

Synthesis of Casein Graft Poly(acrylonitrile)

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ABSTRACT: Casein-graft-poly(acrylonitrile) was synthesized using potassium persulphate as initiator. The effect of monomer concentration on the kinetic parameters such as percent grafting and grafting efficiency were studied. The composition of the graft was analysed using Infrared spectroscopy. The mechanical and thermal stability were also studied and compared with pure casein.

KEY WORDS Casein Grafting / Acrylonitrile / IR Spectroscopy / Physical Properties /

The properties of the polymers can be modified by forming hybrids with other polymers. In general, there are two methods that can be followed to achieve this objective effectively. The first is by physically blending and the other is by chemical blending *viz.* by the formation of copolymers.

Successful formations of copolymers from natural¹⁻¹⁰ and synthetic macromolecules¹¹⁻¹³ have been widely published by various groups. Casein, a milk protein, which finds its application in leather, textiles, and paper industries has poor film forming properties due to the hydrogen bonds between C=O and -NH groups.⁷ This property may be altered by weakening these intermolecular bonds through graft copolymerization of acrylic monomer onto casein.⁷

Casein grafted with acrylonitrile (AN) forms glazable film⁷ when compared to casein grafted with other monomers like ethyl, methyl, and butyl acrylates. At higher temperatures, AN forms into a stable cyclic compound.¹⁴ Therefore, casein grafted with AN is stable even after glazing process. This paper deals with the synthesis and characterization of casein-poly-(AN) grafts.

EXPERIMENTAL

Casein (E. Merck) and potassium peroxydisulphate (E. Merck) were used as such without further purification. Acrylonitrile (BDH) was freed from inhibitor, dried and was finally distilled under vacuum. The middle fraction of the distillate was used for the graft copolymerization reactions.

Graft Copolymerization

Casein (2.5 g) was soaked overnight in water (25 ml). It was then treated gradually with a solution of triethanolamine (0.5 g) in water (10 ml). After the casein has been completely dissolved, the contents were cooled. The pH of this solution was 7.75. This solution was used in the preparation of graft copolymer.

A three necked R.B. flask fitted with a mechanical stirrer, a water condenser, a thermometer, a nitrogen inlet, and two dropping funnels was placed in a water bath maintained at a temperature of $60 \pm 0.5^\circ\text{C}$. Nitrogen was bubbled through the 10% solution throughout the reaction in order to avoid the inhibiting effect of molecular oxygen. The AN monomer was taken in one of the dropping funnels. Potassium persulphate, was

dissolved in water ($9.7 \times 10^{-3} \text{ mol l}^{-1}$) and taken in the second dropping funnel. When the temperature of the contents of the reaction vessel has attained 60°C , the monomer and initiator were added slowly over a period of 10 minutes and the reaction was allowed to continue for three hours in all cases. The reaction was quenched rapidly by immersing the flask in cold water and the graft polymer was precipitated using 10% acetic acid. The homopolymer formed in the reaction precipitates during acid treatment. The concentration of AN monomer studied were 0.8, 1.2, 1.6, 2.0, and 3.0 mol l^{-1} .

The graft copolymers were precipitated using 10% acetic acid solution and centrifuged. The precipitate was washed with water and dried in vacuum. The total polymer formed was calculated from the total weight of the product obtained. For the isolation of grafted copolymer, the ungrafted homopolymer was extracted by solvent extraction in tumble bottle method using dimethylacetamide (DMA) as the solvent for a period of 72 hours. The extracted homopolymer was regenerated using water. The graft and homopolymers were washed with water, dried and weighed separately.

Determination of Percent Grafting and Grafting Efficiency

From the weight of the graft copolymer, the percent grafting and grafting efficiencies were calculated using the following formula

percent grafting =

$$\frac{\text{weight of graft polymer}}{\text{weight of backbone}} \times 100$$

percent grafting efficiency =

$$\frac{\text{weight of the graft}}{\text{weight of the total polymer}} \times 100$$

IR Studies

Infrared studies of the grafted casein were undertaken (by making a KBr pellet with

grafted casein) using Nicolet 20 DXB FTIR spectrometer. The spectra were taken at a resolution of 4 cm^{-1} . In order to improve the spectral quality, 100 scans were taken. For the determination of the composition of copolymer, the following procedure was adopted. Calculated amount of pure casein and pure poly(acrylonitrile) (PAN) were taken separately and ground well to ensure thorough mixing. This mixture was taken with a known amount of KBr and a pellet was formed. IR spectra were taken for the pellets of pure casein and pure PAN. The area of the C=O peak in the casein and that of the C≡N peak for PAN were measured. This area was normalized for 1 g of casein/PAN. From weight and thickness of the pellet, the amount of the pure casein/PAN present in the pellet was calculated.

Casein and PAN were mixed throughly in different ratios (1:4, 2:3, 3:2, and 4:1). Calculated quantities of this casein/PAN mixture were taken, ground well with known quantities of KBr and IR spectra were taken. The area under the C=O and C≡N peaks were measured. Standard curve was made by plotting the area under the peak vs. concentration of PAN taken for a given thickness of pellet (Figure 2).

The experiments were repeated similarly for casein grafted with various concentrations of AN monomer and the area of the peak was measured. From the standard curve, the amount of PAN grafted onto the casein was calculated.

UV/Visible Spectroscopy

Solutions of PAN and pure casein were made by dissolving them in water containing *N*/20 sodium hydroxide. Known proportion of PAN and pure casein as present in grafted casein were mixed (physical mixture) and the mixture was dissolved in *N*/20 NaOH solution. Similarly the solution of grafted casein in the same concentration was also prepared. UV/visible spectra were taken in the range of

250–400 nm using Shimadzu UV 160 A spectrometer. For this study, water containing NaOH, which was used for making the solution was taken in the reference cell to eliminate the influence of water. The physical mixture was taken in the reference cell and the spectrum of grafted casein was recorded.

Thermal Studies

Viscosity studies were carried out at different temperatures for the dilute PAN grafted casein solution using Ubbelohde Viscometer.

Thermogravimetric analysis of the PAN grafted casein was studied using Mettler TA 3000 thermogravimetric analyzer, containing TGA module. The experiments were carried out in nitrogen atmosphere with a bubble rate of about 200 ml min^{-1} and using a program rate of $20^\circ\text{C min}^{-1}$.

RESULTS AND DISCUSSION

Kinetic Parameters

Grafting of vinyl monomers onto a natural and synthetic polymer can be considered as internal plasticization. Grafting in heterogeneous medium is diffusion controlled. Casein is hydrophilic and swells in water extensively. Therefore the rate of diffusion in this case is considerably high. The effect of variation of AN monomer concentration on the kinetic

parameters is presented in Table I.

The percent grafting steadily increases with increase of the monomer concentration. In the case of grafting efficiency, up to 2 mol l^{-1} of monomer concentration, there was no characteristic change. Further increase in the monomer concentration, decreases the grafting efficiency. The results obtained in the case of percent grafting is in accordance with heterogeneous graft copolymerisation.^{9,10,15,16} At higher monomer concentration, viscosity of the medium increases leading to gel effect.¹⁷ Due to this, the termination of the growing polymer chain is hindered, due to the restriction in the mobility of the grafted polymer chain. In addition, the gel effect may cause swelling of casein which assists in the diffusion of monomer to the growing chain and active sites on the casein thereby favouring grafting reaction.

It may be assumed that increased addition of monomer decreased the grafting efficiency by the chain transfer of the growing radicals to the monomers, resulting in higher amount of homopolymer formation. The decrease in grafting efficiency may also be due to the grafted chain acting as diffusion barriers which may impede diffusion of monomer into the backbone. As a result, less monomer would be available, for grafting and most of it may be used for homopolymerization. The percent grafting is dependent not only on the number of grafting sites formed, but also on the length of the grafted chains.

Spectroscopic Studies

IR spectra of pure casein, pure PAN and grafted casein are given in Figures 1a–c respectively. The IR spectrum of casein-PAN grafts (Figure 1c) show absorption at 3300 cm^{-1} (NH/OH), 1660 cm^{-1} (amide I), 1520 cm^{-1} (amide II), 1240 cm^{-1} (amide III), and 680 cm^{-1} (amide IV) in addition to the band at 2244 cm^{-1} ($\text{C}\equiv\text{N}$), characteristic of PAN. Comparison of these spectra with that of PAN homopolymer (Figure 1b) shows that the

Table I. Effect of variation of monomer concentration on percent grafting and grafting efficiency of casein-graft-poly(acrylonitrile)^a

Concn. of monomer	Percent grafting	Grafting efficiency
mol l^{-1}	%	%
0.8	22.47	98.40
1.2	43.04	79.97
1.6	56.20	94.60
2.0	65.00	94.44
3.0	72.20	68.02

^a Volume = 50 ml; initiator $\text{K}_2\text{S}_2\text{O}_8 = 9.7 \times 10^{-3} \text{ mol l}^{-1}$; $t_{\text{em}} = 60^\circ\text{C}$; time = 3 hours.

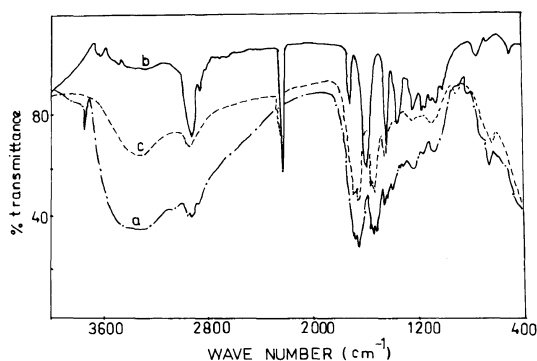


Figure 1. FTIR spectra obtained for: (a), pure casein; (b), pure poly(acrylonitrile); (c), casein-graft-poly(acrylonitrile) (2 mol l^{-1}).

prominent peak at 1660 cm^{-1} is due to amide I ($\text{C}=\text{O}$).

Comparison of Figures 1a and c shows that the strong $-\text{NH}/\text{OH}$ peak around 3300 cm^{-1} , was reduced due to grafting. This qualitatively suggests that the amino acids which contain $-\text{NH}/\text{OH}$ was affected during grafting.

Kulasekaran *et al.*¹⁸ reported, that during AN grafting of casein, amino acids like arginine, cystine, glycine, lysine, serine, threonine, and valine were serving as grafting sites. The mechanism of emulsion graft copolymerization of casein with AN monomer in the presence of redox initiator was reported by Jinyu and Ning.¹⁹ They have also reported the changes in the amino acids in casein, due to grafting of AN onto casein.

The composition of the graft copolymer was measured using IR spectroscopy.²⁰⁻²² When IR is used for this purpose, it is normally assumed, that the absorptivity (absorption coefficient) of the absorption band used for the analysis is the same in the copolymer as it is in the homopolymer.²³ Copolymers of known compositions were required to construct a calibration curve.²¹

Copolymer composition of casein grafts were analysed by monitoring the $\text{C}=\text{O}$ (1660 cm^{-1}) and $\text{C}\equiv\text{N}$ (2244 cm^{-1}) peaks. The areas of the peaks were integrated and a standard curve was drawn by plotting the area under the peak

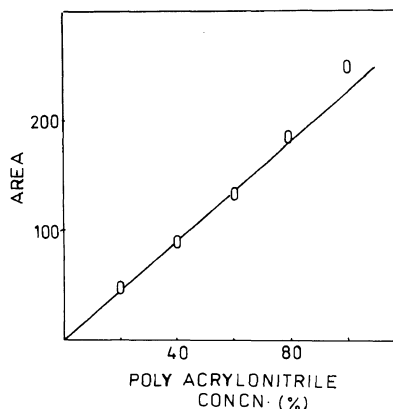


Figure 2. Standard curve showing the relation between the area (arbitrary units) under the $\text{C}\equiv\text{N}$ peak in infrared spectra with concentration of poly(acrylonitrile).

Table II. Percentage of polyacrylonitrile present in casein-graft-poly(acrylonitrile), calculated on the basis of $\text{C}\equiv\text{N}$ peak area

Concn. of monomer mol l^{-1}	% Content of PAN	Percent grafting
0.8	14.0	16.27
1.2	29.0	40.80
1.6	32.2	47.50
2.0	38.3	62.10
3.0	41.0	69.50

vs. concentration of PAN using known mixtures of PAN and casein.

Spectra were taken for grafted casein, prepared with various monomer concentrations. The concentration of PAN in the grafted caseins, were analysed using the area under $\text{C}\equiv\text{N}$ and $\text{C}=\text{O}$ in every case and from the standard curve, the PAN present in the graft was calculated (Table II). The percent grafting values follow the trend, similar to the values obtained from chemical methods (Table I). It is found, that the percent grafting values calculated using IR spectra is less when compared to the values calculated by other method. But, in samples with higher PAN content, the values agree well. The deviation of the data mainly arised from the instrument

Table III. Table showing the characteristic infrared spectral bands of casein-graft-acrylonitrile, at various copolymer compositions

Copolymer	Copolymer composition		Wave number/cm ⁻¹					
	Casein	PAN						
	%	%						
Pure casein	100	—	680	1240	1537	1556	1656	—
Pure PAN	—	100						2244
0.8	86	14	672	1240	1543	1561	1657	2243
1.2	71	29	672	1240	1543	1561	1655	2244
1.6	68	32	675	1240	1539	1563	1653	2243
2.0	62	38	672	1240	1545	1561	1653	2244
3.0	59	41	672	1240	1545	1561	1653	2243
Phys. mix. casein + PAN								
I	80	20	674	1232	1541	1556	1652	2244
II	60	40	671	1240	1541	1559	1652	2244
III	40	60	676	1240	1540	1562	1655	2242
IV	20	80	676	1239	1540	1560	1656	2242

errors due to the low absorption coefficient. This concurs with the findings of Mori *et al.*,²³ who have reported that the standard deviation of this IR method was high, when compared to other spectroscopic techniques like UV, NMR etc. for copolymer composition analysis.

The wave numbers of several characteristic absorption bands, for casein and PAN components with copolymer compositions are listed in Table III. The C=O peak and C≡N peak positions were unchanged with copolymer composition. However, the peaks at 1538 and 1555 cm⁻¹ shifts to higher wave numbers with increasing copolymer content. This is similar to the results obtained by Mori.²³

The marginal shifts in the frequencies of 1537 and 1555 peaks in pure casein on grafting is of interest. These bands are easily assigned to aromatic skeletal vibrations associated with aromatic amino acids such as tyrosine, in casein. The shifts in these frequencies after grafting, may be assigned to one or more of the following reasons *viz.* a) the aromatic residues are potential sites of grafting and b) the grafting of acrylates on to casein alter the inter chain network and local site structures

around aromatic residues.

There have been earlier suggestions in literature^{18,19} and in our own work,²⁴ that aromatic residues may be the potential sites for grafting, based on mechanistic investigations. However, on the basis of the observed experimental data, it is difficult to assign firmly the most probable reasons.

The UV spectra of pure, grafted casein and pure PAN and physical mixture are presented in Figure 3. In these cases, alkaline water was taken as the reference. Physical mixture was taken in the reference cell and the absorption was compared with grafted casein solution. If the grafted casein contains only a physical blend of PAN and casein, there might not be any absorption seen. These results suggest that PAN was not physically bound to casein, but by chemical bonding.

Mechanical Properties

Table IV shows the stress and strain, at break of grafted casein and pure casein films. The strength decreases and extensibility increases considerably, when compared to pure casein, since the grafting of PAN chain acts as an

internal plasticisation process.

Thermal Studies

Thermal stability of the grafted casein was studied using viscosity and TGA studies. The effect of temperature on viscosity of grafted casein solution is presented in Figure 4. The thermodynamic parameters are calculated

using Eyring equation,²⁵ and the results are presented in Table V.

$$\log \eta = \log \left(\frac{hN}{V} - \frac{\Delta S^*}{2.303R} \right) + \frac{\Delta H^*}{2.303R} \frac{1}{T}$$

where η = absolute viscosity; h = Planck's constant; N = Avogadro number; V = molar volume of the solvent; R = universal gas constant; T = absolute temperature.

It is seen from the graph, that there is a linear relationship between $\log \eta$ and $1/T$. The results obtained is similar to pure casein,²⁴ suggesting that there is no transition around this temperature range.

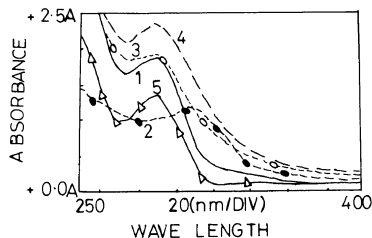


Figure 3. UV spectra obtained for different casein systems: (1), pure casein; (2), pure poly(acrylonitrile); (3), physical mixture of casein and poly(acrylonitrile); (4), acrylonitrile grafted casein; (5), (4)–(3).

Table IV. Tensile properties of casein-graft-acrylonitrile films

	Stress at break	Strain at break	Energy to break
	kg cm ⁻²	%	kcal mol ⁻¹
Acrylonitrile grafted casein (2 mol l ⁻¹)	193.2 ± 12.6	51.4 ± 7.2	9.11
Pure casein	469.0 ± 60.0	7.72 ± 0.8	1.59

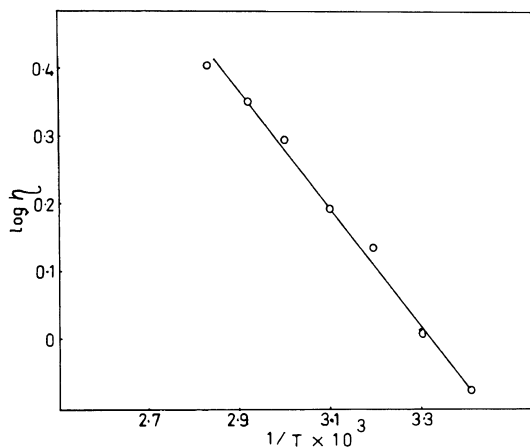


Figure 4. Figure showing the relationship between viscosity and temperature of casein-graft-poly(acrylonitrile) solutions.

Table V. Thermodynamic activation parameters of casein-graft-poly(acrylonitrile) calculated on the basis of viscosity temperature relationship

Temp K	Grafted casein			Pure casein		
	ΔH	ΔG	ΔS	ΔH	ΔG	ΔS
	kcal mol ⁻¹	kcal mol ⁻¹	cal mol ⁻¹	kcal mol ⁻¹	kcal mol ⁻¹	cal mol ⁻¹
293		9.68	8.46		2.22	4.36
303		9.51	7.62		2.16	4.42
313		9.41	7.06		1.16	4.27
323	7.18	9.04	5.71	3.5	2.07	4.44
333		8.70	4.52		2.05	4.34
343		8.68	4.31		2.02	4.32
353		8.53	3.76		1.97	4.34

The Gibb's free energy of activation decreased with the increase in temperature. The decrease clearly indicates, that the work done by the system decreases with the increase of temperature. ΔH and ΔS values were higher in the case of grafted casein, when compared to pure casein. These results suggest that the stability of casein is lowered due to grafting, and the randomness increases in the system. Table V shows that ΔS steadily decreases with the increase of temperature. Variation of $d(\Delta S)/dT$ values suggests that the system is probably more irreversible around 50°C.

The thermograms of grafted casein show that the decomposition takes place at various stages. At 100°C the decomposition level of grafted casein is very much lower (0.58%), when compared to pure casein (6%). At 350°C, the loss in weight for the two systems respectively were 23.3 and 48%. Derivatograms obtained from TGA curves show that in the first stage of decomposition, small quantity of loss in the material was observed (possibly water). Second stage of decomposition starts at 250°C and ends around 520°C. During this stage, 37.6% of the material was lost. In the next stage (551—897°C), about 48.8% of the material was decomposed. In the case of PAN side chains,¹⁴ the degradation and cyclisation starts around 150°C and the product was stable up to 400°C. This is the probable reason for high stability of grafted casein when compared to pure casein. In the case of casein, the amino acids decompose around 350°C. Since the degradation of grafted casein is multiphasic, it is difficult to explain the mechanism of decomposition at different stages.

The activation energies at different degradation stages are determined using the equation suggested by Doyle.²⁶

$$\ln(1-C) = -2.315 A/B + 0.4567 A/BE/R(1/T)$$

where C = concentration of the polymer; E = energy of activation; T = absolute temperature; A and B = constants.

The energy of activation at various deg-

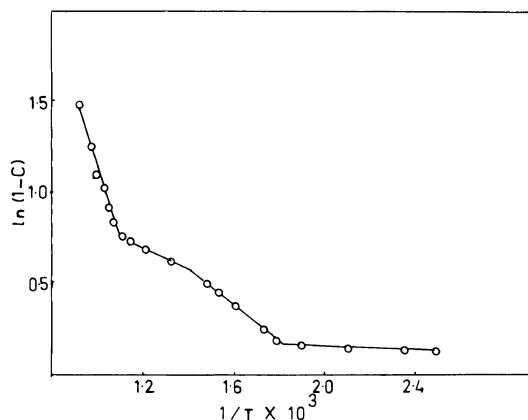


Figure 5. Concentration of casein-graft-poly(acrylonitrile) under various degradation temperatures (2 mol l^{-1}).

Table VI. Energy of activation values for different degradation stages of casein-graft-poly(acrylonitrile), obtained from TGA curves

Grafted casein		Pure casein	
Temp	E_a	Temp	E_a
K	kcal mol ⁻¹	K	kcal mol ⁻¹
379—546	2.18	333—476	3.2
546—714	5.04	476—573	4.3
714—909	4.11	573—666	5.6
909—1075	8.06	666—800	4.7

radation stages was calculated by plotting $\ln(1-C)$ against $1/T$ (Figure 5). The results were compared with that of pure casein (Table VI). At higher temperatures, stability of the grafted casein is very much higher when compared to pure casein. It may possibly be due to the cyclization¹⁴ of the AN side chain. It was reported that for polymers of sufficiently high molecular masses, the activation energy is not dependent on molecular mass.²⁷ The content of polar groups, the regularly or randomly distributed side branches affect the energy of activation.

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