Characterization of the Cross-Linked Structure of Fumarate-Based Degradable Polymer Networks

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ABSTRACT: A new method was developed to examine networks formed with linear macromers of fumaric acid and diacrylate cross-linking agents in order to analyze their cross-linked structure. This method involved the accelerated degradation of the networks and the analysis of the degradation products. Two model networks of poly(propylene fumarate) (PPF) cross-linked with poly(propylene fumarate)-diacrylate (PPF-DA) and oligo(poly(ethylene glycol) fumarate) (OPF) cross-linked with poly(ethylene glycol)-diacrylate (PEG-DA) were evaluated with this method to determine the macromer and cross-linking agent conversions, the network cross-linking density, and an estimate of the molecular weight between cross-links. The validity of the method was confirmed by the analysis of the composition of the un-cross-linked macromers and the correlation of the mechanical properties of the cross-linked polymers with the macromer/cross-linking agent double bond ratio. The results showed that acrylate species had participated more than fumarates in network formation. Furthermore, the structure of PPF/PPF-DA networks was influenced by the amount of cross-linking agent in the polymer formulation, and the OPF/PEG-DA network structure was controlled by the number of repeating fumarate units in the macromer. This method provides a new means to characterize the macromolecular structure of fumarate-based networks.

Introduction

Macromers of fumaric acid are highly unsaturated and therefore can cross-link by themselves or with a cross-linking agent to form polymer networks.[1−5] A number of diacrylate cross-linking agents are currently being used in these systems because they can enhance the polymerization efficiency while imparting specific properties to the network.[1,4,6−8] However, the resulting network structure of these multicomponent systems is highly complex and difficult to characterize.[9] FT-IR spectroscopy to quantify the conversion[10−12] may be problematic because of the overlapping of the C═C stretching bands. Differential scanning calorimetry (DSC), widely used to evaluate reaction and conversion profiles,[13−15] is often difficult for multicomponent networks because of the different reactivities of the functional groups.[16] Mechanical testing and rubber elasticity theory, an effective technique to determine the cross-linking density and mesh size,[4,17,18] generally applies to systems using negligible or zero length cross-linking agents.[19] To our current knowledge, there have been no established means of characterizing the cross-linked structure of these fumarate-based polymer networks prepared with acrylate-based cross-linking agents.

The degradation of these macromers, which breaks them down into simpler, linear constituents, does provide a unique opportunity to analyze the macromolecular network. By examining these degradation products, one can obtain insight into the original cross-linked structure. Fumaric acid has been identified as a product of fumarate-based network decomposition.[1] The presence of this unsaturated unit indicates that not all fumarate double bonds had participated in the cross-linking reaction of the fumaric acid macromer. Quantification of these unreacted groups and a comparison to those of the un-cross-linked macromer can determine the conversion as well as the specific number of cross-linked bonds within the structure, both of which can serve as an index of the degree of cross-linking.

Here, we apply this new approach on two model fumarate-based networks prepared using the following macromer/cross-linking agent pairs: poly(propylene fumarate)/poly(propylene fumarate)-diacrylate (PPF/PPF-DA) and oligo(poly(ethylene glycol) fumarate)/poly(ethylene glycol)-diacrylate (OPF/PEG-DA). The networks were selected because both are comprised of fumarate and acrylate functional groups, but each demonstrates different physical properties. PPF/PPF-DA forms a strong, solid material indicative of a highly cross-linked network,[1] while OPF/PEG-DA forms a very loosely cross-linked hydrogel.[5] Furthermore, the mechanical properties of these networks can be altered by the reagent formulation and most notably by the double bond ratio of their two components.[1,4] This study will characterize the network structure of both PPF/PPF-DA and OPF/PEG-DA networks to establish the relationship between the macromolecular structure and the double bond ratio used to dictate the network composition.

Experimental Section

Materials. Acrylic acid, acryloyl chloride, ammonium hydrogen phosphate, ascorbic acid, benzoyl peroxide, fumaric acid, *N*,*N*-dimethyl-*p*-toluidine, poly(ethylene glycol)-diacrylate (PEG-DA) (M_n 575), and triethylamine were purchased from Aldrich (Milwaukee, WI). Ammonium persulfate was purchased from EM Science (Gibbstown, NJ). Ammonium acetate,

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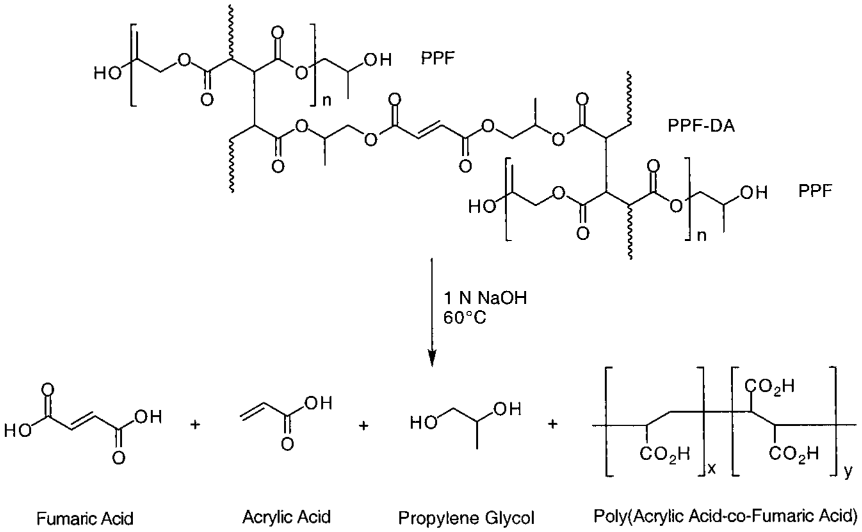

Figure 1. Degradation scheme of PPF/PPF-DA polymer networks.

1 N sodium hydroxide solution, sodium sulfate, and all organic solvents were purchased from Fisher (Pittsburgh, PA). All chemicals were used as received.

Polymer Synthesis. *PPF and PPF-DA Synthesis.* PPF was synthesized in a two-step process as previously described[20] to obtain a number-average molecular weight (M_n) of 1700. The PPF molecular weight was measured by gel permeation chromatography (GPC) using polystyrene standards. ^{1}H nuclear magnetic resonance (NMR) was utilized to confirm the PPF structure.

PPF-DA was synthesized in two reactions. The first reaction to form di-(2-hydroxypropyl) fumarate was carried out as previously described.[1] The product of this first step was then dissolved in methylene chloride in an ice bath under nitrogen. Triethylamine was added to the solution. Acryloyl chloride was then slowly added dropwise so that the reaction temperature did not exceed 0 °C. The molar ratio of di-(2-hydroxypropyl) fumarate, acryloyl chloride, and triethylamine was 1:3:2.1, respectively. After the addition of acryloyl chloride, the reaction was stirred overnight at room temperature. The triethylamine salt byproduct was filtered off, and the methylene chloride solvent was rotary evaporated. The product was extracted from the residue with excess ethyl ether. The solution was then washed with aqueous sodium hydroxide (5% w/w), water, and brine. After drying with sodium sulfate and solvent evaporation, PPF-DA was obtained. The structure of a single fumarate with two terminal acrylate groups (indicating 100% acrylation efficiency) was confirmed by the integration ratio of acryl to fumarate protons in the ^{1}H NMR spectrum as previously described.[1]

OPF Synthesis. Two macromers with PEG of number-average molecular weight 9300 (OPF10K) and 980 (OPF1K) were synthesized as previously described.[5] PEG and OPF molecular weights were determined by GPC using PEG standards. The number of repeating fumarate units present in the macromer was determined from the OPF number-average molecular weight.[5]

Network Preparation. *PPF/PPF-DA Networks.* PPF/PPF-DA networks were formed as previously described[1] with 0.5 wt % benzoyl peroxide (BP) and 0.25 wt % *N*,*N*-dimethyl-*p*-toluidine (DMT). Three formulations of PPF/PPF-DA networks of double bond ratios 0.5, 1, and 2 were used in this study. The double bond ratio is defined as the ratio of acrylates in PPF-DA to fumarates only in PPF and not PPF-DA. The compositions of these formulations are presented in Table 1.

Table 1. Formulations of PPF/PPF-DA Networks Examined in This Study According to the Double Bond Ratio[a]

double bond ratio of PPF/PPF-DA	PPF (g)	PPF-DA (g)
0.5	1.00	2.08
1	1.00	1.04
2	1.00	0.52

[a] The initiator (BP) and accelerator (DMT) content were 0.5 and 0.15 wt % of the total amount of PPF and PPF-DA, respectively.

OPF/PEG-DA Networks. The OPF networks were cross-linked with PEG-DA (M_n 575) as previously described.[4] A 2:1 weight ratio of OPF to PEG-DA was used to obtain fumarate–acrylate double bond ratios of 0.5 and 0.04 for the OPF1K and OPF10K macromers. The cross-linking reaction was carried out in 75 wt % deionized water with an ammonium persulfate and ascorbic acid initiation system. The concentration of the initiator was 0.1 M for both components. Following cross-linking, the formed hydrogels were vacuum-dried for 12 h to remove any water.

Network Degradation and HPLC Analysis of Degradation Products. The polymers were degraded in basic conditions at an elevated temperature to accelerate the degradation process. Figures 1 and 2 illustrate the degradation scheme for PPF/PPF-DA and OPF/PEG-DA networks. Measured amounts of the cross-linked materials were added to 20 mL of 1 N sodium hydroxide (aqueous) in a 26 mL reactor vial with septum. The PPF/PPF-DA networks were ground into a powder to increase the surface-to-volume ratio and further accelerate the degradation. The reactor vials were placed on a Corning hot plate (Fisher, Pittsburgh, PA) and heated to 60 °C with stirring until the polymer was completely degraded. Un-cross-linked polymer formulations, without their respective initiation system, were degraded in a similar fashion. The degradation times, upon which there were no visible particulates in the solution, were different for the two polymeric networks and their un-cross-linked reagents formulations (Table 2). Upon complete degradation, a 1 mL sample was taken for high-performance liquid chromatography (HPLC) analysis.

The concentrations of acrylic and fumaric acid in the degradation solutions were determined by HPLC equipped with a diode-array UV/vis detector (Waters, Milford, MA). Analysis was carried out at room temperature with a Nova-Pak C_{18} column (3.9 mm × 150 mm) (Waters) and a 97:3 0.02 M ammonium hydrogen phosphate/methanol mobile phase at a flow rate of 0.5 mL/min. The pH of the mobile phase was adjusted to 2.35 with phosphoric acid, and 0.03 M ammonium acetate was added to avoid silanol effects with the column.[21] This mobile phase to separate organic acids has been established in the literature.[22] Chromatograms were acquired at a UV absorption of 200 nm. Acrylic and fumaric acid standards of 0, 0.05, 0.15, 0.25, 0.35, and 0.50 mg/mL concentrations in 1 N NaOH (aq) were used to generate calibration curves for

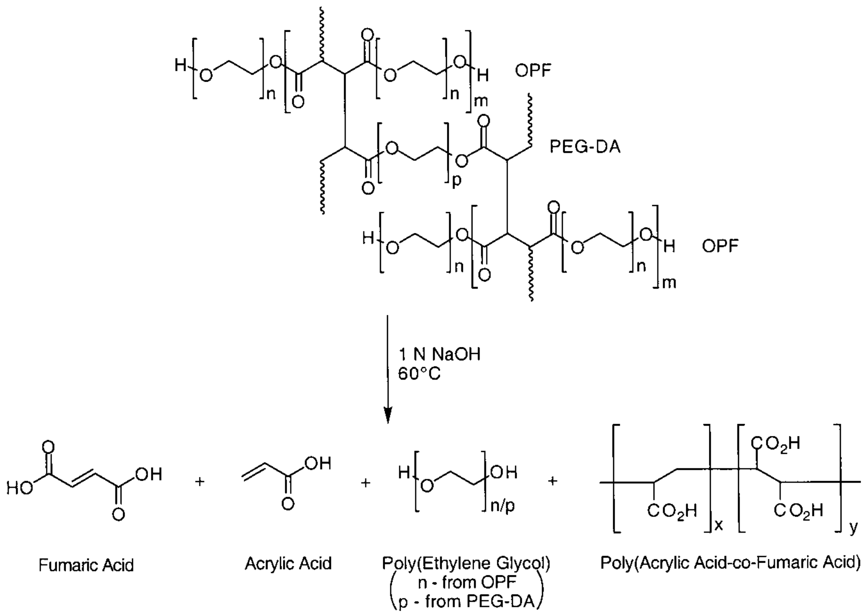

Figure 2. Degradation scheme of OPF/PEG-DA polymer networks.

Table 2. Degradation Time (h) of Polymeric Networks in 1 N NaOH Solution at 60 °C

sample	degradation time
PPF/PPF-DA networks	48
un-cross-linked PPF/PPF-DA formulations	12
OPF/PEG-DA networks	6
un-cross-linked OPF/PEG-DA formulations	2

quantification. The results were normalized to the weight of the polymer sample prior to degradation and are presented in units of [mmol]/[g of polymer].

The HPLC analysis of the degradation products was validated by comparing the experimental findings to theoretical expectations of un-cross-linked PPF/PPF-DA polymer. From GPC and NMR characterization of the polymers following their synthesis, the number-average molecular weight of PPF ($M_n = 1700$), the structure of PPF-DA (one fumarate with two acrylate groups), and the composition of polymers in the double bond ratio formulation (see Table 1) were used to calculate the number of double bonds and hence the expected quantities of fumaric and acrylic acid in the degradation products. PPF/PPF-DA formulations of double bond ratios 0.5, 1, and 2 were examined in triplicate ($n = 3$).

Characterization of Cross-Linked Network. Acrylic and fumaric acid in the degradation products represented their respective double bonds in the PPF/PPF-DA and OPF/PEG-DA networks. The available acrylate and fumarate bonds for cross-linking per mass of polymer (AB_o and FB_o, respectively) were obtained from the un-cross-linked macromers. The number of functional groups that did not take part in the cross-linking reaction (AB_{nox} and FB_{nox}) was quantified from the degraded polymeric networks. With these two components, the reacted acrylate and fumarate bonds (AB_x and FB_x) expressed as moles per unit mass of the polymer ([mmol]/[g of polymer]) were calculated using the following equations:

$$AB_x = AB_o - AB_{nox}$$
$$FB_x = FB_o - FB_{nox} \tag{1}$$

The total number of double bonds that participated in forming the network structure (TB_x) was determined by the sum of these two components.

The exact quantities of fumarate and acrylate functional groups within the network structure can provide the moles of chains occurring between cross-links and in turn the molecular weight between cross-links (M_c). A single fumarate group, which exists within the repeating unit of PPF and PPF-DA, will have two chains extending from its cross-linked bond. The terminal acrylate group will yield only one. It is assumed that these chains exist between two reacted bonds and do not form network defects such as dangling chain ends or loops. The average molecular weight between cross-links was then estimated by

$$\bar{M}_c = \frac{2}{2FB_x + AB_x} \tag{2}$$

The double bond conversions of the individual functional groups were defined as

$$X_{AB} = \frac{AB_x}{AB_o}$$
$$X_{FB} = \frac{FB_x}{FB_o} \tag{3}$$

and the degree of cross-linking for the entire network (regardless of the specific functional group) was calculated by

$$X = \frac{AB_x + FB_x}{AB_o + FB_o} \tag{4}$$

Statistical Analysis. The degradation and corresponding network characterization were carried out on un-cross-linked and cross-linked PPF/PPF-DA and OPF/PEG-DA networks for the double bond ratios described in the preparation section in triplicate ($n = 3$). Results were compared using ANOVA and Tukey's honestly significantly different (HSD) multiple comparison tests with 95% confidence intervals ($\alpha = 0.05$).

Results and Discussion

This study provided a means to characterize the macromolecular structure of fumarate-based PPF/PPF-DA and OPF/PEG-DA model networks and examined how they are influenced by their reagent composition as determined by the cross-linking ratio. These networks are highly complex structures that are formed between polymer chains with repeating fumarate units and a cross-linking agent with terminal acrylate groups. Here, we have used the degradative nature of these materials to break down the network into simple linear chains which can provide insight into its original

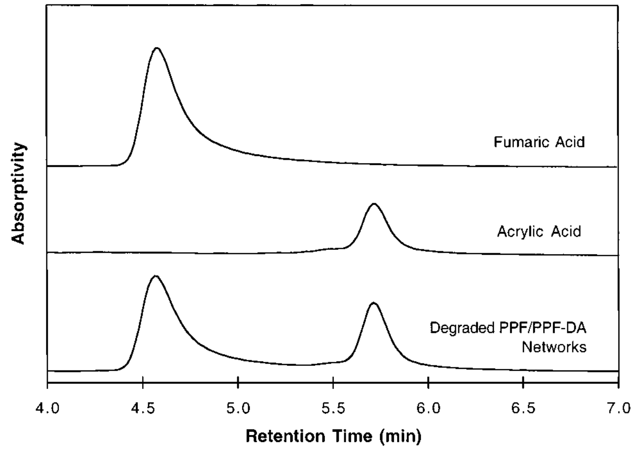

Figure 3. HPLC chromatograms of fumaric acid, acrylic acid, and PPF/PPF-DA degradation products in 1 N NaOH solution. The PPF/PPF-DA chromatogram is representative of OPF/PEG-DA hydrogel degradation products.

structure. Burkoth and Anseth[23] had developed a similar technique to monitor multifunctional monomer reaction behavior, in which polyanhydride networks were degraded and their products were analyzed by mass spectrometry to quantify the kinetic chain length.

These fumarate-based networks are degraded by the hydrolytic cleavage of the ester group, which frees any unreacted double bonds as their corresponding unsaturated organic acid. For the PPF/PPF-DA and OPF/PEG-DA systems, unreacted fumarate and acrylate bonds would be released from the network as fumaric and acrylic acid. Quantifying these components within the degradation products by HPLC and comparing to those of the un-cross-linked macromer lead to the number of double bonds that partake in the network. This enables a unique opportunity to examine the reaction behavior as well as the macromolecular structure of the network. Furthermore, HPLC separation of the fumaric and acrylic acid components allows the analysis of the individual functional groups, providing a very detailed examination of the network. However, it should be clarified that this characterization of the cross-linked network is not a direct inspection of the macromolecular structure, whereby analysis is based upon inferences made from the unreacted double bonds found in the degraded network.

HPLC Analysis of the Degradation Products. Typical chromatograms of the acrylic acid standard, fumaric acid standard, and the PPF/PPF-DA degradation products in 1 N NaOH solution at a UV absorption of 200 nm are shown in Figure 3. The fumaric and acrylic acid peaks occurred at retention times of 4.5 and 6.0 min, respectively. Identical peaks were also observed for OPF/PEG-DA degradation products (not shown). Standards of propylene glycol and poly(ethylene glycol) were not detected with this HPLC method. Also, a standard of poly(acrylic acid-*co*-maleic acid), which was used instead of poly(acrylic acid-*co*-fumaric acid) because the latter was not available commercially, was not detected either.

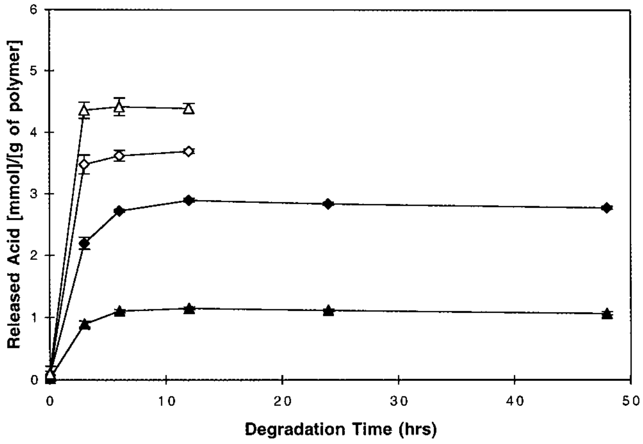

Figure 4. A typical release profile of fumaric (◇) and acrylic (△) acid from degrading cross-linked (filled) and un-cross-linked (open) PPF/PPF-DA in 1 N NaOH solution at 60 °C. Error bars represent means ± standard deviation for $n = 3$.

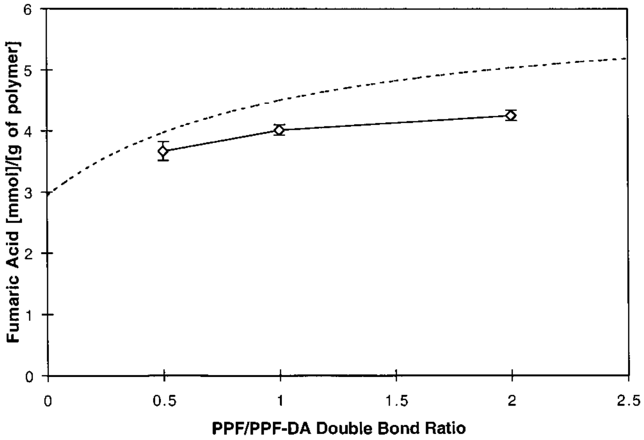

Figure 5. Theoretical curve (dashed line) and HPLC results (◇) of fumaric acid within the degradation products of un-cross-linked PPF/PPF-DA formulations. Error bars represent means ± standard deviation for $n = 3$.

The release profile of fumaric and acrylic acid from degrading cross-linked and un-cross-linked PPF/PPF-DA is illustrated in Figure 4. As expected, the un-cross-linked formulation shows a faster degradation than the cross-linked network. In addition, there were smaller quantities of the unsaturated organic acids released from the cross-linked polymers, indicating that double bonds had been consumed in forming the PPF/PPF-DA networks.

It was assumed that the accelerated degradation conditions employed in this study would completely degrade the polymer without any other reactions to the unsaturated bonds. This was validated with the use of the un-cross-linked PPF/PPF-DA polymers. GPC and NMR characterization of the PPF and PPF-DA polymers enables a theoretical estimate of the fumarate and acrylate double bonds available per gram of polymer, which can then be expressed as a function of the PPF/PPF-DA double bond ratio. This can be compared to the HPLC evaluation of fumaric and acrylic acid in the degradation products of the un-cross-linked polymer (FB_0 and AB_0), where there has been no consumption of the double bonds. Figure 5 shows the theoretical curve and experimental results of the fumaric acid content for the PPF/PPF-DA double bond ratios examined in this study. The corresponding results for acrylic acid are presented in Figure 6. For both components, the experimental values demonstrate similar values and trends as their theoretical estimates. This agreement justifies our assumption that the degradation conditions will completely degrade the polymer while leaving the unreacted double bonds intact. Furthermore, it confirms

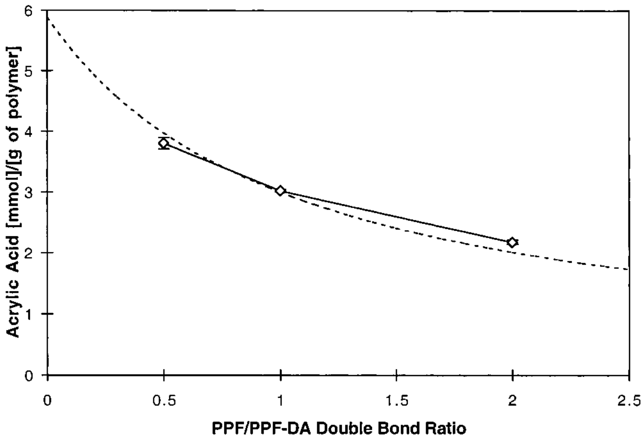

Figure 6. Theoretical curve (dashed line) and HPLC results (◇) of acrylic acid within the degradation products of un-cross-linked PPF/PPF-DA formulations. Error bars represent means ± standard deviation for $n = 3$.

Table 3. Double Bond Conversion of Functional Groups in PPF/PPF-DA and OPF/PEG-DA Networks[a]

polymeric network	double bond ratio	acrylate (X_{AB})	fumarate (X_{FB})	overall (X)
PPF/PPF-DA	0.5	0.70 ± 0.03	0.22 ± 0.01	0.48 ± 0.02
PPF/PPF-DA	1	0.74 ± 0.01	0.24 ± 0.01	0.47 ± 0.01
PPF/PPF-DA	2	0.72 ± 0.02	0.22 ± 0.00	0.41 ± 0.01
OPF1K/PEG-DA	0.5	0.99 ± 0.00	0.93 ± 0.01	0.98 ± 0.00
OPF10K/PEG-DA	0.04	0.99 ± 0.00	1.00 ± 0.00	0.99 ± 0.00

[a] Values represent means ± standard deviations for $n = 3$.

that this HPLC method is appropriate for the separation and quantification of fumaric and acrylic acid.

The values for FB_0 and AB_0 were 3.67 ± 0.15 and 3.81 ± 0.09 [mmol]/[g of polymer] in the PPF/PPF-DA formulations of double bond ratio 0.5. It would appear that these numbers are not representative of a 0.5 ratio. It should be reiterated that the double bond ratio is defined as the ratio of fumarate bonds within PPF to acrylates in PPF-DA. The PPF-DA structure also contains one fumarate group so that the "true" double bond ratio is 1 for a PPF/PPF-DA double bond ratio of 0.5.

HPLC revealed that the degradation products of cross-linked PPF/PPF-DA networks contained both fumaric and acrylic acid components (Figure 3). A previous study using similar accelerated conditions did not detect any acrylic acid with NMR spectroscopy.[1] However, that analysis required acidification of the degradation solution and solvent extraction of the products. This examination of the degradation products is detecting the organic acid salts within the NaOH solution, eliminating the need for any pretreatment that could alter or mask the presence of a constituent.

Characterization of PPF/PPF-DA Networks. The evaluation of the cross-linking structure by examination of its degradation products was utilized to characterize PPF/PPF-DA networks and understand the effect of the PPF/PPF-DA double bond ratio on the cross-linking density. The formulations examined in this study were selected because they have shown significant differences in their mechanical properties that would indicate variations in their cross-linking structures.[1]

The reaction behavior indicates that the acrylate bond participated more in the formation of the PPF/PPF-DA networks (see Table 3). Acrylate conversion (X_{AB}) was greater than its fumarate counterpart by more than 3-fold (~70% compared to ~22%). This increase is attributed to the greater reactivity of the acrylates as well as the biased affinity of both groups toward the acrylate bond. This behavior has been observed with similar fumarate–acrylate radical copolymerizations.[24] There was no apparent effect of the double bond ratio on the reactivity of the individual functional groups; however, PPF/PPF-DA networks of double bond ratio 2 showed a significantly lower overall conversion. Increasing the PPF content within the reagent formulation results in a greater viscosity, which can limit the double bond conversion by diffusion limitations. The overall low reactivity of double bonds in the PPF and PPF-DA polymers can also be attributed to diffusion limitations as these polymers form a rigid solid.

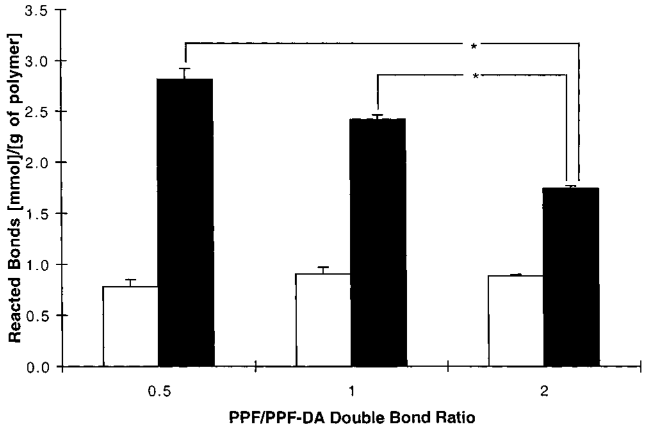

Figure 7. Number of reacted groups (fumarate (FB_x), white bar; acrylate (AB_x), black bar) in PPF/PPF-DA networks of various double bond ratios. Error bars represent means ± standard deviation for $n = 3$. The symbol "*" indicates a significant difference in the number of cross-linked acrylates in each PPF/PPF-DA double bond ratio ($p < 0.05$).

The HPLC analysis enables an exact quantification of fumarate and acrylate bonds that reacted in PPF/PPF-DA networks (FB_x and AB_x). They are presented in Figure 7 as a function of the double bond ratio. These results demonstrate that the networks are composed primarily of cross-links formed with acrylate bonds. FB_x ranged from 0.78 to 0.91 [mmol]/[g of polymer] and did not change with the PPF/PPF-DA formulation while AB_x decreased from 2.82 ± 0.11 to 1.75 ± 0.03 [mmol]/[g of polymer] with increasing double bond ratio. The conversion of acrylate bonds was the same regardless of the double bond ratio, so this trend is related to the amount of the functional group present in the un-cross-linked polymer. As seen in Figure 6, there is a substantial difference in AB_0 from 3.81 ± 0.09 to 3.02 ± 0.02 to 2.18 ± 0.03 [mmol]/[g of polymer] for double bond ratios 0.5, 1, and 2, respectively. The corresponding amount of fumarate species (FB_0) shows a much smaller change (Figure 5), which can account for the invariability between its reacted bonds.

The total moles of bonds participating in the network structure (TB_x), the combination of reacted acrylates and fumarates, are presented in Table 4. This is representative of the cross-linking density but is not indicative of its true value. A cross-link formed by radical polymerization of multifunctional groups consist of a polymer chain made up of a number of bonds. One limitation of this approach to analyze the networks is that while it can identify the specific reacted groups, it cannot distinguish the composition and the number of species within a cross-link. Without knowledge of the

Table 4. Cross-Linking Density and Number-Average Molecular Weight between Cross-Links (M_c) of PPF/PPF-DA and OPF/PEG-DA Networks[a]

sample	double bond ratio	total bonds participating in network (TB_x) ([mmol]/[g of polymer])	M_c (g/mol)
PPF/PPF-DA	0.5	3.60 ± 0.05	457 ± 7
PPF/PPF-DA	1	3.33 ± 0.11	472 ± 20
PPF/PPF-DA	2	2.63 ± 0.02	568 ± 5
OPF1K/PEG-DA	0.5	1.84 ± 0.04	910 ± 25
OPF10K/PEG-DA	0.04	1.36 ± 0.01	1441 ± 16

[a] Values represent means ± standard deviations for $n = 3$.

average number of repeating units per cross-link (i.e., the chain length), the cross-linking density cannot be determined. Here, a greater TB_x would indicate the formation of a densely cross-linked structure. For PPF/PPF-DA networks, a decreasing double bond ratio results in increased cross-linking of the network.

The molecular weight between cross-links, M_c, was calculated on the basis of the number of reacted fumarate and acrylate bonds within the network. The M_c as a function of the PPF/PPF-DA double bond ratio is presented in Table 4. The average length of the chains between cross-links becomes longer with increasing double bond ratio.

Both TB_x and M_c indicated that a more densely cross-linked structure is formed with decreasing double bond ratio. This correlates with the mechanical properties in that the compressive modulus, yield strength, and fracture strength showed similar trends.[1] Since only AB_x varies between formulations (Figure 7), it is evident that this macromolecular structure is solely dependent on the acrylate bonds available in the polymer formulation. PPF/PPF-DA networks composed with a greater PPF-DA composition therefore show a greater cross-linking density.

PPF and PPF-DA provide a unique method to confirm the calculation of M_c since the repeating unit between every unsaturated group is 156 g/mol. This would also be the minimum value of M_c for an ideal network where there is complete conversion of every double bond of the polymers. This value would also be identical for all PPF/PPF-DA networks regardless of the double bond ratio. The experimental minimum M_c can be determined from the number of available fumarate and acrylate bonds found in the degradation products of the un-cross-linked polymer (Figures 5 and 6). Utilizing AB_o and FB_o in eq 2, the minimum M_c of the networks based on the experimental data was 179.51 ± 4.15, 181.00 ± 2.81, and 187.21 ± 3.19 g/mol for PPF/PPF-DA double bond ratios 0.5, 1, and 2, respectively. The similarity within the formulations (ANOVA, $\alpha = 0.05$) as well as the close proximity to the theoretical estimate is a further validation of this analysis for the network characterization of these fumarate-based polymer systems.

Characterization of OPF/PEG-DA Networks. The characterization of the cross-linked network through its degradation products was also applied to OPF/PEG-DA networks in order to observe the effect of the PEG molecular weight in the macromer, which influences the double bond ratio, on its macromolecular structure. The shorter PEG chain length increases the number of fumarate units within the macromer[5] and hence the double bond ratio. Hydrogels of OPF1K and OPF10K were examined because they have shown significant differences in their tensile moduli.[4] In addition, their M_c has also been calculated on the basis of their viscoelastic properties and can further confirm the validity of this characterization.[4] The compositions of the OPF/PEG-DA networks in this study were prepared according to a weight ratio of the two components rather than a double bond ratio in order to compare the properties of these networks to those previously reported.

Table 5. Number of Reacted Fumarate and Acrylate Bonds in OPF/PEG-DA Networks[a]

sample	double bond ratio	reacted acrylate bonds (AB_x) ([mmol]/[g of polymer])	reacted fumarate bonds (FB_x) ([mmol]/[g of polymer])
OPF1K/PEG-DA	0.5	1.45 ± 0.04	0.39 ± 0.00
OPF10K/PEG-DA	0.04	1.33 ± 0.01	0.03 ± 0.00

[a] Values represent means ± standard deviation for $n = 3$.

The double bond ratio has no effect on the cross-linking efficiency of the networks with the individual and overall double bond conversions being greater than 90% (Table 3). There were no detectable amounts of fumaric acid in the degradation products of OPF10K networks, indicating complete conversion of the fumarate bond. OPF/PEG-DA cross-linking occurs with a large water content which can provide a suitable environment for radical chain propagation and eliminate any diffusion limitations.

For OPF1K and OPF10K networks, FB_x decreased and AB_x showed a slight variation with the increased PEG length in the macromer (Table 5). The greater molar concentration of acrylates as expressed in the double bond ratio accounts for the greater participation of acrylates in the network structure. The significant decrease observed in FB_x is attributed to the initial quantities of the species in the polymer formulation where OPF1K, with the shorter PEG length, contains a greater amount of fumarate units. The resulting total bonds participating in the network (TB_x in Table 4) shows that a more densely cross-linked network is formed with increasing double bond ratio. For OPF/PEG-DA networks, the number of fumarate bonds in the macromer and hence the PEG chain length in the macromer is the controlling factor of the macromolecular structure. Reagent formulations of a greater double bond ratio, which therefore consists of macromers with shorter PEG molecular weights, form more enhanced cross-linked structures. These observations were also elucidated from mechanical testing of the hydrogels.[4]

The calculated M_c of OPF/PEG-DA networks is presented in Table 4 and further indicates increased cross-linking with greater double bond ratio. Temenoff et al.[4] utilizing mechanical and swelling data reported a M_c of 2010 ± 116 and 6250 ± 208 g/mol for the OPF1K and OPF10K hydrogels compared to 910 ± 25 and 1441 ± 16 g/mol from this study. It is understood that the M_c determined solely from the number of reacted bonds in a network is an underestimate of the exact value. Equation 2 was developed to represent an ideal network where every chain exists between two distinct cross-links. This assumption excludes network defects such as chain loops or dangling chain ends. There also exists the possibility of an isolated link forming between two terminal acrylate groups which would essentially be a polymerization of a linear chain and hence not an effective cross-link. These imperfections in the network would reduce the number of chains between cross-links and would thereby increase M_c according to eq 2.

Combining this technique with further analysis of the network defects could provide a better understanding of these networks and will be addressed in future work.

An additional source of error in this analysis is that the OPF/PEG-DA networks examined in this study were not swollen after cross-linking to remove the sol fraction. The sol fraction may consist of small separate branched structures that are not part of the overall network. The number of reacted bonds that actually participate in the network would therefore be less, and the resulting M_c would be greater. This error is not likely to affect PPF/PPF-DA because they demonstrate very little water absorption,[1] and other PPF-based materials showed a very high incorporation of the polymer in the network.[25]

This method to analyze the cross-linked structure by examination of its degradation products was developed because there was no established means to evaluate fumarate-based networks formed with acrylate-based cross-linking agents. Numerous techniques for other systems have been described throughout the literature.[10,26] This assay is advantageous in that it is simple to perform and can provide detailed information about the network. The HPLC separation can distinguish between the two reactive species, which is often difficult with other analytical methods. This can be used to show their individual reactivity and participation in the cross-linked structure. This approach can also characterize the network structure by the molecular weight between cross-links (M_c). While the value may be underestimated because of the assumptions used in its calculation, it does consider the influence of the cross-linking agent which is often neglected in other analyses.[4,19]

Most importantly, the data obtained for the PPF/PPF-DA and OPF/PEG-DA networks examined in this study are supported by the physical properties of the materials. This agreement suggests that this is an appropriate method to evaluate and understand the cross-linking of these fumarate-based networks.

Conclusions

The macromolecular structures of PPF/PPF-DA and OPF/PEG-DA fumarate-based networks were characterized with a new technique that degraded the material in order to quantify its unreacted double bond components. This method determined the individual conversion and exact participation of the fumarate and acrylate species in the network. This analysis also provided a representative cross-linking density and average molecular weight between cross-links for the networks. For PPF/PPF-DA, the acrylate species showed a greater conversion than fumarates and in turn formed more cross-links at lower PPF/PPF-DA double bond ratios. The network structure was therefore influenced by the amount of acrylates present in the reagent formulation, which increases with decreasing double bond ratio. For OPF/PEG-DA, acrylate cross-linking was also more predominant, but it was the amount of fumarate species in the network composition that had a greater influence on the cross-linking density. The smaller PEG chain lengths in the OPF macromer, which increased the presence of fumarate bonds and hence the double bond ratio, was attributed to forming more densely cross-linked structures. The trends of the cross-linking density for both PPF/PPF-DA and OPF/PEG-DA networks correlated with the previously reported mechanical properties, confirming the validity of the method for this analysis.

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