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## Induction of morphological changes in soy protein concentrate through pressure induced graft copolymerization and evaluation of chemical and thermal properties

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### Abstract

Modification of natural polymers like protein by graft co-polymerization is an important method to incorporate the desired properties into the backbone. Soy protein concentrate (SPC) was grafted with ethylmethacrylate (EMA) using ascorbic acid /potassium persulphate as redox initiator system under pressure. Different reaction parameters such as reaction time, reaction pressure, solvent amount, initiator ratio, pH and monomer concentration were optimized to get maximum graft yield (59.5%). The optimized reaction conditions were: reaction time; 150 min, reaction pressure; 7.5 psi, solvent; 150ml, AAc: KPS; 1:1.25, pH; 8, [EMA];  $1.99 \times 10^{-3}$  Mol L<sup>-1</sup>. The graft copolymer formed was characterized by FTIR, XRD and SEM techniques. The grafted protein was found to undergo physico-chemical changes on incorporation of polymer chains onto backbone through graft copolymerization which resulted in enhanced resistance towards moisture absorbance and acid-base attack. Thermal analysis showed higher final decomposition temperature of grafted protein as compared to that of ungrafted backbone.

**Keywords:** Biopolymers; graft copolymer; soy protein concentrate; thermal analysis; moisture resistance; swelling studies; redox initiator; pressure induced

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### 1. Introduction

In today's scenario, modification of natural polymers has gained considerable importance as these modifications enhanced the inherent physical and chemical properties of biopolymers (Lopez et al., 2006). Graft copolymerization is one of the techniques employed for modification of polymers (Raju et al., 2007). It is one of most recurrent methods to increase compatibility between synthetic polymers and natural polymer. In-depth study of synthesis, characterization and application of graft copolymers of various natural polymers has been done (Kalia et al., 2008; Lanthong et al., 2006; Mishra et al., 2003). However, only a very few authors have studied soy protein isolate graft copolymers (Xi et al., 2005; Yang et al., 2006).

Soy protein obtained from plant *Glycine max* is an industrial crop cultivated mainly for oil and proteins. It contains about 20% of oil and about 50% proteins. Soy protein has 18 amino acids including polar ones like cysteine, arginine, lysine, aspartic acid and histidine (Kumar et al., 2002). The commercially available varieties of soy protein are soy flour, soy protein concentrate (SPC) and soy protein isolate (SPI). SPC contains about 65% proteins

and 18% carbohydrate. SPC is obtained by removal of soluble carbohydrates from defatted soy flour (Swain et al., 2004). SPC is attracting much attention due to its abundance and relatively low cost but the major drawback is its poor water resistance. Functional properties of soy proteins can be improved with the help of physical, chemical or enzymatic agents. Soy proteins have been modified using alkali, urea and guanidine hydrochloride and sodium dodecylsulphate (Huang and Sun, 2000; Kalapathy et al., 1996; Zong and Sun, 2001). Cross linking (Wang et al., 2007), acylation (Frazen and Kinsella, 1976), blending with other polymers (Zong and Sunan, 2003) and enzymatic modifications (Kumar et al., 2004) are the other methods used to modify the soy proteins.

Literature review reveals that graft copolymerization of soy protein concentrate has not been carried-out till date and therefore, it was thought worthwhile to investigate the graft co-polymerization of EMA onto SPC in aqueous medium under pressure using ascorbic acid (AAc) and potassium persulphate (KPS) as an initiator system and to evaluate the thermal, physical and chemical properties of the candidate polymer. Since graft copolymerization could incorporate water, thermal and chemical resistance in the SPC backbone, it is further planned to use SPC graft copolymers as reinforcing agents in the preparation of green composites.

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## 2. Materials and Methods

Ethylmethacrylate (EMA) used was obtained from e-Merck chemicals. Ascorbic acid (AAc) and potassium persulphate (KPS) were procured from S. D. Fine chemicals LTD. Methanol, butanol and N,N-dimethylformamide (DMF) (S.D. fine chemicals LTD) were used as received.

### 2.1. Graft co-polymerization

SPC was obtained from defatted soy flour after removal of sugar and minor constituents using aqueous alcohol process (Ly et al., 1998). A known weight of SPC (0.5 g) was taken in a flask containing 100 ml distilled water. A definite molar ratio of AAc - KPS was added to the reaction flask and a known amount of monomer was added drop by drop with constant stirring. The reaction was performed under a definite pressure and in a specific time. Homopolymer formed was extracted with acetone for 24 hours and graft co-polymer thus obtained was dried at 40 °C until constant weight was achieved. Optimum conditions of reaction time, pressure, solvent amount, initiator ratio, pH and monomer concentration were worked-out to get maximum graft percentage (Pg). % monomer conversion (Pm), % graft yield (Pg), % graft efficiency (Pe) and % homopolymer (Ph) obtained were calculated as (Princi et al., 2005):

Percentage monomer conversion

$$(Pm) = \frac{(W_2 - W_1)}{W_4} \times 100$$

Percentage graft copolymerization

$$(Pg) = \frac{(W_3 - W_1)}{W_1} \times 100$$

Percentage graft efficiency

$$(Pe) = \frac{(W_3 - W_1)}{(W_2 - W_1)} \times 100$$

Percentage homopolymer (Ph) = 100 - (Pe)

Where  $W_1$  = initial wt. of sample;  $W_2$  = final wt. of sample (before removal of homopolymer);  $W_3$  = wt. of sample (after removal of homopolymer);  $W_4$  = wt. of monomer taken.

### 2.2. Characterization

#### 2.2.1. FT-IR

IR spectra were recorded with Perkin Elmer Fourier transform infrared (FTIR) spectrophotometer using KBr pellets.

#### 2.2.2. X-Ray Diffraction (XRD)

X-Ray diffraction studies were performed on XPERT-PRO X-Ray diffractometer at 40 kV and 35 mA. The samples were scanned from 5° to 50° at 2θ scale using Cu Kα X-Ray radiations of 1.5418 Å.

#### 2.2.3. Scanning Electron Microscope (SEM)

Scanning Electron Micrographs of SPC and its graft copolymer were obtained on LEO 435 VP scanning electron microscope machine.

#### 2.2.4. Thermogravimetric Analysis/Differential Thermal Analysis/Differential Thermogravimetric Analysis (TGA/DTA/DTG)

TGA, DTA and DTG studies were carried-out in the temperature range of 50° -700 °C at a heating rate of 10 °C/minute on TG/DTA 6300, SII XSTAR 6000.

### 2.3. Physio-chemical Properties

Acid and base resistance studies were carried-out as per the earlier reported method (Kaith et al., 2003). Acid resistance of the grafted vis-à-vis ungrafted sample was obtained by putting a known weight of sample (0.1 g) in 25 ml 1N HCl and the weight of each sample was noted at the regular time interval of 6 hours until a constant weight was obtained. Similarly, base resistance was studied with 1N NaOH. % weight loss was calculated as:

$$\% \text{ Wt. loss} = [(W_i - W_f) / W_i] \times 100$$

where,  $W_i$  = initial wt. of sample;  $W_f$  = final wt. of sample.

Moisture absorbance studies were carried out according to ASTM D5229 standard. Percentage moisture absorbance was studied by placing a known weight ( $W_i$ ) of dry grafted and ungrafted samples in the appropriate environment. Weight of the sample was taken at regular interval of time until constant final weight ( $W_f$ ) was obtained and % moisture absorbance was calculated as (Kaith et al., 2011):

$$\% \text{ Moisture absorbance} = [(W_f - W_i) / W_i] \times 100$$

Swelling studies of graft copolymers and ungrafted sample was done by immersing 0.1 g of each sample in a definite volume (50 ml) of water, methanol, butanol and N,N-dimethylformamide (DMF) for 24 hours. Samples were removed from solvent and the excess solvent was removed quickly with the folds of filter paper. Final weight of the sample was taken and the percentage of swelling was calculated as (Chauhan et al., 1999):

$$\% \text{ Swelling} (P_s) = [(W_f - W_i) / W_i] \times 100$$

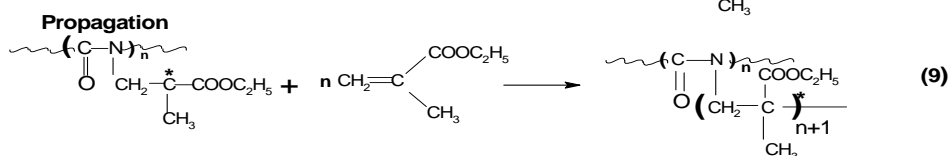
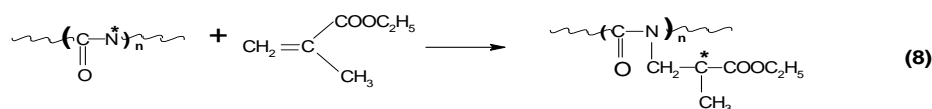
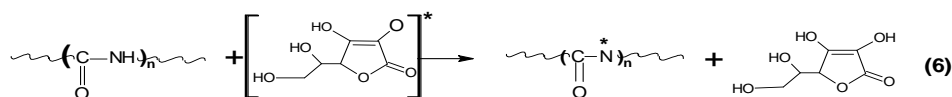
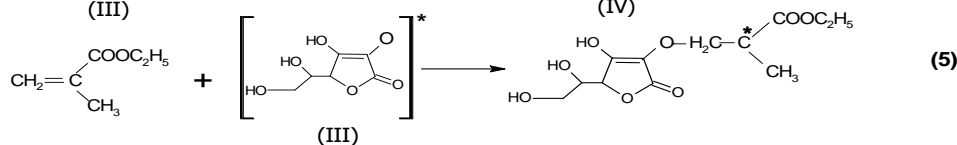
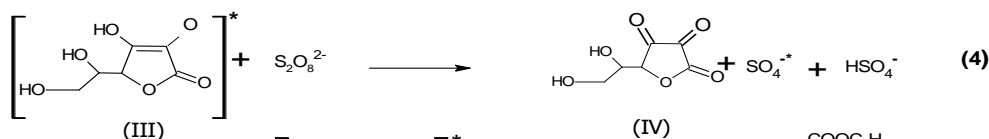
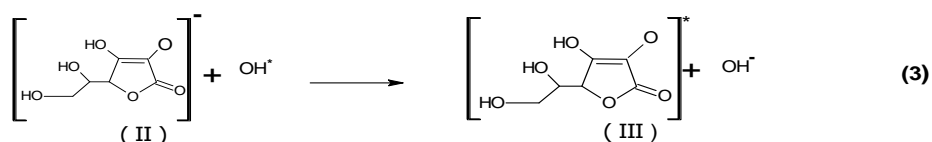
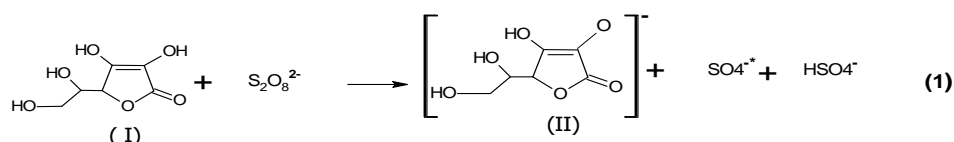
where,  $W_i$  = initial wt. of sample;  $W_f$  = final wt. of sample.

### 3. Results and Discussions

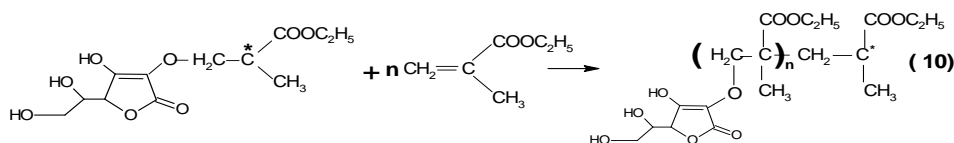
#### 3.1. Mechanism

Potassium persulphate in the presence of ascorbic acid (I) gave  $\text{SO}_4^{\cdot-}$  radicals [Eq. 1] which further in the presence of  $\text{H}_2\text{O}$  generated  $\text{OH}^{\cdot}$  free radicals [Eq. 2].  $\text{OH}^{\cdot}$  on abstraction of hydrogen free radical from ascorbic acid resulted in the formation of ascorbic acid

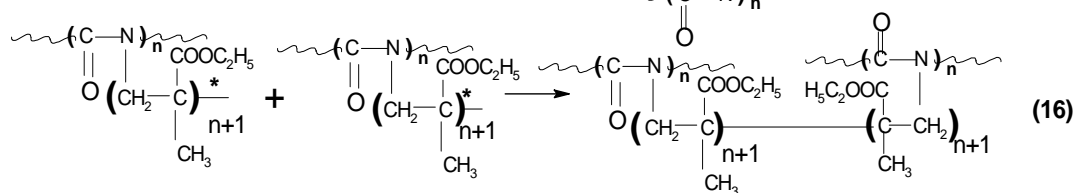
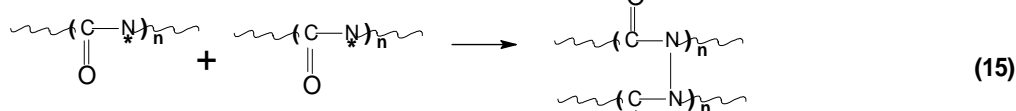
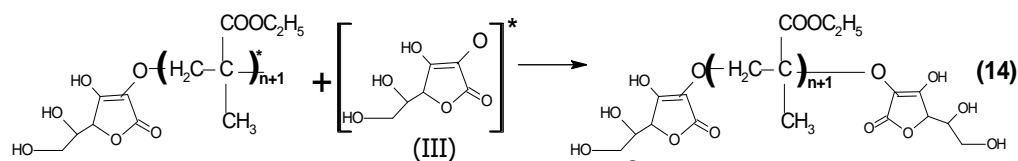
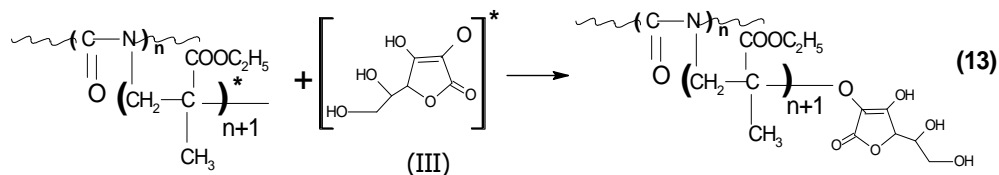
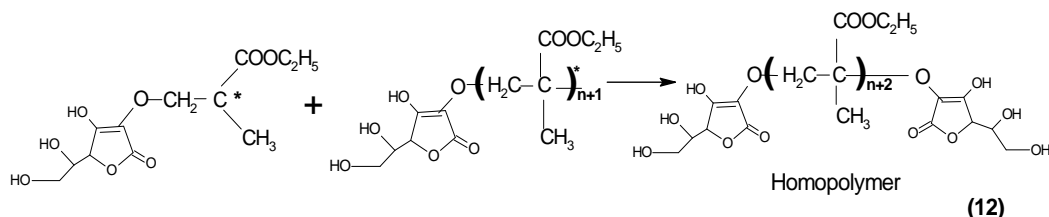
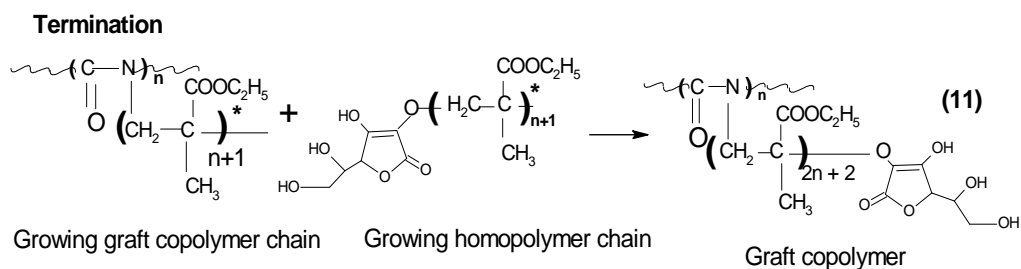
free radical species [(III), Eq. 3], which in the presence of persulphate ion gave  $\text{SO}_4^{\cdot-}$  [Eq. 4]. Thus, these primary free radical species on further reaction with monomer and backbone resulted in the generation of active sites on them [Eqs. 5, 6 and 7] (Liu et al., 2002; Yinghai et al., 2004). Monomer free radicals propagated the chain reaction resulting in growing active chains [Eq. 8].



Growing graft copolymer chain



Growing Active Homopolymer



Reaction between active backbone and growing monomer chains gave graft copolymer [Eqs. 9 - 11]. Termination of the growing chain reactions occurred either by reaction between the two live chains [Eqs. 11 or 12] or due to encounter between the active chains and ascorbic acid free radical [Eqs. 13 and 14]. The mechanism of graft copolymerization is further supported by experimental results obtained. Various reaction parameters affect the reaction mechanism. A reaction condition which favors the initiation and propagation reactions (Eqs. 1-9, 11) increases the graft percentage whereas reaction conditions favors homopolymerization and termination reactions (Eqs. 10, 12-16) decreases the graft percentage. This can

be considered by varying the reaction parameters one by one to get optimum reaction conditions.

### 3.2. Optimization of different reaction parameters

Optimization of different reaction parameters was done by changing one reaction parameter at a time, keeping other parameters constant (Table 1). Six reaction parameters were optimized and optimized reaction conditions were: reaction time (Min.); 150, reaction pressure (psi); 7.5, amount of solvent (ml); 150, pH; 8.0, AAc: KPS (Mol: Mol); 1:1.25, [EMA] (Mol L<sup>-1</sup>); 1.99 x 10<sup>-3</sup>.

### 3.2.1. Effect of reaction time on grafting

Effect of reaction time on graft copolymerization was studied by varying reaction time from 60 minutes to 180 minutes. It was found that Pg increased from 18.9% to 33.3% with increase in the reaction time from 60 minutes to 150 minutes but further increase in the reaction time resulted in decreased graft yield (S. No. 1-5, Table 1). This initial increase in Pg could be due to increase in the interaction of the primary free radicals with the monomer and soy backbone which in turn resulted in the generation of more active sites, thereby resulting in more graft copolymerization [Eqs. 5-7). However, fall of graft yield beyond 150 min time interval could be due to predominance of homopolymerization [Eqs. 10 and 12] over graft copolymerization (Roman-Anguirre et al., 2004).

Graft copolymerization was carried-out at different pressures ranging from 2.5 psi- 12 psi (S. No. 6-10, Table 1). Maximum graft yield (47.3%) was found to be at 7.5 psi. Further increase in pressure resulted in decreased Pg. It could be explained due to the fact that at low pressure, reaction between SPC and initiators was slow and as a result fewer free radical sites were formed. But as the pressure was increased, the reaction accelerated giving rise to more free radical content thereby leading to more Pg. However, increase in pressure beyond 7.5 psi resulted in more homopolymerization which suppressed the graft copolymerization and hence decreased graft yield was found (Singha et al., 2008).

### 3.2.2. Effect of reaction pressure on grafting

**Table 1.** Optimization of reaction conditions in case of SPC-g-poly(EMA)-UP

S. No	Reaction Time (min.)	Reaction Pressure (psi)	Solvent (ml)	pH	KPS:AAC (mol:mol)	Monomer x 10 <sup>-3</sup> (mol/lit)	Pg	Pm	Pe	Ph
1	<b>60</b>	5.0	100	7	1:1	1.99	18.9±2.8	34.9±2.1	9.16±2.3	90.84±1.9
2	<b>90</b>	5.0	100	7	1:1	1.99	25.7±3.2	35.6±2.4	12.15±2.6	87.85±2.4
3	<b>120</b>	5.0	100	7	1:1	1.99	27.6±3.9	37.9±2.2	12.44±2.1	87.56±2.1
4	<b>150</b>	5.0	100	7	1:1	1.99	33.3±3.8	41.7±3.1	13.48±2.7	86.52±2.5
5	<b>180</b>	5.0	100	7	1:1	1.99	30.1±2.7	47.0±2.5	10.78±2.2	89.22±2.8
6	150	<b>2.5</b>	100	7	1:1	1.99	26.1±2.8	41.3±2.2	10.67±2.4	89.33±2.4
7	150	<b>5.0</b>	100	7	1:1	1.99	33.3±3.6	41.7±3.3	13.48±3.2	86.52±3.1
8	150	<b>7.5</b>	100	7	1:1	1.99	47.3±1.5	42.8±1.7	18.63±1.9	81.37±1.9
9	150	<b>10</b>	100	7	1:1	1.99	30.9±2.5	44.6±2.1	11.68±2.0	88.32±2.2
10	150	<b>12.5</b>	100	7	1:1	1.99	26.5±1.6	47.2±1.8	9.48±1.6	90.52±2.1
11	150	7.5	<b>50</b>	7	1:1	1.99	26.3±3.0	35.5±2.9	12.58±2.5	87.42±2.7
12	150	7.5	<b>75</b>	7	1:1	1.99	38.1±2.0	36.0±2.1	17.83±2.1	82.17±2.3
13	150	7.5	<b>125</b>	7	1:1	1.99	47.6±1.6	42.8±1.9	19.72±1.8	80.28±2.1
14	150	7.5	<b>150</b>	7	1:1	1.99	49.6±1.7	43.3±2.1	20.27±2.1	79.73±2.4
15	150	7.5	<b>175</b>	7	1:1	1.99	37.9±4.5	49.3±3.4	12.95±3.1	87.05±2.9
16	150	7.5	150	<b>2</b>	1:1	1.99	23.6±2.3	27.8±2.5	14.32±1.9	85.68±2.2
17	150	7.5	150	<b>4</b>	1:1	1.99	34.4±2.1	36.3±2.2	16.01±2.1	83.99±2.1
18	150	7.5	150	<b>6</b>	1:1	1.99	40.0±3.0	38.8±3.1	17.41±2.6	82.59±2.7
19	150	7.5	150	<b>8</b>	1:1	1.99	51.2±3.3	42.5±2.9	20.34±2.4	79.66±2.3

20	150	7.5	150	<b>10</b>	1:1	1.99	40.4±4.3	50.0±3.7	13.64±3.2	86.36±3.1
21	150	7.5	150	8	<b>1:0.25</b>	1.99	23.3±2.6	40.4±2.3	9.71±2.2	90.29±2.0
22	150	7.5	150	8	<b>1:0.5</b>	1.99	29.3±2.2	41.7±2.1	11.87±1.9	88.13±2.4
23	150	7.5	150	8	<b>1:0.75</b>	1.99	40.6±2.6	44.4±2.1	15.41±1.7	84.59±1.9
24	150	7.5	150	8	<b>1:1.25</b>	1.99	<b>59.5±3.3</b>	46.1±2.8	21.75±2.3	78.25±2.3
25	150	7.5	150	8	<b>1:1.50</b>	1.99	32.2±2.8	48.7±2.5	11.16±2.6	88.84±2.7
26	150	7.5	150	8	1:1.25	<b>1.59</b>	45.6±3.7	55.2±3.2	13.95±3.1	86.05±3.2
27	150	7.5	150	8	1:1.25	<b>2.39</b>	50.0±3.1	67.8±3.1	13.50±2.4	86.50±3.3
28	150	7.5	150	8	1:1.25	<b>2.77</b>	37.1±2.8	75.6±2.4	8.29±2.6	91.71±3.1
29	150	7.5	150	8	1:1.25	<b>3.17</b>	20.5±2.1	78.6±2.1	4.39±2.1	95.61±2.4
30	150	7.5	150	8	1:1.25	<b>3.56</b>	17.0±2.7	113.0±2.5	2.55±2.1	97.45±2.2

### 3.2.3. Effect of amount of solvent on grafting

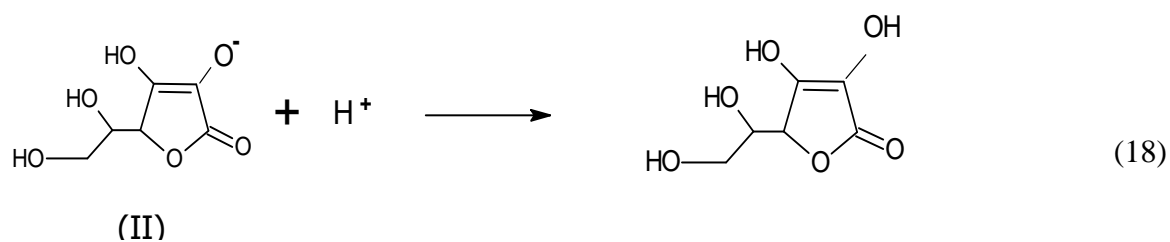
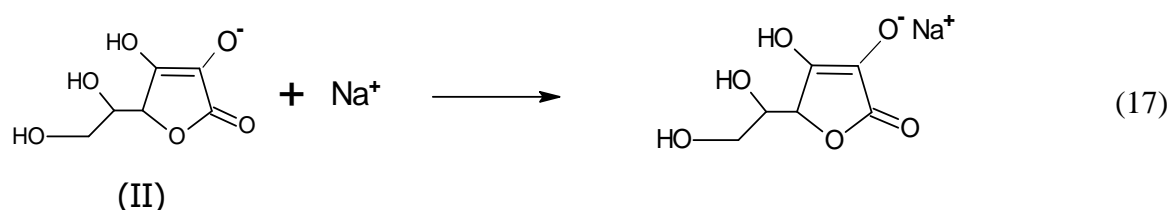
Graft percentage was found to be affected by the amount of the solvent (S. No. 11-15, Table 1). It was found that percentage grafting increased with the increase in amount of solvent. The maximum Pg was found to be 49.6% at 150 ml of solvent, which was due to more availability and access of free radicals. However, further increase in the amount of solvent resulted in decreased graft copolymerization which may be due to decrease in the concentration of primary free radicals per unit volume, thereby giving rise to lower graft percentage (Chauhan et al., 2005).

### 3.2.4. Effect of pH on grafting

Soy protein has both acidic and basic subunits thus pH of the reaction medium affects the configuration of protein. At isoelectric point of soy protein (pH 4.5) electronic screening effects make

the protein folded due to which functional groups required for the reaction remain unavailable (Xi et al., 2005; Yang et al., 2006) and therefore, result in a lower graft yield (34.4%) at pH 4.0. With variation in pH from isoelectric point the unfolding of proteins takes place due to diminishing screening effects and the functional groups become available for grafting. Pg was found to increase (34.4% to 51.2%) on increasing the pH from 4.0 to 8.0 (Table 1). Further increase in pH beyond 8.0 resulted in the increase in concentration of OH<sup>-</sup> and Na<sup>+</sup> ions in the medium which prevented the formation of ascorbic acid free radical species (Eq. 17) and thus generation of primary free radicals [Eqs. 3 and 4] was affected, resulting in decreased Pg.

Initially at pH 2.0 lower Pg (23.62%) was observed which was due to the presence of excess of H<sup>+</sup> ions resulting in the decrease of concentration of ascorbate ions (II, Eq. 18) and hence lower graft yield was observed.



Thus, a moderate alkaline pH (pH= 8.0) is the optimum condition for graft copolymerization as in case of acidic medium ascorbic acid was tended not to release  $H^+$  ion to form ascorbate ions (Eq. 1) whereas highly alkaline conditions hindered the formation of ascorbic acid free radical (Eq. 3).

### 3.2.5. Effect of initiator ratio on grafting

The effect of initiator ratio is presented in Table 1. Initiator ratio was studied in the range of 1:0.5 to 1:1.50. With the initial increase in molar ratio of AAc: KPS from 1:0.5 to 1:1.25 there was an increase in the generation of free radicals which thereby resulted in the more free radical sites on backbone as well as on vinyl monomer and thus an increased graft yield was found. The optimum molar ratio for the maximum Pg (59.5%) was found to be 1:1.25 (KPS:AAc). However, further increase in molar ratio enhanced the termination reactions [Eqs. 13 and 14] and hence a decreased graft yield was observed (Singh et al., 2009). Moreover, increase in molar ratio resulted in increased concentration of  $HSO_4^-$  ions (Eqs. 1-2) which decreased the pH of the reaction medium and resulted in the deactivation of ascorbic acid ionic species (II, Eq. 18) leading to decreased graft yield.

### 3.2.6. Effect of monomer concentration on grafting

It was observed that with the increase in monomer concentration Pg increased and maximum graft percentage of 59.48% was found at  $1.99 \times 10^{-3}$  mol  $L^{-1}$  (Table 1). This could be due to increase in concentration of monomer free radicals in the vicinity of SPC chains giving rise to increased graft yield. However, further increase in monomer concentration resulted in more homopolymerization [Eq. 12] and hence a decreased Pg beyond optimum monomer concentration was observed. Moreover, due to homopolymerization viscosity of the reaction medium increased, which hindered the approach of monomer free radicals towards growing SPC chains (Sun et al., 2003).

## 3.3. Characterization of Graft Copolymer

### 3.3.1. FT-IR Spectroscopy

The FT-IR spectra of SPC and SPC-g-poly(EMA)-UP are shown in Fig. 1. SPC showed broad peak at  $3283.4 \text{ cm}^{-1}$  due to free  $-OH$  and  $-NH$  groups, peak at  $1653.3 \text{ cm}^{-1}$  due to  $C=O$  stretch of amide group (amide-I) and a peak at  $1540.4 \text{ cm}^{-1}$  due to  $N-H$  bending (amide-II). On the other hand, SPC-g-poly(EMA)-UP showed additional peak at

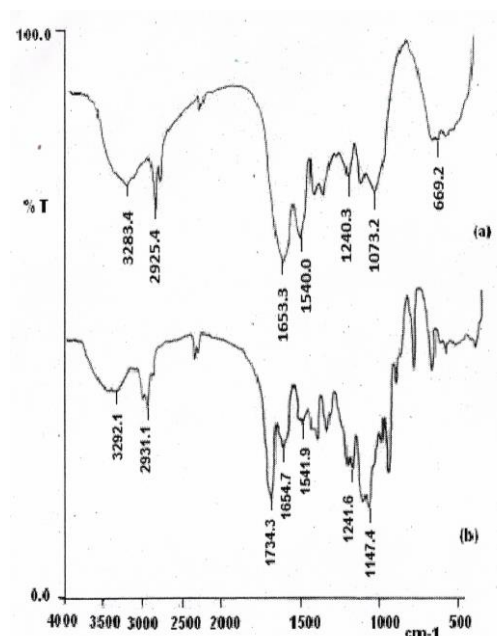
$1734.3 \text{ cm}^{-1}$  due to  $C=O$  stretch and peaks at  $1241.6 \text{ cm}^{-1}$  and  $1147.4 \text{ cm}^{-1}$  due to  $C-O$  stretching of poly(EMA). IR spectra of graft copolymer also showed decrease in the intensity of amide-I ( $1654.7 \text{ cm}^{-1}$ ) and amide-II ( $1541.9 \text{ cm}^{-1}$ ) peaks due to formation of graft chains on  $N-H$  group of soy protein backbone [20]. FTIR data exhibited the evidence for the grafting of poly (EMA) chains on the protein backbone.

### 3.3.2. XRD Studies

When crystallites are less than approximately 1,000 Å in size, appreciable broadening occur in the x-ray diffraction lines. These regions may in fact correspond to the actual size of the particles. At other times, however, these regions form "domains" in the larger particle. The breadth of the diffraction lines can be used to measure the thickness of lamellar crystals using the Scherrer equation. Use of the Scherrer equation is a primary technique to determine lamellar thickness in polymer crystallites (Sarkar et al., 2002).

$$L = 0.9 \lambda / B \cos \theta$$

where  $\lambda$  is the wavelength of X-Ray radiations for  $Cu-K\alpha$ , equal to  $1.5418 \text{ \AA}$ .  $\theta$  is glancing angle in radians and  $B$  is the width of peak at half of the maximum intensity.



**Fig. 1.** FT-IR spectra of (a) Soy Protein Concentrate (b) SPC-g-poly(EMA)-UP

XRD analysis of powdered samples of SPC and SPC-g-poly(EMA)-UP with five different % graft yields is shown in Table 2. XRD pattern of soy

protein showed the amorphous nature of soy protein. Crystal size of the backbone sample was found to increase with the grafting which is

apparent from increase in coherent length along with increase in d-spacing values with increase in Pg.

**Table 2.** X-ray Diffraction Studies of SPC and SPC-g-poly(EMA)-UP

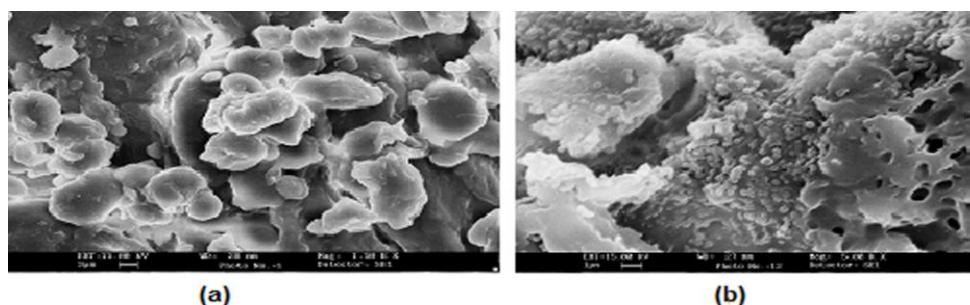
Sample code	% Graft yield	2 $\theta$ ( $^{\circ}$ )	d- Spacing (nm)	Coherent length <sup>a</sup> (Å)
SPC-g-poly(EMA)-UP-1	59.5	19.0200	4.66614	31.784
SPC-g-poly(EMA)-UP-2	47.3	19.0674	4.65656	28.997
SPC-g-poly(EMA)-UP-3	34.4	18.8188	4.61557	25.783
SPC-g-poly(EMA)-UP-4	27.7	19.3869	4.57685	23.376
SPC-g-poly(EMA)-UP-5	17.0	18.7850	4.52007	20.756
SPC	--	19.6995	4.50669	14.154

$$^a \text{Coherent length} = 0.9\lambda / \text{Bcos}\theta$$

The observed difference in the coherent length of SPC and SPC-g-poly(EMA)-UP could be due to contributions from the phase morphology originated in graft copolymers composed by different phases linked through covalent bonds. Moreover, scattering originated from the discontinuity at the interface between SPC backbone and poly(EMA) may lead to increase in coherent length of the grafted polymer. Thus, with increase in Pg, anisotropy continued to increase (Table 2) and SPC became more crystalline in nature on incorporation of poly(EMA) chains with graft copolymerization process. Maximum anisotropy was found with 59.5% graft yield where coherent length was found to be 31.784 Å.

### 3.3.3. Scanning Electron Microscopy (SEM)

A clear cut morphological differentiation has been observed in the scanning electron micrographs of soy protein concentrate and SPC-g-poly(EMA)-UP (Fig. 2). Since on graft copolymerization of EMA the inclusion of poly(EMA) chains onto SPC backbone takes place which resulted in the heterogeneity on the surface of the samples and, moreover, the natural crystal lattice of the backbone polymer also disturbed during the grafting process (Kaur et al., 1998; Saikia and Ali, 2009). Therefore, a significant change in surface morphology of the backbone polymer was observed after graft copolymerization.



**Fig. 2.** Scanning electron micrograph of (a) Soy Protein Concentrate (SPC) (b) SPC-g-poly(EMA)-UP

### 3.3.4. Thermal studies

Thermogravimetric analysis of grafted and ungrafted soy protein concentrate was carried-out as a function of % wt. loss vs temperature. Soy protein has a three dimensional structure involving sequence of amino acids. Covalent bonds present in soy proteins are either peptide bonds between amino acid residues or disulphide bonds. Proteins also have electrostatic-hydrophobic interactions and hydrogen bonding.

In case of SPC three phase decomposition was found (Fig. 3a). First phase decomposition involve

8.8% wt. loss in temperature range of 46.4 $^{\circ}$  – 218 $^{\circ}$ C corresponding to elimination of water and dissociation of quaternary structure of proteins. Second phase of decomposition involved two stages, one with 43.7% wt. loss in the temperature range of 218 $^{\circ}$ –358.9 $^{\circ}$ C due to cleavage of peptide bonds of amino acid residues and second with 19.0% wt. loss in the temperature range of 358.9 $^{\circ}$ –501.4 $^{\circ}$ C corresponding to dissociation of S-S, O-O and O-N bonds (Swain et al., 2005). Third phase of decomposition involved 21.3% wt. loss in the temperature range of 501.4 $^{\circ}$  – 561.7 $^{\circ}$ C due to complete decomposition of proteins, resulting in the liberation of various gases like CO,



CO<sub>2</sub>, and NH<sub>3</sub> (Nanda et al., 2007). In case of SPC-g-poly(EMA)-UP prepared under pressure, a two phase thermal decomposition in the temperature range of 229.1°–387.5°C (66.4% wt. loss) and 387.5°–616.4°C (25.1% wt. loss) was observed (Fig. 3b). A comparative study of TGA of SPC and its graft copolymer in the temperature range from 218.0–387°C showed that SPC lost 43.7% (218–358.9°C) weight whereas its graft copolymer showed 66.4% weight loss (229.1–287.5°C). This sharp weight loss in case of grafted product could be due a significant morphological and chemical transformation which occurred as a result of inclusion of poly(EMA) chains onto backbone during grafting process and this sharp loss in weight could be due to depolymerization of the grafted chains. It is further supported by rate of weight loss (0.958 mg/min) in DTG studies in the same temperature range (372°C). Moreover, in case of graft copolymer at 538°C the rate of weight loss is much lower (0.133 mg/min) whereas a higher weight loss (0.384 mg/min) has been found with SPC at 504.5°C. DTG analysis of SPC, showed other exothermic peaks at 63.2°C (0.0841mg/min.), 320.5°C (0.439 mg/min.) and 496.9°C (0.884 mg/min.). Thus, TGA and DTG data showed that SPC-g-poly(EMA)-UP was thermally more stable than ungrafted SPC. This increase in thermal stability was due to incorporation of poly(EMA) chains onto SPC backbone through covalent bonding.

In case of DTA studies, SPC showed three exothermic peaks at 329.2°C (29.2μV), 500.4°C (152.4μV) and 503.8°C (98.6μV) corresponding to TGA decomposition stages of 218–358.9°C, 358.9–501°C and 501–561.7°C, respectively. In case of SPC-g-poly(EMA)-UP, DTA showed exothermic peaks at 381.2°C (43.05μV) and 531.4°C (22.29μV) corresponding to thermal degradation that occurred in the temperature range of 229.1–387.5°C and 387.5–616.4°C in TGA.

### 3.4. Physio-chemical properties

#### 3.4.1. Acid and base resistance studies

It was observed that acid resistance (Fig. 4a) and base resistance (Fig. 4b) of grafted protein concentrate was found to increase with increase in % grafting. This could be explained due to the fact that poly(EMA) chains grafted onto soy protein backbone being highly hydrophobic in nature, possess less chemical affinity for both acid and base in comparison to backbone (Singha et al., 2004). Thus, incorporation of poly (EMA) chains onto SPC backbone through graft copolymerization resulted in increased acid and base resistance.

#### 3.4.2. Moisture resistance studies

It was observed that graft copolymerization has a great influence on moisture absorbance behavior of soy protein concentrate. Moisture absorbance of soy protein concentrate was found to decrease with increase in % grafting (Fig. 4c). This could be due to insertion of hydrophobic poly(EMA) chains onto SPC backbone and thereby blocking the sites vulnerable for moisture absorbance (Kaith et al., 2011b), resulting in moisture retardancy with increase in Pg.

#### 3.4.3. Swelling studies

Swelling behavior of SPC and its graft copolymers has been studied in different solvents: H<sub>2</sub>O, DMF, CH<sub>3</sub>OH and butanol. SPC showed maximum swelling in water (180.8%) followed by swelling in DMF (148.9%), methanol (121.8%) and n-butanol (110.5%). Whereas graft copolymers have been found to show reverse trend with maximum swelling in butanol followed by methanol, DMF and water. SPC-g-poly(EMA)-UP (Pg =54.6%) showed 45.9%,54.7%, 69.6% and 81.7% swelling in water, DMF, methanol and butanol, respectively. This can be explained on the basis of polarity of the solvents by considering the dielectric constant ( $\epsilon$ ) and polarity index (PI) of

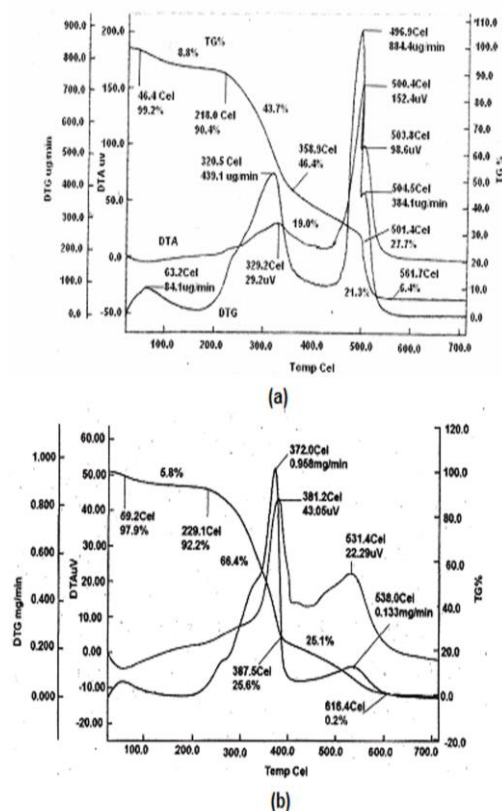
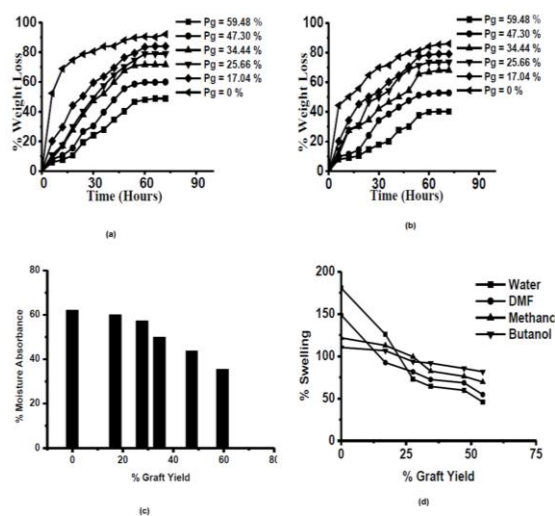


Fig. 3. TGA/DTA/DTG of (a) Soy Protein Concentrate (SPC) (b) SPC-g-poly(EMA)-UP

solvents (Kaur et al., 2010). Water has maximum  $\epsilon$  and PI (78.54 and 10.2, respectively) followed by DMF ( $\epsilon = 38.3$ , PI = 6.4), methanol ( $\epsilon = 33.0$ , PI = 5.1) and butanol ( $\epsilon = 17.8$ , PI = 3.9).



**Fig. 4.** Effect of grafting on (a) Acid resistance in 1N HCl (b) Base Resistance in 1N NaOH (c) Moisture resistance (d) Swelling behavior for different solvent

As soy protein concentrate has polar groups like –NH and –OH, so it has more interaction with solvents of high polarity like H<sub>2</sub>O and DMF. These solvents due to more interactions can penetrate deep into the protein which results in more swelling. Graft copolymerization of SPC with EMA resulted in the incorporation of hydrophobic polymer chains onto SPC.

These hydrophobic chains have less affinity towards polar solvents depending on which graft copolymers show the least percentage swelling in water and maximum percentage swelling in the least polar butanol (Fig. 4d).

#### 4. Conclusion

Thermal stability of soy protein concentrate was found to increase on grafting with ethylmethacrylate in presence of ascorbic acid – KPS initiator. Moreover, the sample was found to undergo physico-chemical changes on graft copolymerization resulting in retardancy towards moisture and acid-base attack. Since incorporation of the hydrophobic properties in SPC make it acid-base resistance the modified SPC could be used as reinforcing material in the preparation of green composites possessing better moisture retardance and acid-base resistance along with better reinforcing phase-matrix interface interactions so that applied load could be transferred to the reinforcing material through the matrix effectively. Thus increase in thermal stability, resistance towards acid-base and moisture retardancy in the

soy protein concentrate on graft co-polymerization with ethylmethacrylate is important from a technological point of view.

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