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To cite this article: Sasmita Pattanaik et al 2018 IOP Conf. Ser.: Mater. Sci. Eng. 410 012011

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Graft copolymerization of Soy Protein Isolate with Polylactide via Ring Opening Polymerization

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Abstract
The present work is devoted to the graft copolymerization of polylactide onto soy protein isolate backbone by ring-opening polymerization in the presence of Sn(Oct)2 catalyst. The effect of various input parameters on the output parameters has been carried out to investigate the required levels to get an optimum product. Maximum graft yield of 81.4% polylactide-g-soy protein isolate could be obtained at 90°C within 12 h and while using SPI amount 1.5g, 1g lactide and preswelling time of 14 h. Characterizations of the grafted polymers were carried out by various methods like Fourier transform infrared (FTIR) spectroscopy, Nuclear Magnetic Resonance (NMR) spectroscopy, thermal (TGA/DTA/DSC), X-ray Diffraction (XRD) and Field emission Scanning electron microscopy (FESEM). The swelling behavior of the SPI and grafted copolymers were studied in binary solvent (water: ethanol) system and the % swelling and % solubility has been determined.

Keywords: Ring opening polymerization, polylactide, soy protein isolate, biodegradable polymer, graft copolymerization.

Introduction
Researchers around the world have made significant technological advance by synthesizing biodegradable polymers like polylactic acid (PLA), polycaprolactone (PCL), poly(hydroxybutyrate) (PHB) and poly(hydroxybutyrate - valerate) (PHBV).1,2 Its demand has increased as a replacement to synthetic thermoplastic for various applications viz. controlled release of medicaments into the human and animal bodies, manufacture of bioabsorbable prostheses or controlled release of insecticides in the agricultural field. However, these polymers are usually more expensive than petroleum - based polymers. Thus, to solve this problem, development of low cost biodegradable polymers has attracted a number of research scientists for last three decades.3-4

Therefore, in the present investigation soy protein isolate (SPI) has been chosen which is a low cost and profusely obtainable agronomic material, which can become a good material for manufacturing biodegradable plastics and food and/or non-food packaging films. However, soy protein could not be processed without a sufficient amount of plasticizers like common thermoplastic materials because of strong intra- and intermolecular interactions, which lead to its low mechanical properties.5 Further, high polarity and hydrophilicity of soy protein molecules restricts the applications of soy protein in plastic materials. Thus, in order to overcome the above mentioned issues, soy protein has been blended with biodegradable polysters and grafted by some polymers such as polyethylene, polylactic acid, polydioxane etc. However, difference in polarities makes most of the polysters, amide and esteramides immiscible with protein.3-4 Subsequently, decrease in the physical behaviour is observed due to thermodynamic incompatibility and poor interfacial adhesion.

Based on literature review, a very few information about graft copolymerization of soy protein with cyclic esters has been found.6 According to Liu research group the aliphatic polyesters such as poly(p-dioxanone) PDO, polycaprolactone PCL, polylactide PLA, etc, has been used in medical application and chemical industries for their biodegradability, biocompatibility and good mechanical behaviour.6-8 Soy protein becomes water resistance with thermoplasticity by grafting/blending with aliphatic polysters due to their hydrophobicity and excellent processability nature.

Therefore, in nutshell, the present work is devoted to the graft copolymerization of polylactide onto soy protein isolate by ring-opening polymerization in the presence of Sn(Oct)2 catalyst. The effect of various input parameters on the output parameters has been investigated so as to control these in required levels to get an optimum product. The structure of graft copolymers were analysed by Fourier transform infrared (FTIR) spectroscopy, 1H NMR, differential scanning calorimetry (DSC), thermogravimetry (TG), and wide-angle X-ray diffraction (WAXD).
Experimental Materials

Soy protein isolate (SPI) with a protein content of 95% (dry basis) was purchased from Adeesh Agrofoods, Haryana, India. 3,6-dimethyl-1,4-dioxane-2,5-dione (L-Lactide) was purchased from Sigma Aldrich (Netherland) and was used as received and were used as monomer. Stannous octoate (Sn(Oct)₂) was purchased from Sigma Aldrich and was used as received. Toluene was received from Himedia Laboratory Pvt. Ltd, India. It was dried by refluxing over a Na/benzophenone complex, and was distilled under nitrogen just before use. Cyclohexane was purchased from Nice Chemicals Pvt Ltd, Cochin and was used without further purification.

Synthesis of polylactide-g-soy protein isolate

The graft copolymerization of polylactide onto soy protein isolate was carried out under purified nitrogen atmosphere. A 50 ml three-necked round-bottomed flask was taken as the reactor, requisite amount of soy protein isolate and Millipore water was poured into the reactor and was placed inside silicone oil bath maintained at required temperature. It was fitted with gas inlet & outlet tubes at 2 extreme necks and the condenser was fixed at the middle neck and purified nitrogen gas was purged through the reactor. Preswelling of the SPI molecules was conducted with stirring of the mixture at 80ºC for particular time period and further refluxed under nitrogen atmosphere by using Millipore water. After removal of the water in the mixture by azotropic reflux with cyclohexane for 4 h, monomer L-lactide and Sn(Oct)₂ (0.3 wt % of total amount of the reactants) was added, and graft copolymerization reaction proceeded at various reaction temperature for varying time period. Finally, after the desired time period, the products were purified in a Soxhlet extractor with acetone for 72 h to remove the unreacted monomers and the oligomers of Polylactide and then dried under vacuum to a constant weight.

The graft yield (%) was calculated as the percentage increase in weight of dry precipitate over the original weight of soy protein isolate. 9 The percentage of grafting was calculated using Fanta’s formula as given in Eq. 1.

\[
\text{Percentage of graft yield} = \frac{\text{Weight of polymer in graft}}{\text{Weight of ungrafted polymer}} \times 100 \quad \text{...(1)}
\]

Characterization Methods

Soy protein isolate and polylactide-g-SPI sgrafted (graft yield-81.4%) samples were characterized by using different analytical instruments such as FTIR (Thermo Nicolet Avatar 370) spectroscopy, H NMR spectroscopy (Bruker Avance 500), X-Ray Diffractometer (XRD) Bruker AXS D8, Thermal analyzer (TGA/DTG) (TGA Q-50 TA Instruments), Differential Scanning Calorimetry (DSC) DSC Q-20 TA Instruments and Field Emission Scanning Electron Microscopy (FESEM) QUANTA 200FEG.

Swelling Study of Polylactide-g-Soy Protein Isolate

Swelling behaviour of neat soy protein isolate and grafted copolymers of polylactide-g-soy protein isolate was studied in a polar solvent to determine the swelling percentage and percent solubility.

Procedure for Swelling Study

1 g sample each of neat SPI and grafted copolymers of polylactide-g-soy protein isolate (graft yield-81.4%) taken separately were suspended in 10 ml of the binary polar solvent system of mixture of ethanol and water in different proportion and the reaction vessel was kept at 40ºC. The samples were filtered after 24 h. The adhered water was dried by pressing the sample gently between the folds of filter paper and weighed immediately. The percent swelling was determined from the increase in the weight of the original sample as follows:

\[
\text{% Swelling} = \frac{W_e - W_o}{W_o} \times 100
\]

Where \(W_e\) is the weight of swollen polymer and \(W_o\) is the weight of original polymer. The swollen sample was dried in an oven till constant weight is obtained and percent solubility was calculated from the following equation:

\[
\text{% Solubility} = \frac{W_d - W_o}{W_o} \times 100
\]
Where \( W_o \) is the weight of original polymer and \( W_d \) is the weight of dried sample after swelling. Dried samples were again placed for swelling study in the solvent for another 24h at the same temperature. Percent swelling and percent solubility was calculated as follows:

\[
\% \text{ Swelling} = \frac{W_{d'}-W_d}{W_d} \times 100
\]

Where \( W_d \) is the weight of dried polymer after 24h and \( W_{d'} \) is the weight of swollen polymer sample after 48h.

\[
\% \text{ Solubility} = \frac{W_{d''}-W_{d'}}{W_{d'}} \times 100
\]

Where \( W_d \) is the weight of dried polymer after 24 h and \( W_{d''} \) is the weight of dried sample after swelling for 48 h.

**Results and Discussion**

**Copolymerization of polylactide-g-SPI**

The total numbers of operating parameters that influence the synthesis of polylactide-g-soy protein isolate grafted copolymers are about six. The effects of various parameters such as reaction time, reaction temperature, preswelling time, concentration of soy protein isolate, lactide, and catalyst stannous octoate on grafting were studied to optimize the reaction conditions for maximizing graft copolymerization. The effects of various parameters were studied by varying one parameter and keeping all other parameters constant. The most significant output parameter for synthesis of polylactide-g-soy protein isolate copolymers is graft yield (wt. %) and it regulate physical, mechanical & biodegradable properties of the polymers, respectively.

**Effect of reaction time on graft yield**

Fig. 1 shows the influence of the reaction time on the graft copolymerization. It has been observed from Figure 1 that with the increase of the reaction time 7-12 h, the percentage of grafting increases and with further increase in reaction time period, graft yield (%) decreases. Thus, the optimum time period for the graft copolymerization process is around 12 h.

The initial increase in graft yield (%) with increasing time period is attributed to the fact that with increasing reaction time, the concentration of initiating species increases and with further increasing in time period, destruction of initiating species occurs and thereby leading to decreasing graft yield (%) with time period.\(^\text{10}\) The process of depolymerization occurs with longer time period and thereby decreasing the graft yield (%) after the optimum time period of 12 h. Beyond the optimum time period, the active sites on polylactide and SPI might be killed leading to decrease in percent graft yield.\(^\text{4}\)

**Effect of reaction temperature on graft yield**

The effect of reaction temperature on graft yield (%) was studied within the temperature range 80-110°C and is depicted in Fig. 2. From the Fig. 2, it is evident that with the increase in temperature from 80°C to 90°C, the graft yield (%) has increased but on further increasing the temperature the graft yield (%) was found to decrease. The percentage of graft yield was maximum at 90°C.

This can be explained by assuming that on increasing the temperature from 80-90°C, solubility of PLA, swellability of SPI, attack of PLA units on the SPI backbone in the solution favors grafting and thereby increases
the graft yield (%). But above 90°C, the interaction of SPI macro radicals with Sn(II) ions destroys the activity of the initiating species and thus leading to decreased graft yield (%) at higher temperatures. 4,5,11

![Diagram](image1.png)

**Fig. 2** Variation of graft yield (%) of Polylactide-g-SPI with Reaction Temperature

**Effect of amount of SPI on graft yield**

The effect of amount of soy protein isolate (SPI) on graft yield (%) is given in Fig. 3. It was observed that on increasing the amount of SPI from 0.5 to 1.5 g the percentage of grafting increases and with further increase in amount of SPI, graft yield (%) is found to decrease.

The increasing trend may be due to increasing concentration of SPI macro radicals at higher concentration and the rate of their combination with lactide units has become faster. However, at further higher concentration of SPI, the graft yield (%) decreases which may be attributed to the fact that the concentration of SPI macro radicals increases further with increase in concentration of SPI and the rate of their combination and disproportion are faster than the rate of their combination with polylactide (PLA) molecules. Further, the rate of diffusion of PLA onto the surface of SPI decreases with further increase in concentration of SPI which hinders the grafting of PLA. Alternatively, when the concentration of the SPI was above a certain value, there were more free radicals attacking the lactide monomeric or polymeric units than the SPI and thus, more PLA homopolymer is formed. 4,5,10

![Diagram](image2.png)

**Fig. 3** Variation of graft yield (%) of Polylactide-g-SPI with Weight of SPI

**Effect of concentration of L-Lactide on graft yield**

The variation plot of concentration of L-Lactide (LA) with graft yield is shown in Figure 4. It was observed from the Figure that on increasing the amount of L-Lactide (LA) from 0.5 to 1.0 g the percentage of grafting increases and with further increase in amount of LA graft yield (%) decreases.
The initial increase in the graft yield percentage was obviously due to the greater availability of monomer in the proximity of the SPI macroradicals. At higher concentration of L-Lactide, the decrease in graft yield may be due to the wastage of LA molecules in the formation of a large amount of homopolymer. Sn(Oct)$_2$ can react with a trace amount of water to form the active derivatives, which promotes the lactide polymerization directly as PLA homopolymer. Moreover, the large molecular weight PLA homopolymer increased the viscosity of the reaction medium. The diffusion coefficient of the monomer molecules to the SPI macroradicals was reduced because of the increase of the viscosity of the reaction system, resulting in the gradual decrease in the percentage of grafting, as well as the percentage of efficiency.

![Graph](image)

**Fig. 4** Variation of graft yield (%) of Polylactide-g-SPI with Weight of L-Lactide

**Effect of amount of catalyst on graft yield**

The effect of amount of catalyst (wt %), stannous octoate (Sn (Oct)$_2$), on graft yield (%) is shown in Figure 5. From the figure, it was observed that with the increase in amount of catalyst from 0.2 wt % to 0.3 wt %, the graft yield (%) increases and with further increasing to 0.4 wt %, the graft yield (wt%) was found to decrease. The graft yield (wt %) was highest when the amount of catalyst was maintained at 0.3 wt % during the reaction.

With the increase in amount of catalyst from 0.2 to 0.3 wt %, a large number of stannous dihydroxide initiator is formed which helps in opening of the lactide ring, which further interacts with the SPI macroradicals and initiate grafting and thereby increasing the graft yield. However, with further increase in amount of catalysts to 0.4 wt %, abundant amount of lactide will undergo ring opening and thus will lead to more amount of homopolymer formation. Some of these active species of lactide monomeric units will also interact with SPI macroradicals and will lead to graft copolymerization. Homopolymer macroradicals formed might terminate the growing grafted chain; the active sites formed on the main chain of SPI might be oxidized and thus terminate the reaction site. The process of graft copolymerization and homopolymerization in such a system is a matter of competition, which depends on the direct attack of active species on SPI or on lactide monomer or polylactide units. When the concentration of the catalyst was above a certain value, they are mainly favoring the homopolymerization reaction instead of graft copolymerization and thus the graft yield (wt %) is found to decrease with the increase in amount of catalyst.
Figure 5 shows the variation of graft yield (%) with preswelling time. It has been observed from the figure and Table mentioned above that with the increase of the preswelling time from 8 to 14 hour, the graft yield (%) increases and then with further increase in the preswelling time, graft yield (%) was found to decrease. It is clear to see that reaction could proceed effectively after the preswelling of protein.

The native globular SPI is an oblate cylindrical structure. Heating the folded SPI causes it to unfold. With the increase of preswelling time, the unfolding structure of SPI increases. These unfolded structures of SPI have more free volumes, chain mobility and exposed active groups, such as -OH, -NH, and so forth, and hence can easily react with PCL. It may further be attributed to the fact that the inter- and intramolecular hydrogen bonding interactions of soy protein decreased after the preswelling and as a result, the soy protein chains became unfolded and therefore promotes the grafting reaction.4,5

Figure 6 shows the variation of graft yield (%) with preswelling time. However, if the preswelling time is too long enough, more SPI main chains assembled or self-assembled. With the unfolding of SPI, -OH groups and -NH groups of globulins such as 2s, 7s, 11s, and 15s formed strong intermolecular hydrogen bonding during the preswelling process. Therefore, these globulins will assemble or self-assemble. Thus, less active radicals were created, which led to the lower percentage of grafting and efficiency.

Characterization Studies of the polylactide-g-SPI grafted copolymer

In this section, the characterizations of pure SPI and polylactide-g-soy protein isolate grafted copolymer by different techniques such as FTIR, NMR, thermal study, XRD and FESEM are discussed.

FTIR Analysis

For the pure soy protein, the strong band at 3463 cm⁻¹ was attributed to the mixed absorption of N-H stretching and O-H stretching. The peak at 1651 cm⁻¹ was the contribution of the carbonyl group stretching
vibration (amide I band). The band at 1531 cm\(^{-1}\) (amide II band) indicated that the N-H had the trans configuration relative to the carbonyl group. In fact, N-H and C=O are trans configured in all peptide units except proline because less energy is needed in the trans configuration.\(^5\) In addition, the absorption bands related to C-H deformation at 1458 cm\(^{-1}\), C-N stretching and N-H bending vibrations at 1237 cm\(^{-1}\), and out-of-plane C-H bending at 1064 cm\(^{-1}\) were also observed.

The FTIR spectrum of L-lactide depicts a new strong peak at 1757 cm\(^{-1}\) for C=O was observed, which was assigned to the absorption of carbonyl in lactide (LA). The peak at 2915 cm\(^{-1}\) and 2950 cm\(^{-1}\) which belonged to symmetric and asymmetric C-H stretching respectively. Further, the peak at 1267 cm\(^{-1}\) is observed for asymmetric stretching vibrations of C-O-C in lactide ring. In FTIR spectrum of Polylactide-g-SPI, characteristic absorption bands at 3305 cm\(^{-1}\) for N-H stretching vibration, 2927 cm\(^{-1}\) for C-H symmetric stretching vibration due to presence of CH\(_3\) group. The peak at 1741 cm\(^{-1}\) for C=O group, and peak at 1384 cm\(^{-1}\) is for symmetric (C-H) bending vibration.

Characteristic FTIR bands of L-lactide, SPI and Polylactide-g-SPI are depicted in Table 1. The presence of peaks both due to functional groups of SPI and lactide/polylactide in the FTIR spectrum of the grafted product Polylactide-g-SPI indicated that Poly lactide chains are present in the copolymer.

### Nuclear Magnetic Resonance (NMR) Spectroscopy

Chemical structures of pure SPI and Poly lactide-g-SPI were also determined by \(^1\)H NMR spectra in deuterated Chloroform (CDCl\(_3\)). The NMR spectrum of SPI and Polylactide-g-SPI are shown in Fig. 7 (a,b). Soy protein isolate has a complex composition that results in a complex solution \(^1\)H NMR spectrum (Fig. 7 (a)). SPI contains many kinds of amino acid, mainly glutamic acid, aspartic acid, arginine, and lysine. The amide signals from peptide groups of SPI are displayed at around 7.5 ppm. The aromatic signals of several amino acids are observed at 7.0 ppm, the olefinic signal at 5.0-5.6 ppm and an aliphatic signal at 0.5-5.0 ppm.\(^5\) In the NMR spectrum of SPI, peaks are observed at around 5.3, 4.3, 4.1, 3.5, 2.3, 1.5, 1.3, 0.8 ppm.

| Wave Number (cm\(^{-1}\)) | Assignment | Ref. |
|---------------------------|------------|-----|
| ~3002                     | -CH asymmetric stretching of CH\(_3\) group in lactide | 15 |
| ~2927, ~2015              | -CH symmetric stretching vibration of CH\(_3\) group in lactide | 16 |
| ~1390, ~1384              | -CH symmetric bending from CH\(_3\) group Lactide and PLA-g-SPI | 5 |
| ~1106, ~1064              | C-H bending in SPI | 5 |
| ~1742                     | C=O stretching vibration for ester group | 17 |
| ~3511                     | O-H stretching in PLA | 17 |
| ~1263                     | C-O-C symmetric stretching vibration in Lactone ring | 5 |

Table 1 Characteristic FTIR bands of L-Lactide, SPI, Polylactide-g-SPI

PLA produces mainly three peaks corresponding to the presence of three different kinds of hydrogen atoms in the PLA solution. The three peaks are observed around chemical shift, δ (ppm, CDCl\(_3\)) 7.25 (s-singlet),
5.15-4.95 (q-quartet, 1H) and 1.60-1.45 (d-doublet, 3H), in the 1H NMR spectrum of PLA. The peaks around 5 ppm and 1.5 ppm are due to the methine (–CH) and methyl (–CH₃) groups of PLA, respectively. The peaks for hydrogen of methine or methyl group of lactide are also observed near the corresponding peak for PLA, but its intensity is very low to be perfectly noticeable.

In the NMR spectrum of Polylactide-g-SPI, the new peaks observed at 5.2 ppm and the complexity of the NMR spectrum near 0.8-2.3 ppm for the amines of SPI and peaks due to polylactide are present. The NMR spectrum of Polylactide-g-SPI (Figure 7) is found to be complex and the peaks present in the grafted are samples are both due the functional groups present in both SPI and polylactide. The new peak at 0.8-1.0 ppm could be attributed to the aliphatic NH-CO group PLA-g-SPI, indicating that the graft mechanism as shown in Scheme 1 is reasonable. The peak at 7.282 ppm (Fig 7(b)) is due to the solvent CDCl₃. The amino acids containing benzene ring in SPI were exposed and gave rise to NMR signals after the graft copolymerization. The above information confirms that PLA-g-SPI was synthesized successfully.

![1H NMR spectrum of pure SPI](image)

**Fig. 7 (a)** 1H NMR spectrum of pure SPI

![1H NMR spectrum of Polylactide-g-SPI](image)

**Fig. 7(b)** 1H NMR spectrum of Polylactide-g-SPI

**Thermal Analysis**

**Thermo Gravimetric Analysis (TGA):**

Primary thermograms of soy protein isolate and Polylactide-g-SPI are presented in Fig. 8 (a and b), respectively. From the figures, it is observed that SPI is stable upto 290°C whereas, PLA-g-SPI is stable upto 147°C. Thus, it can be inferred that graft copolymerization of PLA onto SPI has decreased the thermal stability. The higher IDT of SPI is to its granular structure with long protein chains compactly arranged via alpha-helix and beta-sheet. However, the branched structure of PLA-g-SPI destroyed the regularity of the macromolecule chains to a certain extent, which may have unfolded the SPI backbone, therefore reducing the thermal stability. Further, Initial decomposition temperature (IDT), final decomposition temperature (FDT), and decomposition temperatures (DT) at every 10% weight loss are presented in Table 2. The Percent residue left after 600°C for
SPI is 3.4%. The IDT and FDT of polylactide-g-soy protein isolate are 146.94°C and 541°C. The decomposition temperature at every 10 % weight loss for the grafted SPI is observed to be less than that for neat SPI which clearly indicates that the grafted sample is less thermally stable than the neat SPI. However, the percent residue left for the grafted sample is 5.5% which is higher than that of SPI, which came from the ash decomposed from the protein at high temperature. Thus, slightly higher FDT values and percent residue indicate that grafting of polylactide onto soy protein isolate provides comparable thermal stability to SPI.

![Fig. 8(a) TGA of neat SPI](image)

There were two obvious peaks in the differential thermal gravimetric (DTG) curves of SPI and three peaks for polylactide-g-SPI as can be seen from Figure 8 (a and b) respectively, showing that the thermal degradation could be divided into two and three stages respectively. The first stage for SPI was assigned to the thermal degradation of SPI occurring due to the cleavage of the covalent peptide bonds. During this period, three simultaneous changes occurred: the 7S and 11S protein subunits became dissociated, the secondary structure unfolded, and the denatured subunits reassOCIated with disulfide, hydrophobic, and electrostatic bonds, and so on. In the second stage, further heating caused breakage of S-S, O-N, and O-O linkages in the structure of soy protein, and at last, the protein molecules completely decomposed and formed various gases such as CO, CO₂, NH₃, H₂S, and others. However, in the DTG curve of polylactide-g-SPI, the first stage is corresponding to thermal degradation of grafted polylactide chains, the second stage corresponds to cleavage of the covalent peptide bonds and third stage corresponding to decomposition of S-S, O-N, and O-O linkages in the structure of SPI and polylactide molecules.

![Fig. 8(b) TGA of Polylactide-g-SPI](image)

**Differential Scanning Calorimetry (DSC):**

Further, the DSC plots of SPI and PLA-g-SPI are shown in Figure 10 (a) and (b) respectively. It has been observed from the Figure 9 (a) that SPI shows broad endothermic peak at 52-165°C, whereas, PLA-g-SPI shows two different endothermic peaks. The first endothermic peak at 85.51°C corresponding to the decomposition of SPI and the second peak at 190°C is corresponding to the melting point of PLA. The appearance of these two peaks is at higher temperature than the peak observed for SPI which indicates that % crystallinity of PLA-g-SPI is increasing upon graft copolymerization and is more stable than neat SPI.
Compared to the ungrafted SPI, the grafted SPI shows a glass transition temperature ($T_g$) of PLA at 49°C and melting point of PLA at 190°C as can be seen from Figure 9(b). This transition coincided with the melting point of polylactide homopolymer and would thus be associated with the movement of polylactide side chains, as PLA homopolymer was removed by extraction. Thus, graft copolymerization of PLA onto SPI is confirmed.5

**X-RAY DIFFRACTION (XRD) ANALYSIS**

The powder wide angle X-ray diffraction (XRD) pattern of SPI and Polylactide-g-SPI samples was used to determine the structure for the samples of ungrafted and grafted SPI. As it was observed that the diffraction patterns of the SPI samples exhibited a dominant amorphous halo, a broad band with a maximum at 2θ = 20°, which is characteristic for pure SPI which has 7S and 11S amorphous globulins as the main components.20

However, the graft copolymerization of polylactide onto SPI has increased the crystallinity as can be seen from the area under the crystalline region. The XRD pattern for PLA exhibits characteristic peaks at 2 theta values around 15°, 16°, 18.5° and 22.5° due to the presence of L-isomer and peaks at 12°, 21° and 24° due to the presence of D-isomer.21,22 But WAXD pattern of the grafted sample did not show any sharp peak. This may be due to the fact that the peak for SPI is a broad peak around 20° and the peaks due to polylactide are also around 20° which overlap in the same region and thereby suppressing the sharp peaks.23

When a polymer is in the amorphous state, the macromolecular end groups are distributed randomly in the whole polymer matrix while in a semicrystalline polymer, the macromolecular end groups are mostly distributed in the amorphous phase and mesophase. Therefore, when graft copolymerization takes place, the macromolecular end groups are redistributed and result in a rather high concentration of macromolecular end groups in the amorphous phase and mesophase and thereby increases % crystallinity.24 Therefore, percentage crystallinity of grafted sample is found to increase in comparison to virgin SPI. It is well known that degree of crystallinity is one of the major parameter responsible for tensile strength. Increase in the percentage of
crystallinity increases tensile strength. This attributed to the fact that tensile strength of grafted sample polylactide-g-SPI is more than neat SPI.

**Field Emission Scanning Electron Microscopy (FESEM) Study**

Figures 10 (a) and (b) represents the scanning electron micrographs of SPI and Polylactide-g-SPI, respectively. The comparison of the scanning electron micrographs of the grafted sample with that of ungrafted sample gives a clear indication of change in the topology of the grafted sample. Grafting of polylactide onto Soy protein isolate opens up its matrix and shows considerable deposition of polylactide on the surface of backbone polymer soy protein isolate. It can be observed from Fig 10 (a) that the virgin SPI is neither uniform in size nor uniform in shape. A larger number of reactive sites uniformly created on the SPI molecules led to greater accessibility of the Polylactide and improved considerably the grafting efficiency in the grafting conditions using the swelled SPI. It is evident from Fig. 10 (a) and (b) that the SPI matrix opens up and Polylactide molecules are embedded on the surface of SPI molecules. Thus, on the whole, SEM micrographs have provided substantial morphological evidences in favor of grafting of Polylactide onto SPI.

![Fig. 10 (a) SEM of Soy protein isolate](image1)

**Swelling and Solubility Behavior of Polylactide-g-SPI**

Swelling behavior of SPI and Polylactide-g-SPI in polar solvents was studied in triplicate. The average of the triplicate reading was considered. Samples each of SPI and Polylactide-g-SPI were suspended separately in 10 ml of the ethanol and water in a different proportion. The percent swelling and percent solubility were determined as described in Experimental Section and the results are depicted in Table 2. It is clear that percent swelling of soy protein isolate increases with increasing amount of water in the water-ethanol solvent system both after 24 h and 48 h. Maximum swelling percentage of 526 % and 591 % was observed in neat water (10 ml) in 24 h and 48 h respectively. This swelling behaviour of SPI is observed due to the hydrophilic character of SPI and which was basically due to formation hydrogen bond interaction between them.

Similarly, for the grafted samples of Polylactide-g-SPI, swelling (%) also increases with increase in water content both at 24 h and 48 h. And further, the swelling (%) of Polylactide-g-SPI is found to be less than
neat SPI as expected which is due to the incorporation of hydrophobic polylactide units onto SPI. Maximum swelling (%) of 173 % and 500 % is observed with 10 ml water system at 24h and 48 h respectively. The percent swelling in 5:water: ethanol is intermediate to only water and only ethanol system and the percent swelling in only ethanol system is much lesser than only water system. This might be attributed to the fact that neither SPI nor polylactide are soluble in ethanol. 

Moreover, the solubility of SPI is found to increase with increase in amount of water after 24 h but does not follow a particular trend after 48 h. The maximum solubility observed after 24 h is 54.8 %. The percent solubility with 5: 5 water:ethanol is intermediate (33.33 %) between only water (70.21 %) and only ethanol (64.47 %) solvent system after 48 h. But for the case of Polylactide-g-SPI, solubility (%) is around 52-55 % in all the cases after 24 h and 6-8 % after 48 h. The decrease in solubility (%) with increase in time from 24 to 48 h might be due to unfolding and denaturing of SPI units during the first 24 h and thus losing its capacity to hold water molecules.

A lower value of % swelling and % solubility in case of grafted samples are observed which might be due to the presence of hydrophobic Polylactide units along with hydrophilic soy protein. The solubility of the grafted samples was found to be less than the neat SPI, which might be due to the incorporation of hydrophobic Polylactide units which improved the water resistance. Thus, their mechanical properties will also be higher and thus, materials made from this graft copolymers might be used for food packaging, coating and biomedical materials. Polylactide -g-SPI has shown greater hydrophobicity behaviour than neat SPI. In these determinations, improved water resistance was observed since the graft copolymers were less swell able and less soluble than the neat SPI.27

### Table 2 %Swelling and %Solubility of Soy protein isolate, Polylactide-g- Soy protein isolate in Water: Ethanol system

| Sample                  | Time | Percentage (%) | 10:0 | 5:5 | 0:10 |
|-------------------------|------|----------------|------|-----|------|
| Soy protein isolate (SPI)| 24   | Swelling       | 526  | 427 | 314  |
|                         | 24   | Solubility     | 54.8 | 18.18 | 7.31 |
|                         | 48   | Swelling       | 591  | 544 | 163  |
|                         | 48   | Solubility     | 70.21| 33.33| 64.47|
| Polylactide-g- SPI      | 24   | Swelling       | 173.33| 138.09| 35.78|
|                         | 24   | Solubility     | 55.55| 54.28| 52.63|
|                         | 48   | Swelling       | 500  | 385.41| 82.22|
|                         | 48   | Solubility     | 7.5  | 8.33 | 6.66 |

### Mechanism of Graft copolymerization of Polylactide onto SPI

Polylactide of high molecular weight are exclusively produced by the ring opening polymerization of the corresponding cyclic monomer lactide.28 It is formed when cyclic monomers reacted with a catalyst i.e Stannous Octoate (SnOct2).29 The ring opening polymerization of lactide monomers is based on co-ordination insertion ring opening polymerization, since the propagation is thought to proceed by co-ordination of the monomer to the active species and then insertion of the monomer into the metal oxygen bond by rearrangement of the electrons.7,30 The growing chain remains attached to the metal through an alkoide bond during the propagation. The reaction is terminated by hydrolysis forming a hydroxyl end group with functional alkoxy substituted initiators and further, macromers with active end groups are produced which will further help in production of high molecular weight polymers during post polymerization reactions.

It is well known that during the ring opening polymerization of lactides, the catalyst causes transesterification reactions at elevated temperatures31 or at long time reactions.32 Intermolecular transesterification reactions modify the sequences of copolylactides and prevent the formation of block copolymers.

Scheme 1 describes the grafting as well as ring opening polymerization of L-Lactide.33-36 In step-IV,37 for the Sn(Oct)3-initiated polymerization of cyclic esters, amino group of Soy protein isolate can also serve as co-initiators.38,39 More recently, polymerization of L-Lactide initiated by natural amino acids at elevated temperatures has also been reported.4 These observations open new possibilities, e.g., in the synthesis of polypeptide-poly(aliphatic ester) graft copolymers. On the basis of above discussion the Polylactide grafted Soy protein isolate products are prepared.

### Conclusion

A novel biodegradable copolymer, PLA-g-SPI, has been successfully synthesized via polylactide grafted onto soy protein backbone by ring-opening polymerization in the presence of an azetotropic solvent. Preswelling
played an important role as it could promote the graft polymerization effectively. The coordination insertion mechanism has been proposed for the graft copolymerization of polylactide onto soy protein isolate. The thermal properties of the copolymer differed from those of the original protein. The protein chains did not act as a nucleating agent in the copolymer, and crystallization ability was promoted with the increase of graft length or graft yield (%). In addition, the thermal stability of protein was reduced as the branch structure destroyed the compact three-dimensional organization in the soy protein.

**Scheme 1.** Reaction mechanism of ROP of Polylactide-g-SPI

**Acknowledgements**

The authors are grateful to Ravenshaw University and National Institute of Technology, Raipur for providing research facilities.

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