Acrylonitrile Graft Copolymerization of Casein Proteins for Enhanced Solubility and Thermal Properties

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ABSTRACT: Casein proteins are soluble in 5% aq. ethanolamine, triehtylamine, and triethanolamine, but insoluble in organic solvents. Graft copolymerization of casein (40 g/L) with acrylonitrile (AN) was carried out in 5% w/v aq. triethanolamine at 60°C using potassium persulfate $K_2S_2O_8$ as an initiator. Percent grafting and grafting efficiency increased with increasing initiator concentrations (up to 1.7×10^{-2} mole L^{-1}) and reaction times, but decreasing $[M]/[I]$ ratios. Fourier transform IR spectra confirmed the formation of the acrylonitrile-grafted-casein (AN-g-casein) copolymers. Under the reaction conditions studied, the grafted PAN side chains were characterized by gel permeation chromatography to have M_n between 1.58 and 5.88×10^4 dalton and polydispersities between 2.6 and 4.5. The AN-g-casein copolymers behaved more like a PAN homopolymer in terms of their thermal properties and solubilities. The decomposition temperatures of AN-g-casein copolymers were between 255 and 273°C, closer to the T_d of the PAN homopolymer (275°C) and significantly higher than that of casein (180°C). The AN-g-casein copolymers are soluble in 50% aq. NaSCN and $ZnCl_2$, but are insoluble in 32:28:40 wt % $CaCl_2/CH_3CH_2OH/H_2O$ like PAN and dimethylformamide-like casein. © 2000 John Wiley & Sons, Inc. J Appl Polym Sci 77: 2543–2551, 2000

Key words: casein; free radical initiated grafting; acrylonitrile; solubility; thermal properties

INTRODUCTION

Proteins have limited use as engineered polymeric materials because of the difficulties in processing them from solutions or melt forms and then reconstituting the three-dimensional molecular structure in the solid state. As chemical grafting techniques have been successfully developed for modifying the physical properties of natural polymers, such as collagen,[1] gelatin,[2,3] starch,[4] cellulose,[5,6] silk,[7] and wool,[8] without altering their core structures, various proteins have been modified for improved applications in pharmacological, food, coating, plastics, leather, or other areas.[9] The most important reactions of proteins have included acrylation and related reactions, alkylation, reduction, oxidation, and aromatic ring substitutions. Modifying reagents usually are directed toward side chains of a single type, but few are completely specific. In some cases, modification of a particular side-chain group is possible with different reactions, whereas unrelated different reagents involving the same type of reaction may modify side-chain groups.

Whole milk consists of milk protein (3.1%), fat (4%), lactose (5%), traces of minerals, and water (87%). About 80% of the milk proteins are casein or caseinate with the rest being whey proteins. Caseins are the phosphoproteins that precipitate

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from raw skim milk by acidification (4.6 pH). Caseins are globular proteins whose molecular weights have been reported to be ranging from 19,007 to 25,230 daltons.[17] Over 55% of the amino acids (—NHCHRCOO—) in casein proteins contain polar groups,[16] i.e., 25.8% contains the —COOH group (glutamic acid, R = CH_2CH_2-COOH; aspartic acid, R = CH_2COOH), 15.1% has the —NH_2 group [including lysine, R = $(CH_2)_3$-CH_2NH_2; arginine, R = $CH_2CH_2CH_2NHC(N{=}H)$-NH_2], and 14.6% contains the —OH group [serine, R = CH_2OH; tyrosine R = $CH_2(CH)_6OH$; and theonine, R = $CH(CH_3)OH$]. These polar groups contribute to the hydrophilicity as well as the reactivity of the casein protein molecules.

Casein has long been used as adhesives, glues, coating, and paint binders. The major drawbacks of casein finishes are poor wet-rub resistance and susceptibility to microbial attack. Attempts to improve these characteristics of casein proteins have been reported via side-chain modification or grafting of acrylic,[10] methacrylic,[11] and acrylonitrile,[12–16] monomers. Graft copolymerization of acrylonitrile on casein[15] has also been processed into silk-like fibers. They have been reported to have superior elasticity than silk and cellulose triacetate, and superior electric static property than nylons and polyesters. The fibers can be dyed with acid, basic, direct, and disperse dyes. The fibers, however, are yellowish in color and have much reduced strength under elevated temperatures.

The purpose of this study was to investigate the relationships between grafting reaction conditions and the grafted copolymers. In grafting casein with acrylonitrile, reaction conditions including initiator concentrations, monomer concentrations, and reaction times were varied to find the optimal conditions. Reactions other than leading to grafting, such as those causing the yellow color, are to be avoided. Furthermore, how the grafting reactions affect the physical properties of the acrylonitrile-grafted-casein (AN-g-casein) copolymers was investigated. The thermal and solution properties of AN-g-casein copolymers were characterized to understand how the AN grafts affect the properties of the globular proteins.

EXPERIMENTAL

Solubility of Casein

To select the solvent for copolymerization reactions, solubility of casein (Aldrich Chemical) in several solvents were evaluated. The solvents included $ZnCl_2$ (ACS reagent), urea (99%), NaSCN (98%), triethylamine (99%), triethanolamine (98%), and ethanolamine (99%), all from Aldrich Chemical. The effects of solution concentrations and dissolution temperatures on the solubility of casein were examined at a constant 50 g/L casein concentration. 0.5 g of casein was placed in 10 mL solutions of varying concentrations at 20 and 66°C water baths under constant shaking (75 rpm in a Dubnoff Metabolic Shaking Incubator) over a 24-h period. Casein was found insoluble in 20 and 35% aq. $ZnCl_2$, but became swollen in 50% aq. $ZnCl_2$ (Table Ia). Elevating the temperature to 66°C did not change the solubility of casein in aq. $ZnCl_2$. At 22°C, casein was insoluble in up to 30% aq. urea concentrations. When temperature was raised to 66°C, casein became swollen in 10–30% urea solutions. Aqueous NaSCN up to 30% and CH_3NO_2/H_2COOH (80/20 v/v) caused swelling of casein at 22 and 66°C. Aqueous-organic media containing amines appear to be the best solvents for casein. At 22°C, casein was soluble in 5% solutions of ethanolamine, triethylamine, and triethanolamine. Casein was also soluble in trieth-

Table Ia Solubility of Casein[a]: Effects of Solution Concentration and Temperature

Solution	Concentration (% w/v)	Solubility[b] 22°C	Solubility[b] 66°C
aq. ZnCl2	20	I	I
	35	I	I
	50	sw	sw
aq. Urea	10	I	sw
	20	I	sw
	30	I	sw
aq. NaSCN	10	sw	sw
	20	sw	sw
	30	sw	sw
CH_3NO_2/H_2COOH (v/v)	80/20	sw	sw
aq. Ethanolamine	2	sw	sw
	3.5	sw	sw
	5	S	S
aq. Triehtylamine	2	sw	sw
	3.5	sw	sw
	5	S	S
aq. Triethanolamine	2	sw	sw
	3.5	sw	S
	5	S	S

[a]The 0.5 g casein in 10 mL solution (50 mg/mL concentration) under constant stirring for 24 h.
[b]S: soluble; I: insoluble; sw: swollen.

Table Ib Solubility of Casein[a]: Effects of Time and Casein Concentration

Solution	Time (h)	Casein Concentration (mg/mL) 50	100	150
5% aq Ethanolamine	1	sw	sw	sw
	2	sw	sw	sw
	3	s	sw	sw
	4	s	s	s
5% aq Triethylamine	1	sw	sw	sw
	2	sw	sw	sw
	3	s	sw	sw
	4	s	s	s
5% aq Triethanolamine	1	s	s	sw
	2	s	s	s

[a]Specified amounts of casein in 10 mL solution (i.e., 50, 100, and 150 mg/mL at 60°C under constant shaking).

anolamine at a lower concentration of 3.5% at 66°C.

The solubility of casein in these three aq. amine solutions was examined further at increasing concentrations and reduced lengths of time (Table Ib). At 60°C and a 50 g/L casein concentration, casein is soluble in 5% ethanolamine and 5% triethylamine after 3 h and in 5% triethanolamine in only after 1 h. An additional 1 h was required to dissolve casein in the 100 g/L concentrations, but no additional time is needed at the 150 g/L concentration in these amine-containing solutions. Among these three solutions, the 5% aqueous triethanolamine dissolved the highest quantity of casein in shortest length of time and was chosen as the solvent for the side-chain graft copolymerization. The short dissolution time and low temperature are important to avoid possible hydrolysis of casein.

Graft Copolymerization

Ten grams of casein was immersed in 100 mL distilled water in a 250-mL three-necked flask fitted with a condenser for 8 h. Five milligrams of triethanolamine was added to make a 5% w/v aqueous solution and immediately placed in a 60°C silicon oil bath. A homogeneous casein solution (40 g/L) was obtained in 0.5 h. The hydroquiuone and monomethyl ether hydroquinone inhibitors in AN (99+%, Aldrich Chemical) were removed using an inhibitor removal disposable column (Aldrich Chemical). Graft copolymerization reactions were carried out in N_2 for 3 h. The required amount of initiator $K_2S_2O_8$ (99.8%, J. T. Baker) was dissolved in water. The monomer and initiator were added in two different manners. The "one-step" or "simultaneous" reaction involved the additions of initiator and monomer over a 10-min period. In the "two-step" or "sequential" reaction, the monomer was added 30 min after initiation. The graft copolymerization was stopped by precipitation in 8% glacial acetic acid (99.7%, Fisher Scientific) at 25°C. The crude product was filtered off, washed thoroughly with distilled water, then dried in vacuum at room temperature and weighed. The overall yield (Y) from reaction between casein and acrylonitrile in the presence of the initiator $K_2S_2O_8$ is calculated from the weights of the crude product (W_{CP}), casein (W_C), and monomer (W_{AN}):

$$Y(\%) = [W_{CP}/(W_C + W_{AN})] \times 100$$

Separation and Purification

The crude product was extracted in $CaCl_2/CH_3CH_2OH/H_2O$ (32:28:40 wt %) at 75°C for 24 h. The extraction resulted in only 0.2% weight loss, indicating nearly complete removal of the ungrafted casein in 8% glacial acetic acid. The ungrafted polyacrylonitrile (PAN) homopolymer was removed from the crude product by extraction in 60°C dimethylformamide (99% Aldrich Chemical) for 72 h. The residual solid was repeatedly washed with water, dried in vacuum, and weighed to give the AN-g-C copolymer ($W_{AN\text{-}g\text{-}C}$). The extracted PAN homopolymer was precipitated and washed in water, dried in vacuum, and weighed (W_{PAN}). The percentage of grafting efficiency (E) and homopolymerization (H) was calculated as follows:

$$E(\%) = [W_{AN\text{-}g\text{-}C}/(W_{AN\text{-}g\text{-}C} + W_{PAN})] \times 100$$

$$H(\%) = [W_{PAN}/(W_C + W_{AN})] \times 100$$

Since casein can be hydrolyzed completely in 6N HCl in 24 h and PAN only lost 0.2% under the same condition, the casein backbone of the AN-g-C copolymer was hydrolyzed in 6N HCl for 24 h to release the PAN side chain. The insoluble residue (side chain) was washed with water, dried, and weighed (W_{SC}). The Fourier transform IR (FTIR) spectrum of this residue is identical to homopoly-

mer PAN, confirming complete hydrolysis of the casein backbone. The mass percentage of grafted side chain (G_{SC}) in proportion to the casein backbone was calculated:

$$G_{SC}(\%) = [W_{SC}/(W_{AN\text{-}g\text{-}C} - W_{sc})] \times 100$$

Characterization

The FTIR spectra of PAN, casein, and AN-g-casein copolymer were taken in KBr pellets, using a Nicolet Magna-IR 560 spectrometer. Differential scanning calorimetry (DSC) was performed on PAN, casein, AN-g-casein copolymer, and a physical mixture of PAN and casein (1:1 w/w) using a Shimadzu DSC model 50. The 3-mg samples were heated at a rate of 10°C/min with N_2 flow at 50 mL/min. The solubility and hydrolysis of casein, PAN, and AN-g-casein copolymer in various solvents (HCOOH, 99%; Borax, 99%; $CaCl_2$, 98%; CH_3CH_2OH, 98%; 99%; HCl acid, ACS grade, all from Aldrich Chemical) were observed. 2 g of sample was placed in 100-mL solvent at 75°C under constant shaking (75 rpm in a Dubnoff Metabolic Shaking Incubator) for 24 h. The number average molecular weight (M_n) of the PAN side chains and their molecular weight distribution were determined at 1 g/L concentration using gel permeation chromotography (Waters M208 Gel Permeation Chromotograph). The mobile phase was dimethylformamide (DMF) with 0.05 mole of LiBr at a 0.6 mL/min^{-1} flow rate at 35°C.

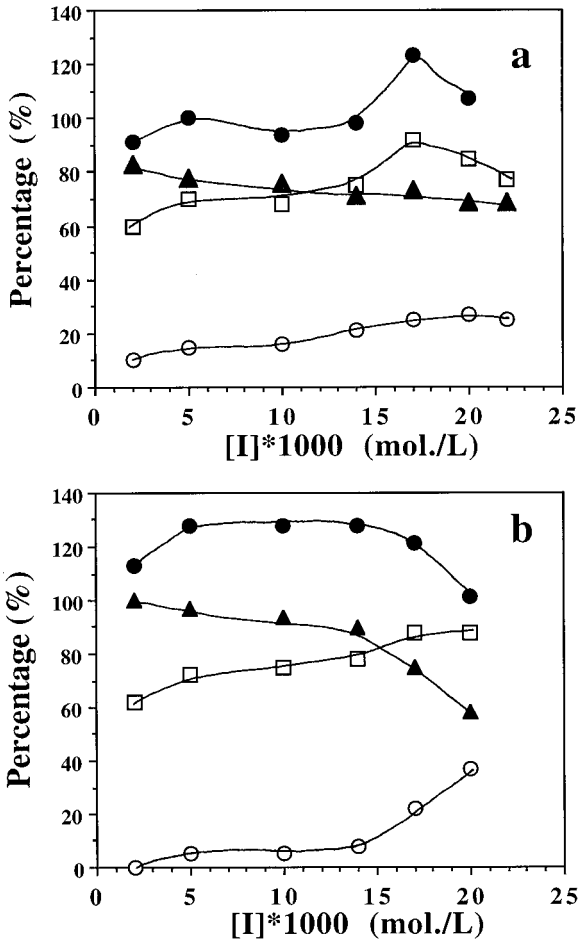

Figure 1 Effects of initiator concentration at constant [M] = 1.566 mol L^{-1} (60°C, pH 7, 3 h): (□) Y—yield; (○) H—homopolymerization; (▲) GE—grafting efficiency; (●) G_{SC}—PAN graft. (a) One-step, or simultaneous, reaction; (b) two-step, or sequential, reaction.

RESULT AND DISCUSSION

Varying Initiator Concentrations: One-Step Reaction

Graft copolymerization of casein with acrylonitrile was performed in 5% aq. triethanolamine at 60°C (7.8 pH). The effects of varying initiator concentrations from 2×10^{-3} mol L^{-1} to 20×10^{-3} mol L^{-1} were studied at a constant monomer concentration [M] of 1.566 mol L^{-1}. These initiator concentrations [I] correspond to decreasing [M]/[I] ratios between 788 and 71. In the one-step reaction, the monomer and initiator were added dropwise simultaneously over a period of 10 min and the reaction lasted 3 h. The yield (Y) of all reaction products increased from 60 to 92% with increasing [I] up to 17×10^{-3} mol L^{-1}, then decreased at a higher [I] of 20×10^{-3} mol L^{-1} [Fig. 1(a)]. Homopolymerization of AN also increases from 10 to 25% with increasing [I] up to 17×10^{-3} mol L^{-1}. The net effect is a slight decreasing trend in grafting efficiency (E) (83–73%), which denotes the extent of AN-g-casein in proportion to the combined AN-g-casein and PAN homopolymer with increasing [I]. The percentage of grafted side chain (G_{SC}) in proportion to the casein backbone varied between 91 and 123%, but with no specific trend in respect to the increasing [I]. The highest Y (92%) and G_{SC} (123%) were observed at a [I] of 0.017 mol L^{-1}. At [I] = 0.02 mol L^{-1}, slightly lower Y and G were observed at similar levels of homopolymerization.

The average molecular weights of (M_n) of the PAN side chains decreased from 4.08 to 3.25 $\times 10^4$ dalton with increasing [I] up to 0.014 mol

Table II Effects of Addition Procedure and Initiator Concentration on Side-Chain Length and Color of AN-g-Casein

Procedure	$[I]$ ($\times 10^3$ mol L^{-1})	$[M]/[I]$	M_n ($\times 10^4$ dalton)	Polydispersity	Color
Simultaneous	2	718	4.08	4.5	White
one-step	5	313	3.88	4.2	White
	10	156	3.78	4.3	White
	14	112	3.25	3.8	White
	17	92	3.85	2.8	White
	20	78	1.58	3.5	Cream
Sequential	2	718	5.88	3.8	White
two-step	5	313	5.56	3.9	White
	10	156	5.18	3.5	White
	14	112	4.98	3.2	White
	17	92	5.18	2.6	White
	20	78	3.60	3.8	Cream

[a]Ten grams of casein in 110 mL triethylamine, $[M] = 1566 \cdot 10^{-3}$ (mol L^{-1}), 60°C, pH 7.8, 3.5 h.

L^{-1}. The side chain M_n then increased slightly to 3.85×10^4 dalton at $[I] = 0.017$ mol L^{-1} (Table II). At this range of $[I]$, these slight variations in average side-chain lengths or M_n is accompanied by lowered polydispersities from 4.5 to 2.8. At $[I] \leq 0.014$ mol L^{-1}, a similar G_{SC} coupled with slightly lowered side chain M_n suggests increased numbers of grafted sites on the casein molecules with increasing $[I]$. The lowered polydispersity also indicates more uniform chain lengths. At $[I] = 0.017$ mol L^{-1}, the highest grafting is coupled with longest and most uniformly distributed chain lengths.

At $[I] = 0.02$ mol L^{-1}, the M_n of PAN side chains reduced to a lowest 1.52×10^4 dalton. In comparison with the grafting characteristics at $[I] = 14 \times 10^{-3}$ mol L^{-1}, the significantly reduced M_n of the side chain (by 50%) coupled with a 10% increase in G_{SC} suggests much greater numbers of grafted sites were formed on the casein molecules, but the grafted chain lengths were significantly shorter. At this high $[I]$, the excess of initiator may cause chain transfer reactions, terminating chain growth. The creamy colored AN-g-casein copolymers also suggest oxidation of casein at the highest initiator concentration.

Varying Initiator Concentrations: Two-Step Reaction

In the sequential two-step reactions, the general trend in Y was similar to that from the one-step procedure, but homopolymerization (≤8%) was considerably lower at $[I] \leq 14 \times 10^{-3}$ mol l^{-1} [Fig. 1(b)]. At $[I] = 0.017$ mol L^{-1}, homopolymerization increased substantially to 22%, similar to that in the one-step process (25%). As $[I]$ increased to 0.02 mol L^{-1}, homopolymerization in the two-step procedure exceeded that in the one-step counterpart. The extent of grafted side chain G_{SC} and the grafting efficiency were also drastically reduced.

At $[I] \leq 10 \times 10^{-3}$ mol L^{-1}, the two-step procedure had higher grafting efficiencies ($E \geq 90\%$) on casein and much lower homopolymerization than the one-step procedure. This is likely due to the creation of the active centers on the casein protein molecules in the absence of monomer. These activated sites then initiate and propagate when the monomer is introduced, reducing homopolymerization. Significantly higher H at the higher $[I]$ indicates that the initiators are in excess and remain active, 30 min after their introduction, to initiate homopolymerization.

The M_n of the side chains in AN-g-casein copolymers from the two-step procedure ranged from 3.6 to 5.88×10^4 dalton, 35–53% higher than the counterparts in the one-step method at the same $[I]$ range (Table II). The polydipersities of the grafted side chains produced by the two-step procedure were slightly lower. At $[I] = 20 \times 10^{-3}$ mol L^{-1}, the AN-g-casein copolymer produced by the two-step procedure also appeared creamy in color.

With either reaction procedure, overall yield of reaction product (Y) and homopolymer (H) increased with $[I]$ at a fixed $[M]$. Proportion of AN to the casein backbone (G_{sc}) peaked at 123% and 128% at $[I] = 0.017$ mol L^{-1} for the one-step and at $[I] = 0.005$ mol L^{-1} for the two-step procedures,

respectively. Further increases in [*I*] usually lead to significantly increased H and reduced grafting efficiency (E). The grafted PAN side chains have M_n between 1.58 to 5.88 $\times$ 10^4 dalton and polydispersities between 2.6 and 4.5. The side chains from the sequential two-step procedures are longer and more uniform in lengths. The AN-g-casein copolymers produced at [*I*] < 0.02 mol L^{-1} are white with either method.

Varying Monomer Concentration and Reaction Time: One-Step Reaction

The effects of reaction time were studied with the one-step reaction at a constant [*I*] of 0.017 mol L^{-1} at 60°C. Figure 2(a) shows that *Y*, *E*, and *H* increase with longer reaction time. The induction time of grafting and homopolymerization is about 20 min under this condition. The effects of monomer concentrations were further examined under the same one-step reaction condition and a 3-h reaction time. It was observed that overall yield (*Y*), grafting (*G*), and grafting efficiency (*E*) increased with increasing [*M*] initially [Fig. 2(b)]. As [*M*]/[*I*] exceeds 180, not only the yield and homopolymerization decreased significantly, grafting and grafting efficiency also reduced dramatically. The highest yield (96%) and grafting efficiency (91%) was obtained at a [*M*] of 3.06 mol L^{-1} or a [*M*]/[*I*] of 180. No clear trend was observed on the M_n of the side chains, whereas a slight increase in polydispersity is observed with increasing [*M*] (Table III).

Structure and Properties of AN-g-Casein Copolymers

Figure 3 shows the FTIR absorption spectra of PAN homopolymer, casein, and AN-g-casein copolymer. The FTIR spectrum of PAN homopolymer shows the characteristic C≡N stretching vibration at 2260 cm^{-1}. The FTIR spectrum of casein shows absorption bands at 3400, 1650, 1520, and 1230 cm^{-1}, corresponding to the N—H stretching and amide bending vibrations. The FTIR spectrum of the AN-g-C copolymer shows absorption bands at 2260 cm^{-1}, characteristic of C≡N stretching as well as those at 3400, 1650, 1520, and 1230 cm^{-1} characteristic of N—H stretching and amide bending vibrations. The presence of these intense absorption bands confirms the structure of the AN-g-casein copolymer.

The DSC thermogram of casein shows that an endotherm from evaporation of water in casein and a decomposition exotherm over a broad range of temperatures (120–220°C) with the plateau centering between 170 and 190°C (Fig. 4). PAN homopolymer, on the other hand, has a very sharp decomposition exotherm at a much higher temperature of 275°C. The physical mixture of casein and PAN homopolymer exhibits two distinctively separated and much smaller decomposition exotherms at the corresponding decomposition temperatures of casein and PAN. The AN-g-casein copolymer ([*M* = 1.566 mol L^{-1}, [*I*] = 0.017 mol L^{-1}, G_{sc} = 123%) has a single decomposition exotherm 265°C with little evidence of the endotherm from the decomposition of casein. At G_{sc} = 123 %, this AN-g-casein copolymer contains 45% casein and clearly exhibits much improved thermal stability than casein.

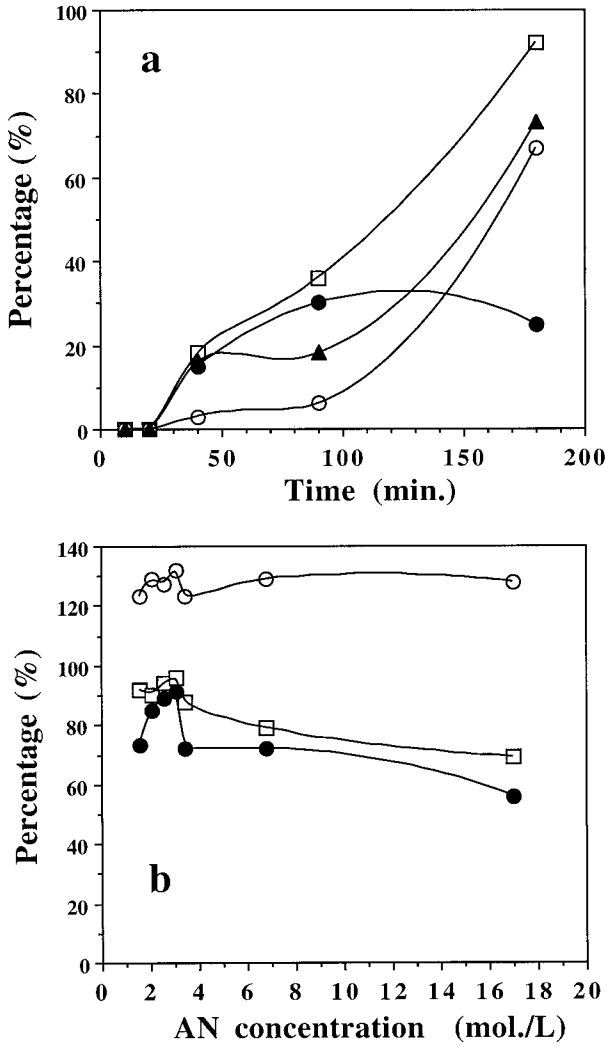

Figure 2 Effects of time and monomer concentration at constant [*I*] = 0.017 mol L^{-1} (60°C): (□) *Y*—yield; (□) *H*—homopolymerization; (▲) *GE*—grafting efficiency; (●) G_{SC}—PAN graft. (a) Effects of reaction time ([*M*]/[*I*] = 92); (b) effects of monomer concentration (3 h).

Table III Effects of Monomer Concentration ([*I*] = 17 × 10^3 mol L^{-1}, 60°C, pH: 7.8, 3.5 h) on Side-Chain Length and T_d of AN-g-Casein Copolymers

[*M*] (mol L^{-1})	[*M*]/[*I*]	M_n (×10^{-4} dalton)	Polydispersity	T_d (°C)	Color
1.57	92	3.85	2.80	272.7	White
2.04	120	4.18	3.18	270.8	White
2.55	150	4.18	3.18	270.8	White
3.06	180	4.38	3.34	268.7	White
3.40	200	4.26	4.50	254.5	White
6.80	400	4.30	4.40	273.3	White
17.0	1000	4.38	5.02	270.3	White

The molecular weights of caseins, i.e., α, β, and K caseins according to their primary sequence homology, have been reported to be ranging from 19,007 to 25,230 daltons.[17] The M_n of the side chains in AN-g-casein copolymers is in the 10^4 dalton range, basically in the same magnitude as the protein backbone (Table III). The absence of casein decomposition exotherm suggests that the PAN grafts protect the main protein backbone from thermal decomposition. This absence of casein decomposition coupled with nearly equal molecular mass of the grafts supports the idea that the PAN grafts or side chains are long branches extending from and surrounding the globular proteins.

DSC of AN-g-casein copolymers produced at varying [*I*] by the two procedures (Fig. 1) and

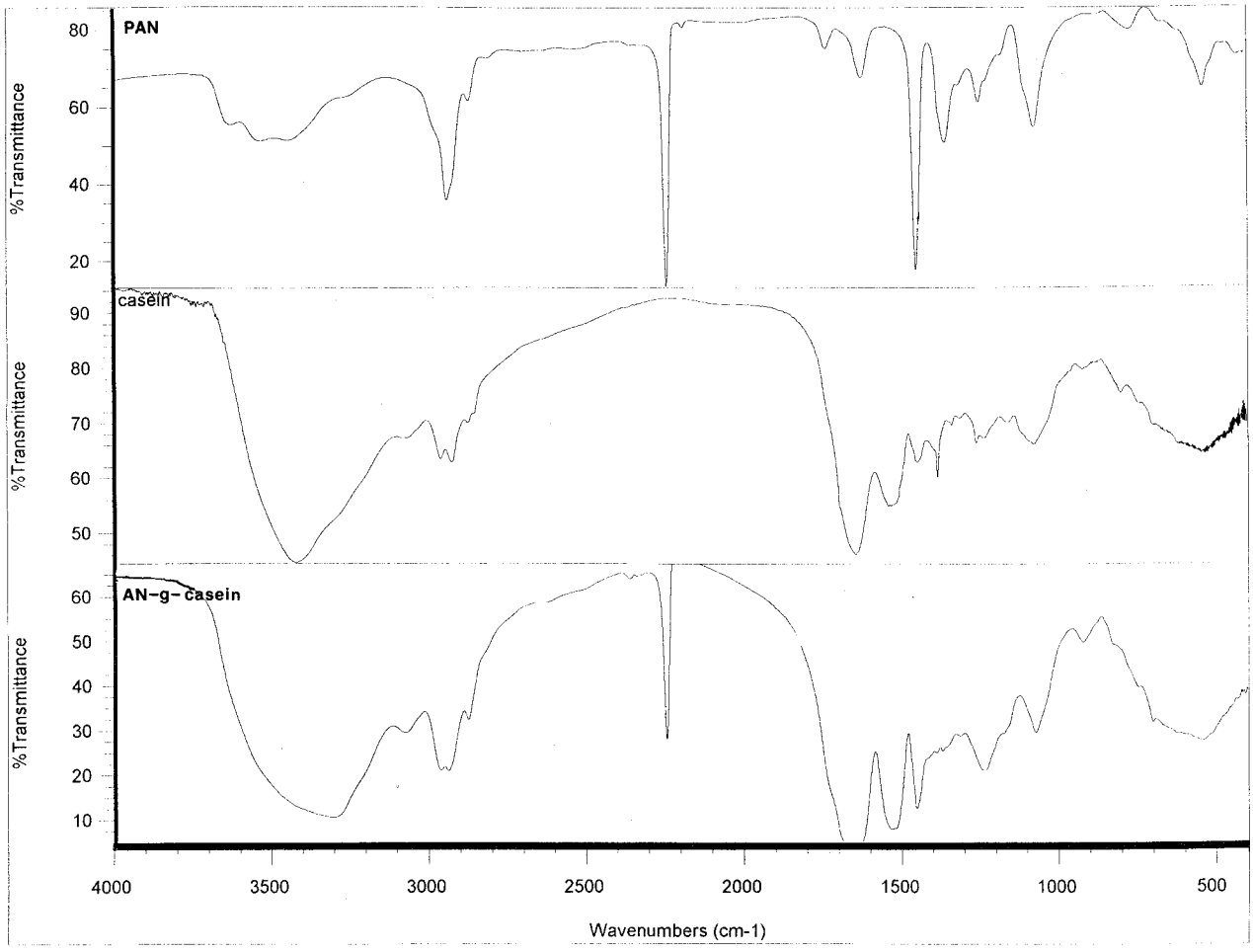

Figure 3 FTIR spectra of PAN, casein, and AN-g-casein copolymer.

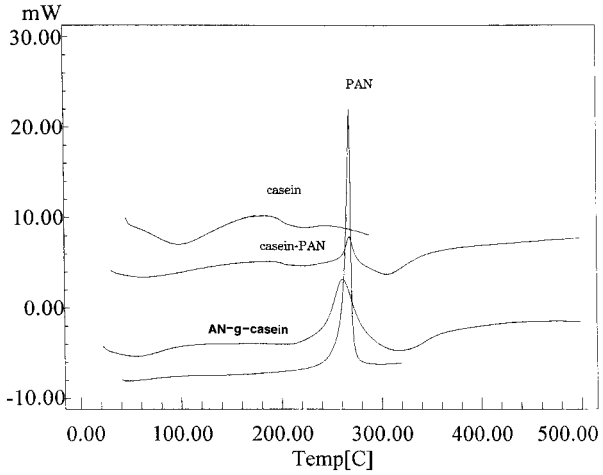

Figure 4 DSC of casein, PAN, casein-PAN mixture, and AN-g-casein copolymer.

those produced at varying [*M*] by the one-step method (Fig. 3) was performed. The T_ds of these three series of AN-g-casein copolymers were all above 247°C, significantly higher than the T_d (180°C) of casein. The T_d of the AN-g-casein copolymers increases proportional to the extent of grafted PAN side chain (Fig. 5). The highest T_ds of AN-g-casein copolymers was 273°C, very close to the T_d (275°C) of PAN.

The solubility of AN-g-casein copolymers is more like that of the PAN homopolymer. Casein is soluble in $CaCl_2/CH_3CH_2OH/H_2O$ (32:28:40 wt %) and Borax solutions (Table IV).The PAN homopolymer is soluble in DMF, 50% aq. NaSCN, and $ZnCl_2$. The AN-g-casein copolymers are soluble in 50% aq. NaSCN and $ZnCl_2$ and insoluble in $CaCl_2/CH_3CH_2OH/H_2O$ (32:28:40 wt %) like PAN. On the other hand, the AN-g-casein copolymers are insoluble in DMF like casein. The casein protein backbone in the AN-g-casein copolymers also remains hydrolyzable by acetic acid and HCl. These similarities in solution properties between the AN-g-casein copolymers and casein suggest minimal, if any, hydrolysis of casein proteins caused by the solvent, triethaolamine. The similarity between the solubility of AN-g-casein copolymers and PAN homopolymer is also consistent with the notion that PAN grafts extend and surround the globular proteins.

CONCLUSION

Solubility studies of casein in varying solutions clearly show aqueous organic media containing amine are better solvents for casein proteins. Among triethylamine, triathanolamine, and ethanolamine, casein had the best solubility in aqueous triethanolamine, which was used in the graft copolymerization of casein with acrylonitrile. Two procedures were examined for initiating grafting of AN to casein. The simultaneous one-step reaction involved the dropwise additions of initiator and monomer into casein solution over a 10-min period. The sequential two-step reaction involved introducing the monomer 30 min after the initiator was added in the casein solution.

With either reaction procedure, overall yield of reaction product (*Y*) and homopolymer (*H*) increased with [*I*] at a fixed [*M*] of 1.566 mol L^{-1}. Grafting (*G*) of AN on casein peaked at 123 and 128% at [*I*] = 0.017 mol L^{-1} for the one-step and at [*I*] = 0.005 mol L^{-1} for the two-step procedures, respectively. Further increases in [*I*] usually lead to significantly increased homopolymerization and reduced grafting efficiency (*E*). In the one-step reaction, the numbers of grafted sites on the casein molecule increase slightly with increasing [*I*]. At [*I*] = 0.017 mol L^{-1}, the highest grafting is coupled with longest and most uniformly distributed chain lengths. The sequential two-step reaction produces much higher grafting efficiencies on casein ($E \geq 90\%$) and much lower homopolymerization ($H \leq 8\%$) at [*I*] $\leq$ 0.01 mol L^{-1}. The side chains from the sequential two-step reactions are longer and more uniform in lengths. This is likely due to the creation of the active centers on the

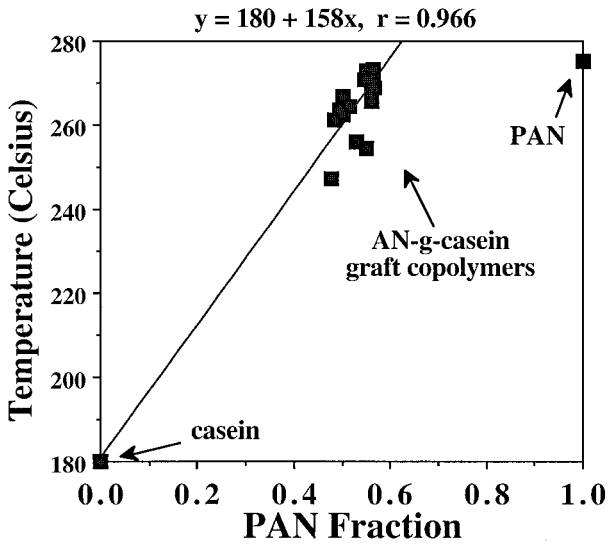

Figure 5 Relationship between decomposition temperature (T_d) and AN fraction in AN-g-casein copolymers.

Table IV Solubility[a] and Hydrolysis of PAN, Casein, and AN-g-Casein Copolymer[b]

Solvent	Casein	PAN	AN-g-Casein
DMF	I	S	I
$CaCl_2/CH_3CH_2OH/H_2O$ (32:28:40 wt %)	S	I	I
Acetic acid (pH 2.9)	S	I	H (5 wt %)
Sat. borax	S	I	I
HCl (6N)	H	I	H (backbone)
50% aq. NaSCN	SW	S	S
50% aq. $ZnCl_2$	SW	S	S

[a]S: soluble; I: insoluble; H: hydrolyzable; SW: swollen.
[b]Two gram sample in 100 mL solvent at 75°C for 24 h.

casein protein molecules in the first place. These activated sites then initiate and propagate when monomer is introduced, reducing homopolymerization. The grafted PAN side chains have M_n between 1.58 and 5.88 $\times$ 10^4 dalton and polydispersities between 2.6 and 4.5. With either method at $[I] < 0.02$ mol L^{-1}, the AN-g-casein copolymers produced are white. Either gradual addition of both initiator and monomer or delayed addition of monomer at low initiator concentrations was essential to reduce homopolymerization of AN and free radical attacks of the casein protein, thus prevent yellowing.

The FTIR spectrum of AN-g-casein copolymers confirmed the occurrence of grafted structure. The thermal property of AN-g-casein copolymers was significantly improved with T_ds of all AN-g-casein copolymers above 247°C, significantly higher than the T_d (180°C) of casein. The T_ds of the AN-g-casein copolymers increases proportional to the extent of grafted PAN side chain in the copolymers. The highest T_d of the AN-g-casein copolymers (273°C) is very close to the T_d (275°C) of PAN. The solubility of the AN-g-casein copolymers is more like that of the PAN homopolymer. AN-g-casein copolymers are soluble in 50% aq. NaSCN and $ZnCl_2$, and insoluble in $CaCl_2/CH_3CH_2OH/H_2O$ (32:28:40 wt %) like PAN homopolymer. On the other hand, the AN-g-casein copolymers are insoluble in DMF-like casein. The casein protein backbone in the AN-g-casein copolymers also remains hydrolyzable by acetic acid and HCl. These AN-g-casein copolymers have improved solubility and thermal properties that offer more options for processing and applications.

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