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SYNTHESIS OF GRAFT COPOLYMER OF CASEIN WITH ACRYLAMIDE

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Acrylamide has been graft copolymerized onto casein in phosphate buffer medium. The grafting was characterised by elemental analysis, solubility, FTIR, intrinsic viscosity and refractive index measurements. It was observed that grafting ratio increases with increasing concentration of acrylamide and decreases with increasing concentration of casein. The effects of concentration of acrylamide and casein on grafting ratio, grafting efficiency, rate of conversion of monomers and rate of graft copolymerization have been discussed.

Key words: Grafting efficiency, Degradation, Backbone radicals.

Introduction

A significant amount of literature refers to the grafting technique for the modification of natural and synthetic polymers like collagen [1,2], gelatin [3] and casein [4,5] with suitable vinyl monomers without affecting the overall properties of the backbone chain. These graftings by use of radical forming initiator like sodium or potassium persulfate are important method since low degradation of the pure polymer takes place. But these reactions are hetrogeneous in nature resulting in phase separation [6]. The degradation of casein during reaction and problem of phase separation can be minimised by accomplishing the grafting reactions in dilute solutions (pH>7) and at low temperature (≤60°) [7]. This paper deals with the graft copolymerization of acrylamide onto the backbone of casein in dilute solutions using phosphate buffer medium.

Experimental

Casein (E.Merck, alkali soluble), sodium hydroxide (BDH) potassium phosphate (BDH) and isopropyl alcohol were used without further purification. Acrylamide (BDH) was recrystallised twice from toluene-acetone mixture solution. All other organic solvents were used after distillation.

Polymerization reactions were carried out in a flange flask fitted with stirrer, condenser, separating funnel, thermometer and nitrogen inlet. Known quantity of casein was dissolved in 45 ml phosphate buffer of pH 8.23 in the reaction flask with constant stirring under nitrogen atmosphere. Required amount of acrylamide (in 25 ml buffer) and potassium persulfate were first dissolved in buffer and then added simultaneously to the reaction flask in 10 mins with constant stirring, the reaction was accomplished at 60°±1° for 50 mins. After required reaction time, the resulting product was precipitated with ice cold isopropyl alcohol to quench the reaction. Then it was filtered and dried in vacuum to a constant weight. The unbound polyacrylamide was separated by Soxhlet extraction using water as solvent for 60 hrs. The resulting graft copolymer thus obtained was dried in vacuum at 50° to a constant weight. The product was analysed for nitrogen on an elemental analysis equipment. FTIR of the product was recorded to identify the grafting of growing polymer chain of acrylamide on the backbone of casein from the appearance of characteristic absorption bands which were not present in the spectrum of pure casein.

The intrinsic viscosity of grafted product was measured at 30° using Ostwald's type viscometer. The refractive index of a dilute solution of the product was also measured on Refractometer No.122894 of Zeiss Opton Germany.

Results and Discussion

Data regarding the graft copolymerisation of acrylamide onto casein, in phosphate buffer medium of pH 8.23 using potassium persulfate as initiator at 60° are depicted in Table 1 and 2 and shown in Figs. 2 and 3. The graft copolymer was identified by elemental analysis, solubility, FTIR spectral studies, intrinsic viscosity, refractive index etc. The elemental analysis of the product was carried out for nitrogen. The ten samples of the product contain 14.88-15.65% nitrogen whereas casein and polyacrylamide contain 14.52 and 19.60% nitrogen respectively. The percentage of nitrogen increases with increasing concentration of acrylamide in feed (Table 1) and it decreases with increasing concentration of casein in feed (Table 2). This difference in percentage of nitrogen and increase or decrease of nitrogen contents in product samples are simply due to the attachment of growing polymer chains of acrylamide onto the backbone of casein. Solubility is also one of the important parameter which helps in the identification and ensuring the addition of acrylamide molecules on the activated sites of casein backbone. Both product and casein dissolve in phosphate buffer (pH 8.2). Casein swells in

TABLE 1. EFFECT OF MONOMER CONCENTRATION IN GRAFT COPOLYMERIZATION OF ACRYLAMIDE ON TO CASEIN (1.67 x 10^{-4} MOLE) USING POTASSIUM PERSULFATE (1.48 x 10^{-3} MOLE) AS INITIATOR AT 60 ± 1° FOR 50 MINUTES.

Expt. No.	Acrylamide (mole)	Total conversion of monomer		Elemental analysis	Grafted monomer	GR	GE	Rg x $10^{-5}ms^{-1}$	Rp x $10^{-5}ms^{-1}$
		(mole)	(%)	N(%)	(mole)		(%)		
1	0.0704	0.0473	67.2	15.13	0.0349	0.41	74	1.16	1.58
2	0.1126	0.0929	82.5	15.15	0.0728	0.86	78	2.43	3.10
3	0.1400	0.1028	73.0	15.17	0.0838	0.99	81	2.79	3.43
4	0.1690	0.1310	77.5	15.62	0.1098	1.30	84	3.66	4.37

TABLE 2. EFFECT OF BACKBONE CONCENTRATION IN GRAFT COPOLYMERIZATION OF ACRYLAMIDE (0.1126 MOLE) ON TO CASEIN USING POTASSIUM PERSULFATE (1.48 x 10^{-3} Mole) AS INITIATOR AT 60°± 1° FOR 50 MINUTES.

Expt. No.	Casein x 10^{-4} (mole)	Total conversion of monomer		Elemental analysis	Grafted monomer	GR	GE	Rg x $10^{-5}ms^{-1}$	Rp x $10^{-5}ms^{-1}$
		(mole)	(%)	N(%)	(mole)		(%)		
1	1.11	0.0707	62.8	15.32	0.0546	0.97	77	1.82	2.36
2	1.67	0.0929	82.5	15.15	0.0778	0.86	78	2.43	3.10
3	2.22	0.0944	83.8	14.94	0.0761	0.68	81	2.54	3.15
4	2.78	0.0958	85.1	14.88	0.0775	0.55	81	2.58	3.19

ethylene glycol with a little solvent uptake (16%) whereas the product swells with appreciable solvent uptake (66%). Furthermore, casein swells in water with a solvent uptake of 50% whereas the product swells upto saturation and disperses into pieces. It is certainly an evidence of the attachment of growing polymer chain of acrylamide on the backbone of casein since acrylamide is soluble in water. An FTIR spectrum of a product is shown in Fig 1. The absorption band at about 1735 cm^{-1} wave number indicates the characteristic of the ester carbonyl group which supports the formation of acrylamide-g-casein.

The total conversion, grafting ratio and grafting efficiency are calculated by using the following simple relations,

$$\text{Total conversion \%} = \frac{\text{total weight of vinyl polymer formed}}{\text{weight of vinyl monomer used}} \times 100$$

$$\text{Grafting ratio} = \frac{\text{weight of vinyl polymer in grafts}}{\text{weight of backbone}} \times 100$$

$$\text{Grafting efficiency \%} = \frac{\text{weight of vinyl polymer in grafts}}{\text{total weight of vinyl polymer formed}} \times 100$$

The effect of concentration of acrylamide on the grafting of acrylamide onto casein backbone is given in Table 1 and Fig.1. These results show that with an increase in acrylamide concentration, the rate of conversion of monomer (Rp), rate of graft copolymerization (Rg), grafting ratio (GR) and grafting

TABLE 3. INTRINSIC VISCOSITY [η] NUMBER AVERAGE MOLECULAR WEIGHT, $\overline{Mn}$ AND REFRACTIVE INDEX OF POLY (ACRYLAMIDE-G-CASEIN) AND CASEIN.

Extp. No.	[η] dl/g	$\overline{Mn}$	Refractive index
1	1.90	232420	1.337
2	1.27	154729	1.337
3	1.52	185518	1.335
4	2.48	304187	1.338
5	1.84	225010	1.335
6	1.27	154729	1.337
7	1.44	175658	1.337
8	1.48	180587	1.336
9	0.30*	36000	1.336

* Casein

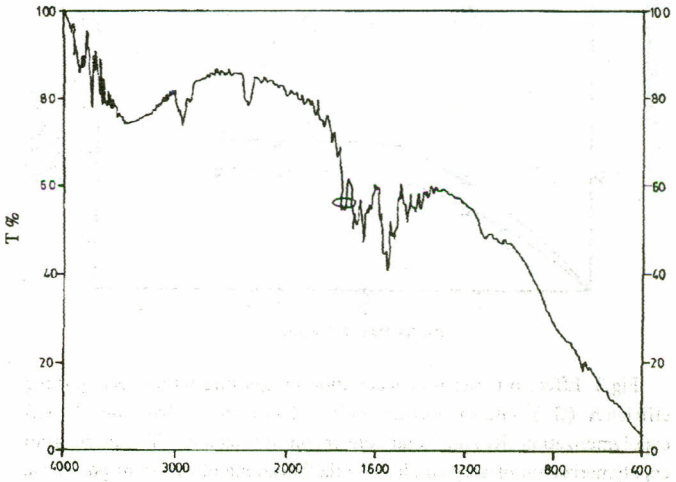

Fig.1. FTIR spectrum of graft copolymer of casein and acrylamide.

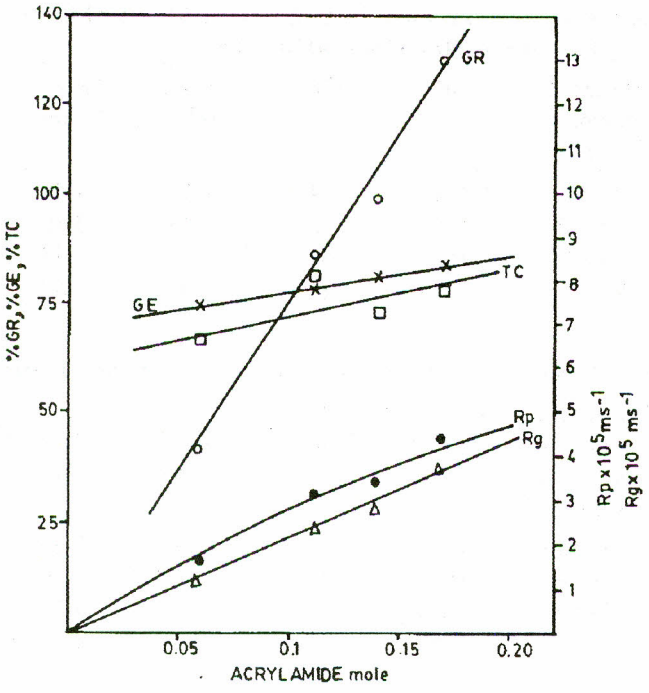

Fig.2. Effect of acrylamide concentration on grafting ratio (GR), grafting efficiency (GE), rate of polymerization of monomer (Rp), rate of graft copolymerization (Rg) and total conversion of monomer (TC) in the graft copolymerization of acrylamide onto the backbone of casein in phosphate buffer using potassium perrsulfate as initiator at 60°.

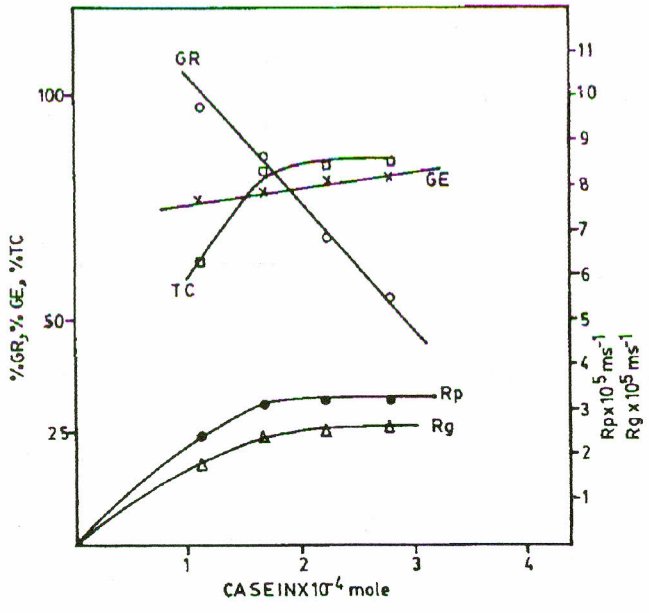

Fig.3. Effect o f casein concentration on grafting ratio (GR), grafting efficiency (GE), rate of polymerization of monomer (Rp), rate of graft copolymerization (Rg) and total conversion of monomer (TC) in the graft copolymerization of acrylamide onto the backbone of casein in phosphate buffer using potassium persulfate as initiator at 60°.

efficiency (GE) get increased. It might be due to higher availability of the monomer molecules in the vicinity of casein macroradicals. The more growing polymer chains of monomer are available which attach onto the activated sites of casein resulting more grafting. The rate of conversion of acrylamide is greater than the rate of grafting since at the early stage of reaction homopolymerization of acrylamide also takes place. The same thing happens in the graft copolymerization of different acrylates on casein [5,7], wool [8], silk [9] and Nylon [10].The rate of conversion of monomer and the rate of graft copolymerization increase (Fig. 1) progressively with monomer concentration in feed as shown in Table 1.

Table 2 includes the results of the effects of backbone concentration on the graft copolymerisation of acrylamide onto casein. These results indicate that Rp, Rg and GE increase whereas grafting ratio (GR) decreases as casein concentration increases (Fig. 2). The increase in concentration of casein produce large number of grafting sites along backbone, resulting in an increase of grafting efficiency. The decrease in grafting ratio might be due to the absolute decrease in the monomer to backbone concentration ratio. The number of grafting sites on the backbone of casein is more than that of growing polymer chains and radicals. The growing polymer chains utilize the grafting sites of backbone partially and some unbound casein radicals are left behind which cause mutual termination between backbone radicals [7]. Similar results have been obtained in the graft copolymerisation of butyl acrylate on gelatin [11].

Intrinsic viscosities [η] in dl/g of the grafted casein were determined at 30° in an Ostwaldes type viscometer using buffer of pH 8.23 as solvent. Number average molecular weight $\overline{Mn}$ was determined by using the following relation. The value of K and a of this equation was determined by use of standard samples of casein.

$$[\eta] = 9.25 \times 10^{-6}\ Mn^{-0.99}$$

Intrinsic viscosity was obtained as the intercept of the plot η_{sp}/C Vs C where C is the concentration of the solution and η_{sp} is its specific viscosity. The intrinsic viscosities of the product samples were 1.27–2.48 dl/g. The number average molecular weights calculated from these values were 154724-304187 (Table 3). The molecular weight of casein is 36000 ([η] = 0.3 dl/g). The fact that the molecular weights of the product samples, are higher than that of casein means that both acrylamide and casein are participated in the reaction and growing polymer chains of acrylamide are attached to the available activated sites of backbone. Further, the increase in molecular weight shows that there also is possibility of mutual termination of backbone radicals resulting in macromolecules of casein. The product obtained after Soxhlet extraction is the

mixture of grafted casein and grafted casein macromolecules.

When the binary mixture of casein and acrylamide in phosphate buffer is heated in the presence of potassium persulfate, three types of radicals are formed, persulfate radicals, monomer radicals and casein radicals. Persulfate radicals creat the activated centres on the backbone of casein to form casein radicals and react with acrylamide resulting in monomer radicals. At the early stage of reaction mutual termination of monomers radicals begin to take place forming polyacrylamide and then mutual terminations of monomer radicals to casein-radicals and casein radicals to casein radicals occur producing graft copolymers and macromolecules of casein. The termination race between the homopolymer radicals is faster than that between casein and homopolymer radicals. This is because the homopolymer radicals are almost immobile [7] due to reduced segment movement. Homopolymer radicals couple to all available activated sites of casein. Some times if more casein is available in the system, the homopolymer radicals can't turn each activated site of casein and then casein radicals mutually terminate themselves forming casein macromolecules. It results decrease in grafting ratio.

Some physical parameters such as refractive index, swelling properties, heating effect have also been carried out. The refractive index of product samples (Table 3) was found to be in the range 1.335-1.337 at 30° whereas the refractive indices of casein and buffer (solvent) were 1.334 and 1.333 respectively. This difference in refractive indices evidences the attachment of acrylamide molecular units to the backbone resulting in graft copolymer.

The swelling tests of casein and grafted casein were carried out in different solvents at room temperature. The results indicate that a grafted casein shows a little solvents uptake in acetone(22%), ethylacetate (4%) and tetrahydrofurane (9%) whereas in ethylene glycol the solvent uptake is increased from 16 to 66%. Casein swells in water with solvent uptake 50% but the grafted casein makes paste in it. It may be due to the attachment of acrylamide molecules to the backbone of casein and acrylamide is soluble in water. Further the product does not show any tendency to swell in solvents like benzene, xylene, CCl$_4$, chloroform DMF, alcohols, methylene chloride etc.

All the grafted casein samples are creamy tough substances which may be powdered. One of them starts decomposing above 150°. It becomes brown when temperature reaches to 166°.One furhter heating it melts at 221.5° and becomes dark brown material at 234° which does not dissolve in any solvent. The insolubility might be due to the intermolecular and intermolecular rearrangements of the amide groups in the polymer chains.

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