Tethered pH Responsive Biomaterials for Mucoadhesive Oral Controlled Release Drug Delivery Systems

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Summary

Smart biomaterials composed of pH responsive polymers, poly((meth)acrylic acid), were synthesized using a precipitation polymerization technique. The microparticles were grafted with poly(ethylene glycol) (PEG) chains that are capable of complexing with the hydroxyl groups of the polyacid and interpenetrating into the mucus gel layer upon entry into the small intestine. Upon introduction of an alkaline solution, these materials imbibe a significant amount of water and create a highly viscous suspension. These materials have the necessary physicochemical properties to serve as mucoadhesive controlled release drug carriers for the oral delivery of drugs.

Introduction

Tethered polymer networks have received considerable attention for their use in modifying surface adhesion and friction, drug delivery systems, and bioadhesives[1-4]. Biomimetic systems, biomaterials that mimic a biological environment to elicit a desired cellular response, have also utilized tethered macromolecules to effectively enhance cellular adhesion[5] or prevent protein adsorption. Certain tethered polymer surfaces have been theorized to act as adhesion promoters by interpenetrating into the mucous gel layer, bridging the interface between the hydrogel-based drug delivery system and the absorption site[16].

The incorporation of a (meth)acrylic monomer possessing a carboxylic acid moiety imparts pH responsive behavior into the hydrogel network. The anionic behavior of the polymer allows for ionic repulsion to occur along the polymer backbone, causing the hydrogel networks to expand and collapse depending on whether the pH of the environment is above or below the pK_a of the polymer. The addition of a polymer tether capable of participating in interpolymer complexation may affect the swelling behavior, mechanical properties, and solute transport characteristics dependent upon the state of the network, complexed or uncomplexed.

In our research, we studied the synthesis and characterization of microparticles composed of polymers possessing the ability to form interpolymer complexes stabilized by hydrogen bonding. These tethers are also present to act as adhesion promoters between the hydrogel and other gel networks such as the mucous gel layer covering the epithelium of the gastrointestinal tract. The conditions necessary for a successful polymerization are elucidated. The effects of the concentration and size of the polymer tether on the dynamics of the polymer networks are evaluated. Also, the behavior of two different polyacids in conjunction with the polymer tether is studied.

Experimental Part

Synthesis Methacrylic acid (MAA), anhydrous potassium carbonate (K_2CO_3), and ethyl acetate (EtAc) were obtained from Fisher Scientific and used as received. Acrylic acid (AA, inhibited with 200 ppm hydroquinone), poly(ethylene glycol) methyl ether methacrylate (PEGMMA, M_n ~ 300, 1100) and PEGMMA solution (M_n ~ 2080, 50 wt % in H_2O) was obtained from Sigma Aldrich (Milwaukee, WI). The PEGMMA 50 wt % in H_2O solution was freeze dried to obtain anhydrous PEGMMA M_n ~ 2080.

Allyl pentaerythritol (APE), pentaerythritol triacrylate (PETA), and di(4-tert-butylcyclohexyl) peroxydicarbonate (BCHPC) were kindly supplied by Perstorp Polyols (Toledo, OH), Sartomer (Exton, PA), and Degussa Initiators (Elyria, OH), respectively. All other chemicals were of reagent grade and used as received.

In a typical thermally-initiated free radical precipitation polymerization, MAA, K_2CO_3, PEGMMA, and deionized distilled water (ddH_2O) were combined and mixed to form a homogeneous mixture, allowing the escape of the neutralization by-product carbon dioxide[17]. The crosslinking agent, APE or PETA, was dissolved in ethyl acetate. The monomer mixture and crosslinking agent were added to a four-necked round bottom flask equipped with an overheard stirrer, nitrogen purge, and condenser containing the polymerization solvent EtAc. Following a 20 minute purge with nitrogen, the initiator BCHPC dissolved in the polymerization solvent was added to the vessel and further purged for an additional 10 minutes. The vessel was placed in a thermostatic bath at 50°C ± 0.5°C where precipitation was evident in a matter of minutes. The reaction was allowed to proceed for 16 hours to ensure a high percentage of monomer conversion. Following the polymerization, the particle slurry was centrifuged and washed with fresh ethyl acetate and dried using a rotary evaporator at elevated temperature and reduced pressure (90°C/40 mmHg).

Characterization Discs containing 1 mg of sample and 150 mg of KBr were prepared on a Carver laboratory press using a 15,000 lb compression force. Infrared spectra of the microparticles were obtained in the wavenumber range of 400-4000 cm_{-1} on a Fourier transform infrared spectrophotometer (FT-IR, Thermo Mattson Infinity, Thermo Electron Corp., Waltham, MA) in transmission mode equipped with a KBr beamsplitter and DTGS detector. Each spectrum is an average of 64 scans at a resolution of 1 cm_{-1}.

The thermal properties of the microparticles were characterized using a differential scanning calorimeter (DSC, MDSC 2920, TA Instruments, New Castle, DE). Approximately 10-15 mg samples were analyzed at a sample rate of 10°C/min over the range of 80°C to 160°C for poly(acrylic acid) and 80°C to 300°C for poly(methacrylic acid) using a heat/cool/heat method to erase the thermal history.

The equilibrium weight swelling ratio of the polymeric hydrogel microparticles was determined by carefully weighing 50 mg of dried particles and combining them with 35 mL of $NaHCO_3$ solution (1.5 g / 100 mL). The suspension was agitated for 60 min and then centrifuged for 60 min at 2000 rpm, carefully discarding the supernatant. The pellet was resuspended in an additional 35 mL of $NaHCO_3$ solution and agitated for 60 min. The suspension was centrifuged at 2000 rpm for 60 min, carefully removing the supernatant, and the weight of the gelled mass was determined. This procedure was also carried out in a 0.1 N HCl solution.

Results and Discussion

Precipitation Polymerization for Synthesis of Tethered Microparticles

The main requirements for a precipitation polymerization are the presence of an inert diluent which dissolves the monomer but precipitates the polymer as it is formed. In the formation of the tethered microparticles, the monomer, crosslinking agent, tether, and initiator are dissolved in ethyl acetate which is a non-solvent for the formed polymer[18]. Polymer precipitates out and forms agglomerated microparticles. The resultant microparticles are composed of primary polymeric particles which have agglomerated together.

For the P(AA-g-PEG) polymerizations, the PEG did not affect the solubility of the formed microparticles. With PEG being soluble in ethyl acetate, its incorporation resulted in a higher propensity for salvation of the growing polymer chains. This effect can be kinetically controlled by decreasing the reaction temperature after initiation to decrease the extent of agglomeration that occurs. For P(MAA-g-PEG) polymerizations, this effect is negligible due to large extent of the insolubility of PMAA in ethyl acetate.

Characterization Figure 1 shows the IR spectrum for crosslinked poly(acrylic acid) (PAA) microparticles containing the PEGMMA tether at various concentrations and potassium acrylate (P(AA-g-PEG)). All spectra exhibit the characteristic C=O stretching, with crosslinked PAA stretching occurring at 1710 cm^{-1} and moving to a slightly higher wavenumber with increasing PEG tether concentration (1740 cm^{-1} for AA:EG 50:50). This indicates a higher prevalence of obtaining cyclic hydrogen-bonded COOH groups in dimeric form with a decreasing concentration of the PEG tether. As the concentration of PEG increases in the network, free (non-hydrogen-bonded) COOH groups increases due to the disruption or prevention of dimeric formation with the addition of the PEG macromolecule into the structure. The asymmetric vibration of the methyl group present at the end of the PEG tether is exhibited on the FT-IR spectrum at approximately 2890 cm^{-1}. The characteristic C-O-C stretching of the PEG tether is clearly evident at approximately 1110 cm^{-1}.

The glass transition temperature, T_g, of the dry crosslinked PAA was approximately 131°C, which is significantly higher than that reported for linear PAA. This can be attributed to the presence of the pentaerythritol crosslinking agent and potassium acrylate. Upon incorporation of small amounts of the PEG tether (AA:EG 98:2 and 90:10), the C_p decreased with no change or appearance of a second T_g observed. It was not until the ratio of AA:EG is increased to 83:17 that a second T_g was observed. This was due to a heterogeneous network consisting of crosslinked PAA rich domains and domains containing both crosslinked PAA and the PEG tether. Upon further increase of the PEG concentration, a single lower T_g is observed, indicating the return to a more homogenous network.

We evaluated the swelling properties of the PEG-tethered hydrogel networks. The molecular weight between crosslinks, $\overline{M_c}$, was calculated according to the Flory-Rehner equation, (1), where $\overline{M_n}$ is the number average molecular weight of the uncrosslinked polymer (20,000 for PMAA, 50,000 for PAA), $\bar{v}$ is the specific volume of the polymer (0.71 cm^3/g), V_1 is the molar volume of the swelling medium (18.1 cm^3/mol), and is the Flory polymer-solvent interaction parameter in water which is calculated as a weighted average for the values PMAA, = 0.5987, PAA, = 0.495, and PEG, = 0.55.

$$\frac{1}{\overline{M}_c} = \frac{2}{\overline{M}_n} - \frac{\frac{\bar{v}}{V_1}\left[\ln\left(1-v_{2,s}\right)+v_{2,s}+\chi v_{2,s}^2\right]}{\left(v_{2,s}^{\frac{1}{3}}-\frac{v_{2,s}}{2}\right)} \qquad (1)$$

The mesh size of the hydrogel network, , was determined according to equation (2).

$$\xi = v_{2,s}^{-1/3}\left(\overline{r_o^2}\right)^{1/2} \qquad (2)$$

The results for the swelling dynamics of PAA microparticles are summarized in Table 1. Loosely crosslinked PAA (0.43 mol % APE) imbibed a significant amount of water in the sodium carbonate buffer (pH~9) as compared to 0.1 N HCl. As the amount of crosslinking agent increases, the degree of swelling in the carbonate buffer decreases. The results for the swelling dynamics of PMAA microparticles (Table 2) show a similar trend. At similar crosslinking levels, PMAA microparticles imbibe a significantly less amount of water due to the presence of the methyl group along the backbone of polyacid. Swelling for both networks occurs due to deprotonation of the carboxyl group resulting in ionic repulsion of the neighboring chains. This causes the network to expand and imbibe more water. In the 0.1 N HCl buffer, the carboxylic acid groups remain protonated and water uptake is minimal. This expansion is also evident through the significant increase in the mesh size for the polymers in the carbonate buffer as compared to the 0.1 N HCl solution. As crosslinking is increased, the mesh size decreases which corresponds to the lower solution uptake.

Upon incorporation of PEG, the swelling dynamics of the PMAA microparticles changes significantly (Table 3). The swelling capacity of the tethered hydrogels is significantly lower as

compared to crosslinked PMAA microparticles containing no tether. The degree of swelling is similar with a slightly smaller mesh size. The ratio of the mesh size in carbonate buffer versus 0.1 N HCl is significantly greater for tethered hydrogels. This allows for a dramatic increase in the amount of water uptake in higher pH solutions compared with lower pH ones providing a stimuli responsive material.

Crosslinked PAA hydrogel microparticles, upon neutralization, produce a highly viscous suspension at low concentrations (1 wt % polymer). This viscous gel suspension possesses tack which is capable of adhering to other hydrogel materials. This characteristic is exhibited in Figures 2-4 or crosslinked PAA microparticles containing a PEG tether. The gel adhesion remains relatively unchanged upon incorporation of low amounts of the PEG tether (AA:EG 83:17). However, as the concentration is increased to a ratio of AA:EG 60:40, a significant decrease in the adhesive characteristics is obtained. The viscosity of the neutralized hydrated gel is the key factor that attributes to the ability of the gel to act as a bioadhesive. With higher concentrations of PEG, the amount of ionizable gropus present in the network are decreased which leads to a significant reduction in the viscosity of the gel. The lowered viscosity in turn causes the adhesive characteristics to be lower than hydrogels containing lowered amounts of PEG.

Conclusions

The fundamental understanding of the behavior of tethered gel networks has implications in tissue engineering, adhesion, and drug delivery. In this research, ionizable hydrogel networks containing a PEG tether were successfully synthesized using a thermally initiated free radical precipitation polymerization. The effects of the PEG tether on the structure of the microparticles were evaluated. The addition of the PEG was shown to cause disruption of the dimer formation of PAA. Differential scanning calorimetry exhibited a concentration dependent decrease in the T_g of xerogels. At low concentrations, the network exhibits a heterogeneous behavior which is evident through a lowered C_p and the presence of two distinct T_g's.

The gel adhesion of a neutralized gel was shown to be dependent on the amount of PEG incorporated into the network, which in turn affected the viscosity of the gel. These materials possess interesting swelling properties which can beneficial in the development of pH responsive drug delivery systems.

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Table 1 Equilibrium swelling ratio of crosslinked PAA microparticles.

	0.1 N HCl		$NaHCO_3$ Buffer	
Theoretical	Weight	Mesh Size	Weight swelling	Mesh Size

Crosslinking Ratio (X_{theo})	swelling ratio (q)	(ξ, Å)	ratio (q)	(ξ, Å)
0.0043	17.0 ± 0.3	420	91.4 ± 0.8	780
0.0075	15.0 ± 0.2	400	64.6 ± 1.7	700
0.0148	14.4 ± 0.2	390	47.6 ± 0.2	630
0.0309	16.5 ± 0.7	420	34.7 ± 2.2	561

Table 2 Equilibrium swelling ratio of crosslinked PMAA microparticles.

	0.1 N HCl		$NaHCO_3$ Buffer	
Theoretical Crosslinking Ratio (X_{theo})	Weight swelling ratio (q)	Mesh Size (ξ, Å)	Weight swelling ratio (q)	Mesh Size (ξ, Å)
0.0043	11.6 ± 0.6	270	40.5 ± 0.4	350
0.0079	11.9 ± 0.5	270	34.8 ± 0.8	340
0.0148	11.8 ± 0.6	270	26.6 ± 0.6	310
0.0309	11.2 ± 0.7	260	20.8 ± 1.1	290

Table 3 Equilibrium swelling of crosslinked PEG-tethered PMAA microparticles (PEG-1000).

	0.1 N HCl		$NaHCO_3$ Buffer	
MAA:EG	Weight swelling ratio (q)	Mesh Size (ξ, Å)	Weight swelling ratio (q)	Mesh Size (ξ, Å)
83:17	6.1 ± 0.2	61	41.6 ± 0.9	300
69:31	5.8 ± 0.3	54	42.0 ± 0.5	290
50:50	5.3 ± 0.4	45	43.4 ± 1.2	270

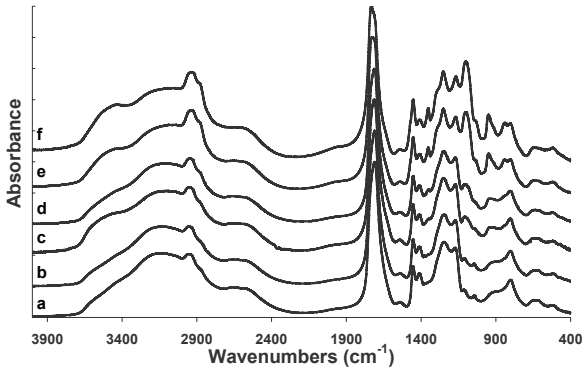

Figure 1. FT-IR spectrum of (a) crosslinked PAA (0.75 mol % APE), (b) P(AA-g-PEG), PEG-1000, AA:EG 98:2, (c) 90:10, (d) 83:17, (e) 60:40, (f) 50:50

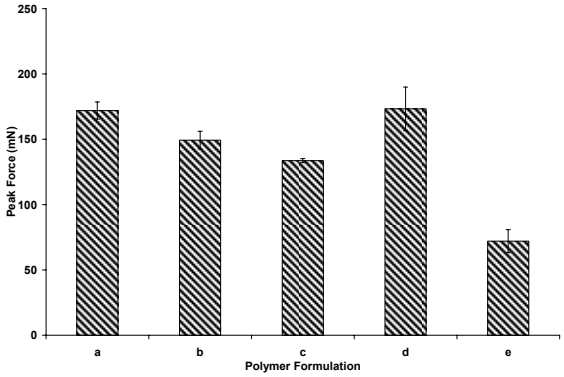

Figure 2. Evaluation of the peak force obtained from neutralized hydrated gels containing (a) crosslinked PAA (0.75 mol % APE), (b) PAA-g-PEG, PEG-1000, AA:EG 98:2, (c) 90:10, (d) 83:17, (e) 60:40, (f) 50:50.

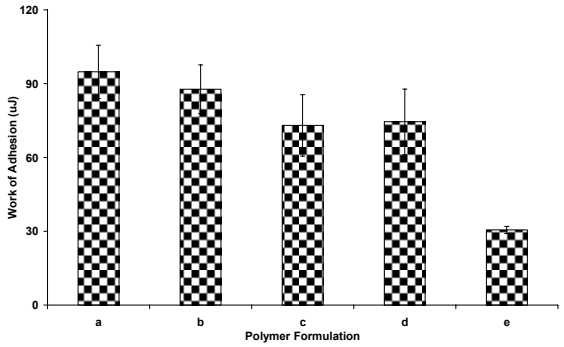

Figure 3. Evaluation of the work of adhesion of neutralized hydrated gels containing (a) crosslinked PAA (0.75 mol % APE), (b) PAA-g-PEG, PEG-1000, AA:EG 98:2, (c) 90:10, (d) 83:17, (e) 60:40, (f) 50:50.

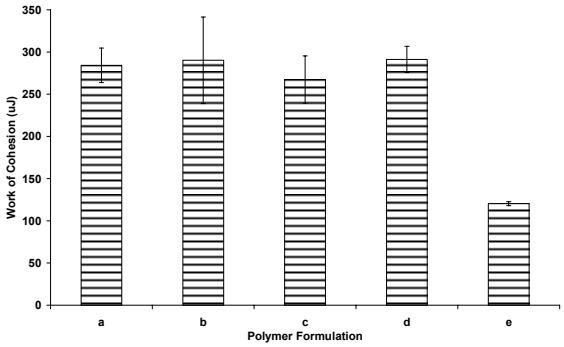

Figure 4. Evaluation of the work of cohesion of neutralized hydrated gels containing (a) crosslinked PAA (0.75 mol % APE), (b) PAA-g-PEG, PEG-1000, AA:EG 98:2, (c) 90:10, (d) 83:17, (e) 60:40, (f) 50:50.