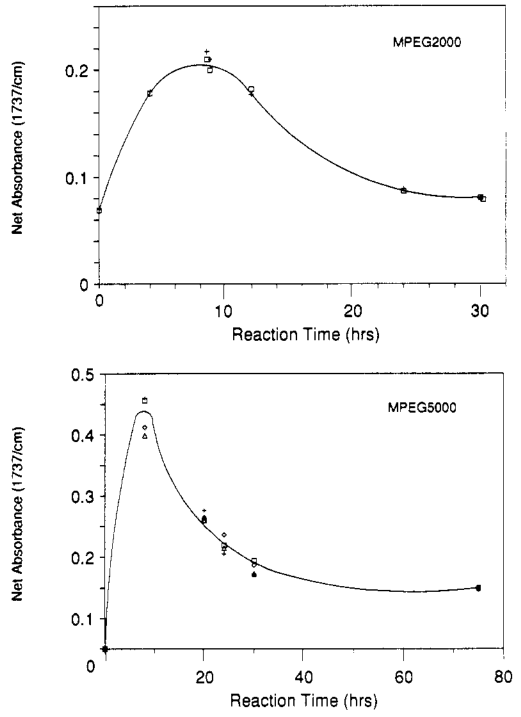

Figure 4. Reaction profiles for the synthesis of aldehyde-terminated monomethoxypoly(ethylene glycol) molecular weight 2000 (MPEG2000) and 5000 (MPEG5000). Reaction was carried out at room temperature with a 20:1 mole ratio of acetic anhydride to hydroxyl end group. Net absorbance was obtained by subtracting base-line absorbance from total absorbance at 1737 cm^{-1}. Symbols in the figures are for individual experiments.

Modified PEG. The terminal hydroxyl group of PEG was oxidized to an aldehyde so as to immobilize the PEG molecule to PVA, via a cyclic acetal functionality. The extent of reaction with time was followed by FTIR, by monitoring the change in absorbance of the carbonyl stretch at 1737 cm^{-1}. Figure 4 shows that the oxidation proceeded through a maximum at 8 h and then decreased. These results were confirmed by the Schiff test for aldehydes that showed a maximum color change for the 8-h sample. PEG of both molecular weight 5000 and 2000 gave similar reaction time profiles, indicating that the reaction was independent of molecular weight in the range studied. Harris et al.[28] also observed a similar trend for PEG of molecular weight 3400. They also reported that increases in the reaction temperature over room temperature or acetic anhydride concentration over a 1:1 mole ratio with hydroxyl groups did not result in an increase in the aldehyde concentration. However, initial experiments following their methods resulted in a lower conversion to aldehydes, reflected by a lower absorbance value at 1737 cm^{-1}, than was achieved using a 20:1 mole ratio of acetic anhydride. Albright and Goldman[31] reported that

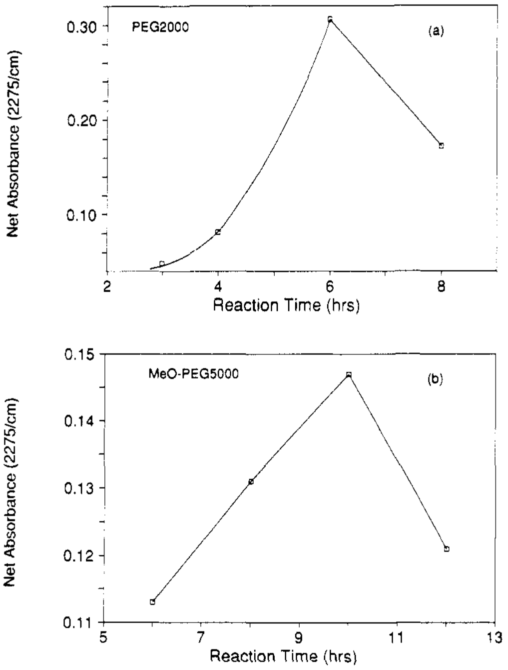

Figure 5. Reaction profiles for poly(ethylene glycol isocyanate) synthesis (single experiment). Reaction was carried out at 90 °C with a PEG solution concentration of 0.5 g/mL in DMSO for MW 2000 (a) or with 0.25 g/mL for MW 5000 with a monomethoxy end group (b).

a 20:1 mole ratio excess of acetic anhydride was required to oxidize primary alcohols to aldehydes.

Similarly, the modification of PEG with diisocyanatohexane was followed by measuring the change in absorbance of the isocyanate (N=C=O) moiety at 2275 cm^{-1} with time; it was determined that maximum conversion occurred at 6 h for MW 2000 (Figure 5a) and 10 h for MW 5000 (Figure 5b). The reaction was carried out only at 90 °C with an excess of isocyanate, following the suggestions of Santerre and Brash.[32] To ensure that there was always a large excess of isocyanate, the PEG solution was added dropwise to the diisocyanate solution. In preliminary experiments it was observed that rapid addition of the PEG solution resulted in an increase in the cross-linking of PEG. This was suggested by the increase in viscosity of the reaction solution as well as the increase in the absorbance value of the urethane carbonyl stretch (1688 cm^{-1}) over that for isocyanate.

Immobilization of PEG onto PVA Hydrogel. The determination of the reaction profile for PEG immobilization onto PVA film via a cyclic acetal (MPEG-CHO-PVA) was carried out with MPEG5000-CHO only. It was assumed that MPEG2000-CHO would have a similar profile. Although the IR spectrum of MPEG-CHO-PVA had no distinguishing absorbance band when compared with that of PVA, closer examination of both spectra revealed an enhanced absorbance for the ether group (~1100 cm^{-1}), with a corresponding reduction in that for the hydroxyl (~3300 cm^{-1}) for MPEG-CHO-PVA. Thus the change in the ratio of ether absorbance to hydroxyl absorbance (A_{CO}/A_{OH}) with reaction time was followed to determine the extent of the reaction. The results, shown in Figure 6a, indicate that the amount of PEG immobilized on the PVA increased over the 8-h period investigated. This suggested that a reaction time greater than 8 h would be required for complete coverage.

Subsequent examination of these modified gel films by XPS revealed that maximum immobilization was attained after 4 h at 70 °C (Figure 6b). As XPS was a more reliable technique for quantification than ATR-FTIR, these reaction conditions were used to bind MPEG5000-CHO to the hydrogel-coated surfaces. The results indicated an oxyethylene content of 71 ± 8% (six samples). High-resolution C 1s spectra for PVA and MPEG5000-CHO-modified PVA are shown in parts a and b of Figure 7, respectively. Gaussian-Lorentzian (80% Gaussian) statistics were used to peak-fit the high-resolution spectra,

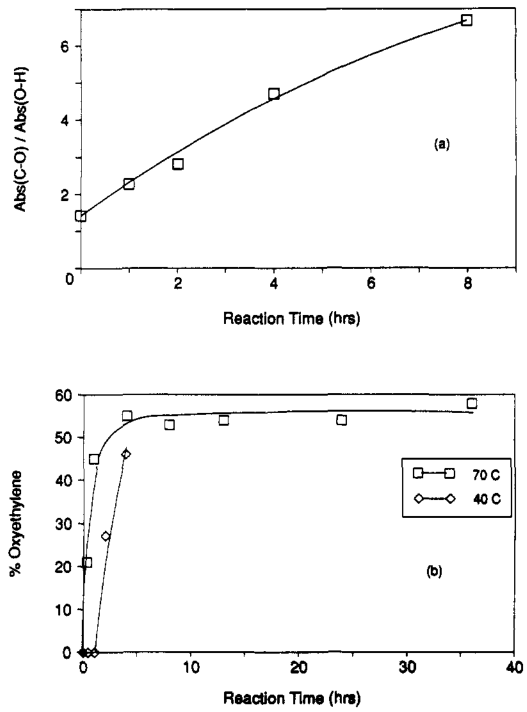

Figure 6. Reaction profile for the immobilization of PEG onto a PVA hydrogel via a cyclic acetal. Determined by using (a) FTIR and (b) XPS. In a, PEG-CHO solution (0.5 g/mL in DMSO) was heated in PVA-coated PE tubing (2.69 mm i.d.) at 70 °C, while in b fragments of dried PVA gel were added to the reaction solution.

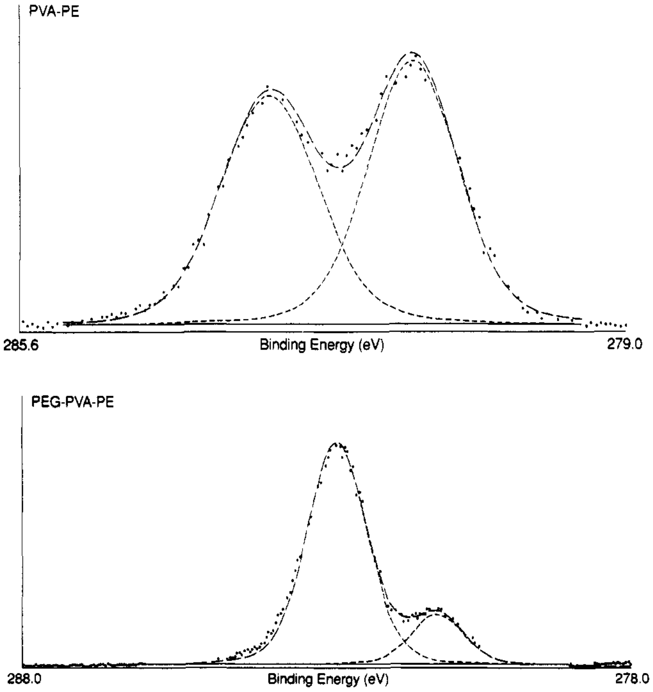

Figure 7. XPS high-resolution spectrum of the luminal surface of (a) PVA-PE tubing and (b) monomethoxy-PEG5000CHO-PVA-PE tubing (4 h/70 °C). The peak area ratio of CC(H) to CO was 52:48 (a, top) and 17:83 (b, bottom).

Table II
XPS Depth Profile of MPEG5000–CHO–PVA-Coated[a] PE

takeoff angle, deg	% C 1s 285.0 eV	 286.6 eV	% oxyethylene[b]
15	34	66	48
35	29	71	59
90	23	77	70

[a] Reaction conditions: 4 h at 70 °C. [b] % oxyethylene = [% C 1s (286.6 eV) − % C 1s (285.0 eV)]/% C 1s (286.6 eV).

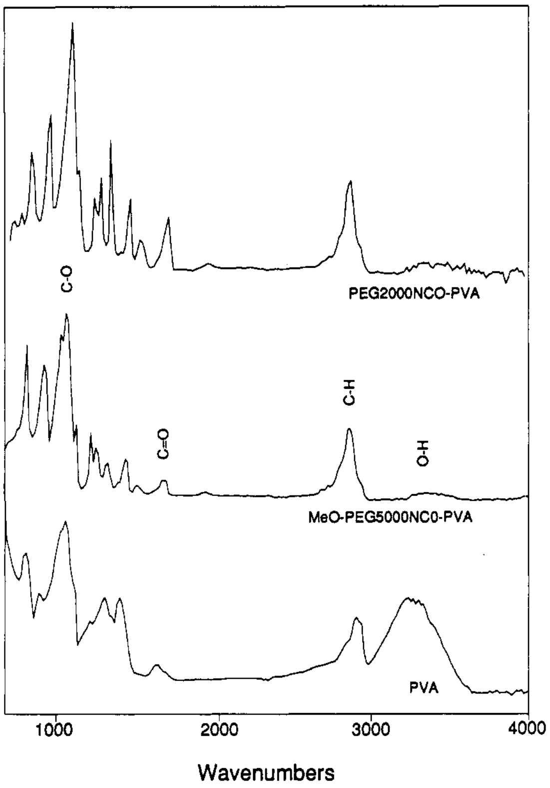

Figure 8. Infrared spectra of PEG2000NCO–PVA (top), monomethoxy-PEG5000NCO–PVA (center), and PVA hydrogel (bottom). Immobilization of PEG2000 was performed at 70 °C for 4 h and MeO–PEG5000 at 90 °C for 12 h.

and the respective peak areas were determined. The error associated with the curve-fitting techniques resulted in an overall error of about 1% in the oxyethylene content calculation. The percent oxyethylene on the surface was determined from the difference between the total CO peak area and the CC peak area since the CC peak area, contributed entirely by PVA, is equal to its contribution to the CO peak area. A depth profile study was also performed on the luminal surface on one of the tube samples, to determine the extent of PEG immobilization into the bulk. Results for this sample showed that the oxyethylene content increased from about 50% at a takeoff angle of 15° (~10-Å depth) to 70% at 90° (~50 Å) (Table II). The low-resolution spectra of all of the surfaces revealed that silicon contamination was less than 2%. This may account for the small increases in the C/O ratio over the theoretical value of 2:1 that were observed.

Typical infrared spectra for PEG immobilized by the isocyanate group are shown in Figure 8. The reaction was followed by determination of the change in the ratio of the absorbance at ~1100 cm^{-1} (ether group) to that at ~3300 cm^{-1} (hydroxyl group) with reaction time, at 70 and 90 °C (Figure 9). For R–PEG2000 negligible absorbance of the hydroxyl moiety remained in 4 h at a temperature of 70 °C. Similar results were obtained for MPEG5000 in 12 h at 90 °C.

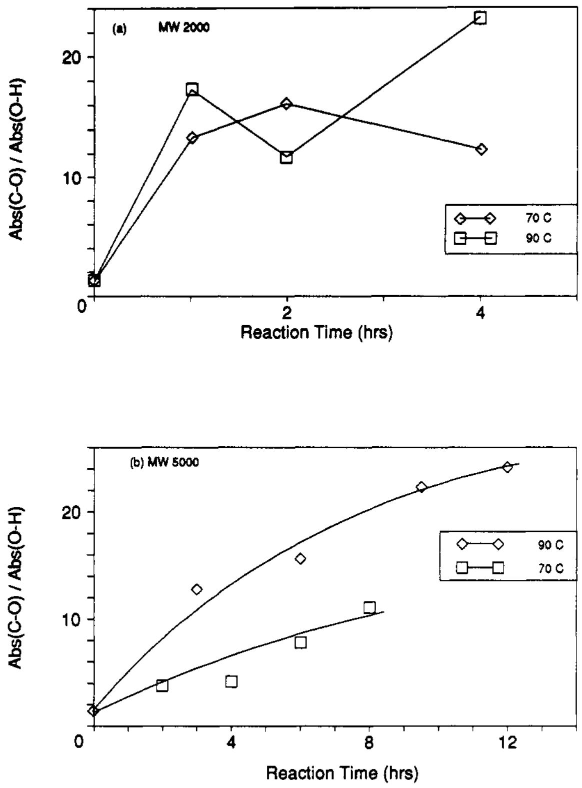

Figure 9. Reaction profile for immobilization of poly(ethylene glycol isocyanate) onto PVA hydrogel. PVA (30 mg/mL) was heated in a DMSO solution of PEG (0.5 g/mL for MW 2000 (top) or 0.25 g/mL for MW 5000 (bottom)).

Table III
XPS Analysis of MPEG$_x$–NCO–PVA–PE

surface	reaction temp, °C	reaction time, h	% oxyethylene (±SD; n = 3[a])
x = 2000	70	4	74 ± 5
	80	4	66 ± 10
	90	6	49 ± 4
x = 5000	70	8	69 ± 3
	80	8	73 ± 1
	90	12	13 ± 2

[a] Three different samples (from different batches).

Results of XPS analysis of the lumen of PVA-coated PE tubes, on which the immobilization reaction was carried out under a variety of conditions, are given in Table III. Maximum coverage of PEG (74% oxyethylene) was attained for MW 2000 at 4 h/70 °C. Similarly, a surface oxyethylene content of 73% was obtained for MW 5000 at 8 h at 80 °C. Significantly lower surface concentrations of PEG were obtained under more severe reaction conditions, i.e., at a temperature of 90 °C and reaction times longer than 4 h.

Contact Angle Studies. The dynamic advancing and receding angles for the PVA–PE and PEG–PVA–PE surfaces were derived from the hysteresis curves, examples of which are shown in Figure 10. The advancing and receding angles were calculated from the forces at zero depth of immersion, and the results are summarized in Table IV. PVA surfaces gave an advancing angle of 54.2° (±2.9°) and 56.5° (±0.4°) for water-based and DMSO-based systems, respectively. These values are in close agreement with the value of 54° reported by Pritchard[33] for poly(vinyl alcohol) homopolymer. The receding angle for both hydrogel surfaces was zero, indicating complete wetting of the surfaces by water. These results indicate that there were no differences in the surfaces derived from either solvent system.

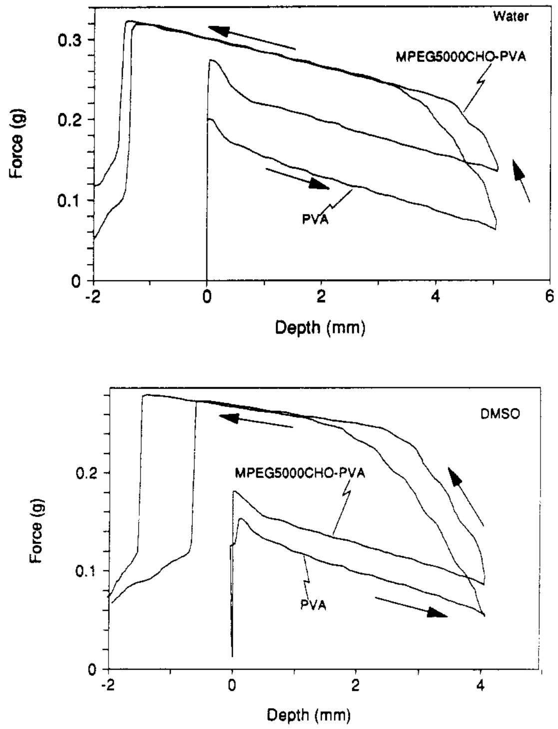

Figure 10. Typical contact angle hysteresis curves for water-based (top) and DMSO-based (bottom) gels.

Immobilization of MPEG5000–CHO to the DMSO-based PVA surface under optimum reaction conditions did not result in any change in either the advancing or receding angles. However, a similar degree of PEG immobilization onto water-based PVA surfaces resulted in a decrease in the advancing angle to 29.0° (±1.2°). Similarly, immobilization of MPEG5000 via a urethane moiety resulted in a lower advancing angle (34.3° ± 2.1°) with no effect on the receding angle.

Discussion

Solvent Choice. DMSO was selected as an alternative solvent to water for preparing the gel because of the need to maintain anhydrous conditions during the subsequent immobilization of the PEG–CHO. DMSO proved to be a better solvent than water, as PVA dissolved more readily in it. The cross-linking reaction, in the presence of toluenesulfonic acid catalyst, in DMSO proved to be more facile than that in the water-based system, which uses magnesium chloride as the catalyst. Another advantage encountered in using DMSO-based PVA solution was in the coating of PE surfaces. Coating of the surfaces with this PVA formulation proved to be less time consuming than with the aqueous system, as no drying stages were required. Drying periods were critical when coating PE with water-based PVA solution, since the water needed to be removed prior to curing in this case.

Hydrogels produced by using DMSO had lower compression moduli than their water-based counterparts even if both had the same water content. This difference was attributed to the presence of crystallites of PVA in the water-based hydrogel network. The drying periods that were employed in the water-based process are favorable to the formation of crystallites,[34] while the DMSO gels remain fully solvated throughout their preparation, thereby minimizing the formation of crystallites.

Immobilization of PEG. Two methods were used to covalently attach PEG onto PVA hydrogel surfaces. The first approach involved the oxidation of the terminal hydroxyl groups to an aldehyde followed by reaction with the hydroxyl groups of PVA to form a cyclic acetal (Figure 11). Under optimum reaction conditions an oxyethylene content of 71 ±8% was obtained with this method. Theoretically if all the hydroxyl groups that were not involved in forming cross-links in the gel matrix react with aldehyde-terminated MPEG5000, an oxyethylene content of greater than 97% would be obtained. Experimentally this is difficult to achieve because of steric limitations. For the formation of a cyclic acetal link the close proximity of two hydroxyl groups is required (Figure 10). However, the reaction is likely to occur at random intervals along the PVA molecule, resulting in isolated hydroxyl groups that are unable to be involved in cyclic acetal formation. Limited mixing between the PEG and the PVA (see below) may also play a role.

One side reaction that is important in immobilizing PEG through an acetal is the cleavage of the ether bond of the PEG backbone by the acid catalyst. This can result in the formation of low molecular weight PEG species having an aldehyde end group that can in turn react to form a cyclic acetal. This latter scenario would give rise to immobilized PEG with a wide distribution of molecular weights. This may be important as the biological activity of covalently bound PEG may be a function of its molecular weight.[18]

As the theoretical oxyethylene content (i.e., greater than 97 atomic %) was not realized using the aldehyde method, the alternative isocyanate route was devised. This involved modification of the hydroxyl moiety of PEG to produce the more reactive isocyanate terminus. Immobilization of the poly(ethylene glycol isocyanate), however, resulted in a hydrogel with an oxyethylene content similar to that produced by the first method, still lower than expected. Unlike immobilization via a cyclic acetal, covalently linking PEG–NCO to PVA is not limited by the location of the hydroxyl group on the PVA chain but rather by the probability of interaction between this functional group and the terminal isocyanate moiety of the PEG molecule. This is controlled in part by the degree of mixing between PEG and PVA. Since the degree of mixing between these two polymer species presumably increases with decreasing molecular weight, then the lower molecular weight PEG (i.e., M_n 2000) is more likely to be immobilized onto the hydrogel in greater amounts. However, XPS results show that the ether content of the PEG-modified hydrogel was the same for both molecular weight species. Perhaps this reflects similarities in the extent of reaction within the low molecular weight tail of the two PEGs. If the extent of the reaction is controlled by the limited mixing between the two polymers, then the immobilization of higher molecular weight PEGs would be precluded.

Ether- or Hydroxyl-Rich Surface. XPS provided quantitative information about the PEG-modified PVA hydrogel surface in a high-vacuum environment and not in an aqueous environment such as blood. This is significant as PEG and PVA may behave differently when placed in water. Contact angle studies were, therefore, performed to ascertain whether PEG or PVA dominated the surface of the hydrogel under aqueous conditions.

PVA hydrogels prepared from DMSO or water had similar contact angles despite possible differences in crystallinity, suggesting that crystalline regions contributed little to the interfacial free energy. Comparison of both the advancing and receding angles of the PVA hydrogel showed that the surface was "hydrophobic" when interfaced with air. In an aqueous environment the pendant hydroxyl groups reorient toward the water,

Table IV
Water Contact Angles

surface	advancing angle (±SD[c])
PVA[a]	54.2 ± 2.9
PVA[b]	56.5 ± 0.4
MPEG5000ALD–PVA[a,d]	29.0 ± 1.2
MPEG5000ALD–PVA[b,d]	53.4 ± 1.9
MPEG5000NCO–PVA[b,e]	34.3 ± 2.1

[a] Water-based PVA. [b] DMSO-based PVA. [c] n = 3–5 samples. [d] PEG immobilization reaction conditions: 4 h at 70 °C. [e] PEG immobilization reaction conditions: 8 h at 80 °C.

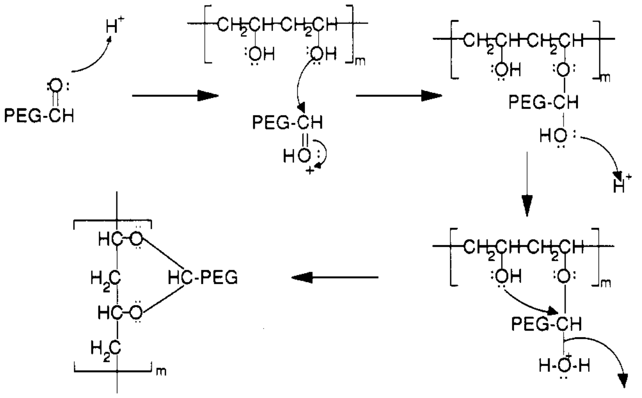

Figure 11. Reaction mechanism for cyclic acetal formation between aldehyde-terminated PEG and PVA.

accounting for the extremely low receding angle. In the PEG molecule the hydrophilic oxygen group is part of the polymer backbone, and therefore PEG presumably cannot exhibit quite the same behavior as PVA. Once immobilized in sufficient concentration to the PVA hydrogel network, the advancing angle of the resulting surface is reduced. This is probably because the PEG ether groups are more abundant at the surface than the CH_x groups of the PVA.

In an aqueous environment the PVA and PEG–PVA surfaces exhibit total wetting. Since both PEG and PVA freely hydrogen bond with water and would display a low interfacial free energy in water, the hydrated hydrogel surface may be dominated by a mixture of both species. The distribution of the two cannot be deduced from the available data. This complicates the interpretation of the effect of immobilized PEG on thrombogenicity.[26]

Prudence must be exercised when interpreting the contact angle results. Close examination of the hysteresis curves revealed that the advancing slope and the receding slope for all the surfaces were not parallel. This indicated that the surfaces were not undergoing true thermodynamic hysteresis but rather kinetic, time-dependent hysteresis, indicative of some type of physical change with time. Andrade et al.[35] pointed out that, with a hydrogel of very high water content and low modulus, there is the possibility of the surface being deformed by the forces acting on it at the three-phase boundary. This results in a deformation ripple moving along the liquid front as the sample is immersed. A different deformation ripple exists with the receding liquid front. The extent of the surface deformation is inversely proportional to the compression modulus of the hydrogel network. It was observed that the difference in the slopes was greatest on the DMSO-based surfaces and less on the stiffer water-based surfaces.

Conclusions

PEG was immobilized onto PVA hydrogel to a maximum oxyethylene content in the range of 70–75%, independent of whether PEG was covalently linked via a cyclic acetal or a urethane moiety. This suggested that the immobilization reaction was limited by the degree of mixing of PVA and PEG and not by the reactivity of the end groups of PEG. This may be significant as fewer PEG molecules would be covalently bound to the surface as its molecular weight increased, adversely affecting the biological properties of the surface.

PVA hydrogels, prepared by using water or DMSO as solvents, possessed different mechanical properties. Fully hydrated gels prepared from DMSO had a lower compression modulus than those prepared from water although both materials possessed similar water contents. This difference in mechanical integrity was probably due to differences in the higher degree of crystallinity in the water-based PVA hydrogel.

Acknowledgment. We are grateful for the financial support of NHLBI (HL24020), the Medical Research Council of Canada, and the Ontario Centre for Materials Research. We thank B. D. Ratner and D. Castner of the NESAC–BIO Center, University of Washington, for performing the XPS analyses and for their helpful discussions.

References and Notes

(1) Peppas, N. A., Ed. *Hydrogels in Medicine and Pharmacy Vol. III: Properties and Applications*; CRC Press: Boca Raton, FL, 1987.

(2) Sefton, M. V. In *Hydrogels in Medicine and Pharmacy Vol. III: Properties and Applications*; Peppas, N. A., Ed.; CRC Press: Boca Raton, FL, 1987; pp 17–51.

(3) Sefton, M. V.; Cholakis, C. H.; Llanos, G. In *Blood Compatibility. Vol. I.*; Williams, D. F., Ed.; CRC Press: Boca Raton, FL, 1987; pp 151–198.

(4) George, J. N. *Blood* **1972**, *40*, 862–874.

(5) Hiatt, C. W.; Shelvkov, A.; Rosenthal, E. J.; Galimore, J. N. *J. Chromatogr.* **1971**, *56*, 362–364.

(6) Wasiewski, W.; Rasco, M. J.; Martin, M. B.; Detwiler, J. C.; Fenton, J. W. *Thromb. Res.* **1976**, *8*, 881–886.

(7) Grainger, D. W.; Okano, T.; Kim, S. W. *Polym. Mater. Sci. Eng.* **1985**, *53*, 21–25.

(8) Grainger, D. W.; Kim, S. W.; Feijen, J. *J. Biomed. Mater. Res.* **1988**, *22*, 231–249.

(9) Grainger, D. W.; Nojiri, C.; Okano, T.; Kim, S. W. *J. Biomed. Mater. Res.* **1989**, *23*, 979–1005.

(10) Brash, J. L.; Uniyal, S. *J. Polym. Sci., Polym. Symp.* **1979**, *66*, 377–389.

(11) Whicher, S. J.; Brash, J. L. *J. Biomed. Mater. Res.* **1978**, *12*, 181–201.

(12) Bots, J. G. F.; van der Does, L.; Bantjes, A. In *Polymers in Medicine. II: Biomedical and Pharmaceutical Applications*; Chiellini, E., Giusti, P., Migliaresi, C., Nicolias, L., Eds.; Plenum Press: New York, 1986; pp 223–224.

(13) Helmus, M. N.; Malhotra, O. P.; Gibbons, D. F. In *Biomaterials: Interfacial Phenomena and Applications*; Cooper, S. L., Peppas, N. A., Eds.; Advances in Chemistry Series 199; American Chemical Society: Washington, DC, 1982; pp 81–93.

(14) Okkema, A. Z.; Grasel, T. G.; Zdrahala, R. J.; Solomon, D. D.; Cooper, S. L. *J. Biomater. Sci. Polym. Edn.* **1989**, *1* (1), 43–62.

(15) Sa Da Costa, V.; Brier-Russeli, D.; Salzman, E. W.; Merrill, E. W. *J. Colloid Interfaces Sci.* **1980**, *80*, 445–452.

(16) Sa Da Costa, V.; Brier-Russell, D.; Trudel, G.; Waugh, D.; Salzman, E. W.; Merrill, E. W. *J. Colloid Interface Sci.* **1980**, *76*, 594–596.

(17) Takahara, A.; Jo, N. J.; Kajiyama, T. *J. Biomater. Sci. Polym. Edn.* **1989**, *1* (1), 17–29.

(18) Nagaoka, S.; Mori, Y.; Takiuchi, H.; Yokota, K.; Tanzawa, H.; Nishiumi, S. In *Polymers as Biomaterials*; Shalaby, S., Hoffman, A. S., Ratner, B. D., Horbett, T. A., Eds., Plenum Press: New York, 1984; pp 361–371.

(19) Sun, Y.; Hoffman, A. S.; Gombotz, W. R. *Polym. Prepr. (Am. Chem. Soc., Div. Polym. Chem.)* **1987**, *28* (1), 292–294.

(20) Gombotz, W. R.; Guanghui, W.; Hoffman, A. S. *J. Appl. Polym. Sci.* **1987**, *37*, 91–107.

(21) Park, K. D.; Okano, T.; Nojiri, C.; Kim, S. W. *J. Biomed. Mater. Res.* **1988**, *22*, 977–982.

(22) Lui, S. Q.; Ito, Y.; Imanishi, Y. *J. Biomater. Sci. Polym. Edn.* **1989**, *1* (2), 111–122.

(23) Kim, S. W.; Jacobs, H.; Lin, J. Y.; Nojiri, C.; Okano, T. In *Blood in Contact with Natural and Artificial Surfaces*; Leonard, E. W., Turitto, V. T., Vroman, L., Eds.; *Ann. N.Y. Acad. Sci.* **1987**, *516*, 116–130.

(24) Han, D. K.; Jeong, S. Y.; Kim, Y. H. *J. Biomed. Mater. Res., Appl. Biomater.* **1989**, *23* (A2), 211–228.

(25) Han, D. K.; Park, K. D.; Ahn, K.-D.; Jeong, S. Y.; Kim, Y. H. *J. Biomed. Mater. Res., Appl. Biomater.* **1989**, *23* (A1), 87–104.

(26) Llanos, G.; Sefton, M. V. *J. Biomed. Mater. Res.*, in press.

(27) Evangelista, R. A.; Sefton, M. V. *Biomaterials* **1986**, *7*, 206–211.

(28) Harris, J. M.; Struck, E. C.; Case, M. G.; Paley, M. S.; Yalpani, M.; Van Alstine, J. M.; Brooks, D. E. *J. Polym. Sci., Polym. Chem. Ed.* **1984**, *22*, 341–352.

(29) Vogel, A. I. *Textbook of Practical Organic Chemistry*; Longman: Birmingham, AL, 1956; pp 330–331.

(30) Cluff, E. F.; Gladding, E. K.; Pariser, R. *J. Polym. Sci.* **1960**, *45*, 341–345.

(31) Albright, J. L.; Goldman, L. *J. Am. Chem. Soc.* **1965**, *87*, 4214–4216.

(32) Santerre, P.; Brash, J., personal communication, 1987.

(33) Pritchard, J. G. *Polyvinyl Alcohol: Basic Properties and Uses*; Polymer Monograph 4; Gordon and Breach: New York, 1970.

(34) Peppas, N. A. *Eur. Polym. J.* **1976**, *12*, 495–498.

(35) Andrade, J. D.; Smith, L. M.; Gregonis, D. E. In *Surface and Interfacial Aspects of Biomedical Polymers, Vol. 1: Surface Chemistry and Physics*; Andrade, J., Ed.; Plenum Press: New York, 1985; pp 249–292.

Registry No. (PEG)(VA)(OHC$(CH_2)_3$CHO) (graft copolymer), 135928-59-5.