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PREPARATION AND PARTIAL CHARACTERIZATION OF COMPOSITE FILMS CONTAINING SOYA PROTEIN AND SAGO STARCH

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ABSTRACT

The aim of the present study is to develop a novel biomaterial from natural resources which can be used for various biomedical applications. In this work, composite films containing soya protein (SY) and sago starch (SG) were prepared by solvent casting methodology; these films were cross linked with glutaraldehyde to improve their mechanical properties. The films prepared were characterized for their Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), mechanical properties and thermo gravimetric Analysis (TGA). The results obtained showed that with the increase of soya protein in the composite, there was a decrease in the water absorption capacity. The IR and SEM studies have shown the composite nature of the films prepared. The thermal analysis has shown the stability of the composites at higher temperature which is an added advantage to biomaterials. The scanning electron microscope studies have shown a smooth surface of the SG-SY-G film which is a prerequisite for a wound dressing material.

KEYWORDS: Sago starch, Soya protein, Composite film, Biomaterial

1. INTRODUCTION

There is an increasing need to develop new biodegradable materials to be used in skin tissue engineering, wound cover or as dressings and barrier–membranes since there is a high demand for skin replacements and skin repair treatments. For instance, an ideal material to be used as a wound dressing should associate availability with minimal storage requirements, long shelf life, versatility and biocompatible behavior[1] .Soya protein is an edible product of soya bean oil industry and production of biodegradable films has the potential to add value to soy protein[2]. Renewable polymers, soy protein, the major component of the soya bean, has the advantages of being economically competitive and present good water resistance as well as storage stability. The combination of these properties with a similarity to tissue constituents and reduced susceptibility to thermal degradation makes soy an ideal template to be used as a biomaterial for skin tissue engineering[3]. Soy protein produces flexible, smooth and clear films compared to films from other plants protein sources[4]. The use of soya protein as film forming agent, alone[5], or in blend with other polymers[6], can add value to soya bean besides producing environmentally friendly biodegradable films. Soya protein composite films are usually obtained by casting method[7]. Silva *et al* prepared blended membranes composed by chitosan and soya protein by solvent casting methods[8]. The thermal stability of films was improved by the addition of sodium dodecyl sulfate SDS molecule attached onto hydrophobic amino acid residues within the film to active[2]. Rayner *et al* developed and evaluated to reduce fat transfer in deep fried foods. Soy protein isolate solution (10% SPI) with 0.05% gellan gum a plasticizer cooled after being held at 80°C for 20min provided suitable films[9]. Soy protein films provide limited resistance to water vapor transfer due to inherent hydrophilicity of proteins[10]. Some studies reported that the combination of soy protein with other proteins such as wheat gluten[11] or, casein[12], may promote physical and chemical interactions which improve properties of soy protein. Combination of polysaccharides such as carrageenan, xanthan[13], dialdehyde starch[14] with soy protein has also been investigated.

Starch, is one of the most abundant and inexpensive polysaccharides sources with unique characteristic of biodegradability and easily dissolve in water[15]. Edible films prepared from polysaccharides, proteins, and lipids have a variety of advantages such as biodegradability, edibility, biocompatibility, esthetic appearance, and barrier properties against oxygen and physical stress[16]. Biomaterial such as starch satisfies the requirement of adequate thermal stability, minimum interference with flow properties and minimum disturbance of product quality[17].

Lutfor *et al* used vinyl monomers onto starch for preparing composites of starch with synthetic polymer[18]. Maizura *et al* prepared edible films with partially hydrolyzed sago starch and alginate (SA). Lemon grass oil (0.1% to 0.4% v/w) and glycerol (0% and 20%, w/w) were incorporated in the films to act as natural antimicrobial agent and plasticizer[19]. Fasihuddin *et al* determined the swelling characteristics and rheological properties of a range of sago starches obtained from different sources[20]. Gangwar *et al* studied starch obtained from sago palm (Metroxylan sagu), which was used as a binder in different concentrations in paracetamol tablets[21].

This study evaluates the influence of chemical cross-linking in the water uptake, degradation rate and biocompatibility of the blend system composed by sago and soy protein.

2. MATERIALS AND METHODS:

Soya seeds and Sago rice were purchased from nearby local retail market and other chemicals used in this study were of laboratory grade.

2.1 Preparation (SY) of Soya Solution

The soya seeds were powdered using a domestic mixer and sieved to a particle size of 50-200µm, 2g of soy powder was boiled in 100 ml water for 30mts. The boiled solution was cooled and filtered through a cotton cloth. This solution was used for further experiments. The solid content of the extract was found to be 1.7%

2.2 Preparation of Sago Solution (SG)

Sago was powdered using a domestic mixer and sieved to a particle size of 50-200µm .10g of Sago powder was boiled in 100 ml water till it becomes a homogenous solution. The solid content of the solution was found to be 13.23% and this solution was used for further experiments.

2.3 Optimization of Sago film (SG)

As the film formed by drying SG solution was very brittle, EG was used to get the flexibility to the film. Keeping the amount of sago solution constant, the amount of EG added was varied and the details are given in table 1a. The resultant solutions were poured in polythene tray having measurements 10×10 cm, and dried at 30-35° C .The dried films were stored in polythene covers for further use.

2.4 Optimization of Sago-Soya film (SG-SY)

The stochiometric ratio which gave better tensile strength to the SG film was used for the preparation of SG-SY composite. Keeping the amount of sago solution and EG (in this case (table 1-a, sample No-3)**,** the amount of soya solution was varied and the details are given in table 1b. The resultant solution was poured in polythene tray having measurements 10×10 cm, and dried at 30-35°C .The dried films were stored in polythene covers for further use.

2.5 Glutaraldehyde cross-linked SG-SY film (SG-SY-G)

The stochiometric ratio of SG-SY which gave better mechanical properties (table 1b, sample 3) was used for the preparation of cross linking with gluteraldehyde. Here keeping this particular stochiometric ratio constant, the amount of gluteraldehyde was varied .The details are given in the table 1C .The resultant solution was poured in polythene tray having measurements 10×10 cm, and dried at 30-35° C .The dried films were stored in polythene covers for further use.

3 Characterization:

3.1Water absorption capacity

Estimation of water absorption capacity was done by the method explained by Rao *et al.* [22]. The water absorption capacity of samples prepared was determined by swelling small pieces of each sample of known weight in distilled water at room temperature. The swollen weights of the samples were determined by first blotting the samples with filter paper followed by accurately weighing the sample. The weights of the swollen pieces were recorded every 1h, 2 h, and 3 h, and after 24 h. Percentage swelling of the samples at a given time was calculated from the formula

$$ES = \frac{Ws - Wo}{Wo} X 100$$

Where Ws is the weight of the sample (moist) at given time, Wo is the initial weight of the sample, Es is the percent of swelling at a given time. The results given are average of three samples.

3.2 Tensile strength of the sample

Three dumbbell-shaped specimens of 4mm wide and 10mm length were punched out from the prepared films using a die. Mechanical properties such as tensile strength (MPa) and percentage of elongation at break (%) were measured using a universal testing machine (INSTRON model 1405). The results given are an average of three specimens.

3.3 Infrared Spectroscopy

FTIR spectra of the samples prepared were taken using Nicollet impact 400 FTIR spectroscopy by preparing a 500 mg KBr pellet containing 2-6 mg of the sample.

3.4 Scanning Electron Microscopy

The sample was coated with gold ions using an ion coater (fisons sputter coater) under the following conditions: 0.1 Torr pressure, 20 mA current and 70 s coating time. Surface structure was visualized by scanning electron microscope (JSM 5300 Scanning microscope) using a 15 KV accelerating voltage.

3.5 Thermal gravimetric analysis

The thermal stability was determined with a thermo gravimetric (TG) analyzer (Perkin-Elmer TGA) over a temperature range of 37 °C to 800 ^{o}C at a heating rate of 20 ^{o}C / min under nitrogen atmosphere.

4. RESULTS AND DISCUSSION:

Sago starch solution when dried forms a film. However, the mechanical properties of the film formed are not suitable for biomedical applications. Hence a plasticizer has to be used to get the required mechanical property to this film. In the present study, ethylene glycol was used to get the flexibility to the sago film. Among the various compositions studied sample no 3 in **Table 1a** gave better tensile strength and elongation properties. With the increase in the amount of ethylene glycol the tensile strength is increased up to addition of 4 ml of ethylene glycol and later decreased. The decrease in tensile strength may be due to the excess of ethylene glycol molecules impregnated between the starch molecular backbones. This composition (sample No.3-**Table 1a**) was used to prepare SG-SY composites. Among various SG-SY composites prepared (**Table 1b**) sample no 3 gave better mechanical properties. With increasing the soya solution the tensile strength of the composite initially increased and later decreased. The stochimetric composition of 60 ml sago, 10 ml soya and 4 ml ethylene glycol gave better tensile strength. However, the elongation of break is decreased. Similarly SG-SY-G composites were prepared by varying the concentration of gluteraldehyde. The stochimetric ratio of sample no 2(**Table 1c**) gave better mechanical properties. The stochimetric ratio of 60 ml sago, 10 ml soya, 4 ml ethylene glycol and 1µl gluteraldehyde gave better tensile strength compared to other samples. The increase in the gluteraldehyde decreases the tensile strength of the composites. This may be due to the homopolymerisation of gluteraldehyde in the samples. This sample was used for its further characterization such as IR, TGA, SEM and water absorption studies.

Table-1a
Mechanical properties of SG film

Sample No	Sago (ml)	EG (ml)	Elongation at break (%)	Tensile strength (MPa)
1	60	2	59.17	2.30
2	60	3	45.94	2.45
3	60	4	62.22	2.84
4	60	5	54.17	1.90
5	60	6	41.94	1.45

Table-1b

Mechanical properties of SG-SY film

Sample No	Sago (ml)	Soya (ml)	EG (ml)	Elongation at break (%)	Tensile strength (MPa)
1	60	6	4	5.94	1.99
2	60	8	4	22.50	2.32
3	60	10	4	4.30	6.02
4	60	12	4	31.22	3.14
5	60	14	4	22.33	3.61

Table 1c
Mechanical properties of SG-SY-G film

Sample No	Sago (ml)	Soya (ml)	EG (ml)	Gluteraldehyde (µl)	Elongation at break (%)	Tensile strength (MPa)
1	60	10	4	0.5	20.28	5.07
2	60	10	4	1	27.78	6.04
3	60	10	4	2	33.61	4.35
4	60	10	4	3	32.22	3.74
5	60	10	4	4	23.33	3.71

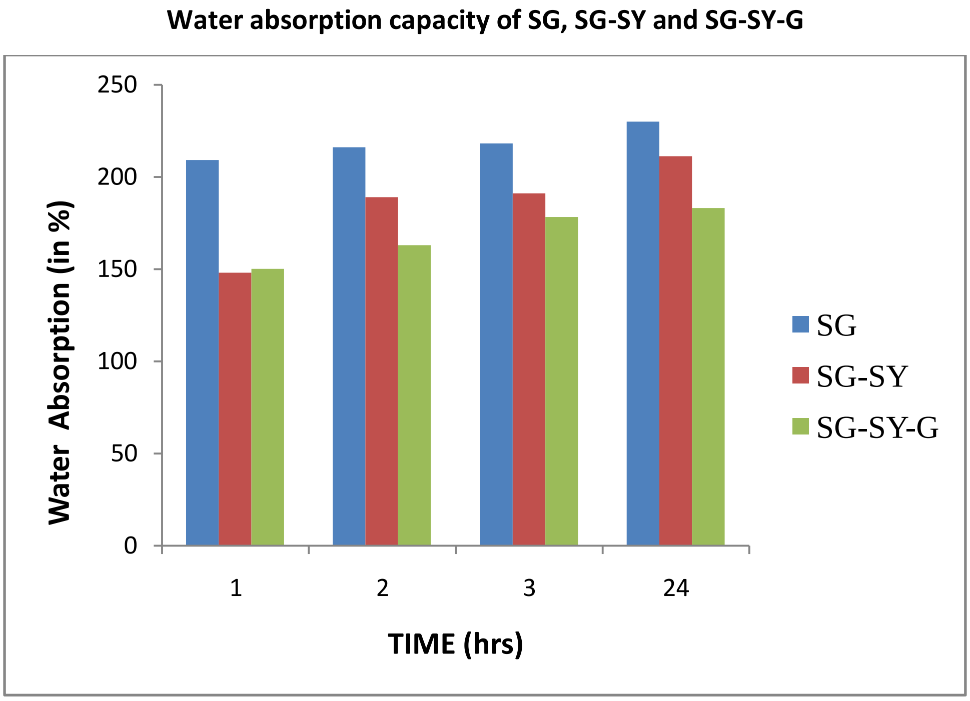

Figure 1.Water absorption capacity of samples SG, SG-SY, SG-SY-G; the SG-SY-G composite has shown lesser water absorption capacity compared to those of SG and SG-SY films.

4.1 Water absorption studies

Water absorption capacity studies of samples, SG, SG-SY and SG-SY-G which gave better tensile strength. SG film has shown increased water absorption capacity with increase in the time, a maximum of 230% water absorbing capacity was observed after 24 hours. SG-SY has also shown increased water absorption capacity with increase in time. However, the water absorption capacity was less at all time periods compared to SG films .This may be explained as the sago contains more hydroxyl groups on its polysaccharides backbone compared to SG-SY. SY contains other functional groups such as NH2, COOH apart from OH groups on its backbone. As hydroxyl groups are more hydrophilic, the increased water absorption capacity of SG was observed. Similarly SG-SY- G composites have shown lesser water absorption values compared to SG and SG-SY. This decrease may be explained as follows, the amino groups on the backbone of SG-SY are cross linked with aldehyde groups of gluteraldehyde and blocking the hydrophilic groups on the backbone, due to this phenomenon lesser water absorption of SG-SY-G was observed.

4.2 SCANNING ELECTRON MICROSCOPY:

Scanning electron microscope pictures exhibit the surface morphology of biomaterials. In the present study Scanning electron microscope pictures were taken for SG (**Figure.2a**), SG-SY (**Figure.2b**) and SG-SY-G (**Figure. 2c**).The SG film has shown porous and rough surface, similar surface characteristics were observed for SG-SY film. However, the surface of the SG-SY-G film was smoother comparative to the above to films. Porous nature of the film was also observed in SG-SY-G film. Smooth surface of the film is mandatory for a wound dressing material, because the material has to stick to contours of the wound surface and the patients also feel comfortable with a biomaterial having a smooth surface.

SEM image of SG, SG-SY and SG-SY-G

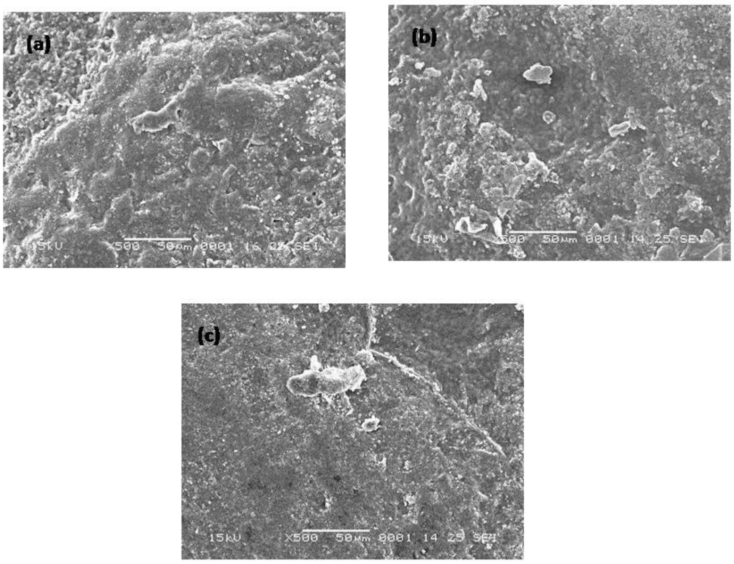

Figure 2.Scanning electron micrograph of SG (a), SG-SY (b), SG-SY-G(c); SG and SG-SY films have exhibited porous and roughness surface. But, SG-SY-G surface was smoother compared to SG and SG-SY films.

4.3 THERMO GRAVIMETRIC ANALYSIS:

In thermo gravimetric analysis weight loss of the material due to increase in the temperature, is recorded. Normally, when a biomaterial is heated to higher temperature, it decomposes into CO_2, CO, NO and H_2O. Composites containing chitosan and soya bean were prepared and their susceptibility to thermal degradation was described in the earlier studies[8]. In the present investigation, the biomaterials prepared were heated in a nitrogen atmosphere from 30^{o} -750 ^{o}C. A major single step weight loss was observed in all the materials. However, SG (**Fig. 3a**) has lost 81% of its weight at 372 ^{o}C, SG-SY (**Fig. 3b**) and SG-SY-G (**Fig. 3c**) samples lost 88% of the weight at the same temperature. The decrease in the thermal stability of these two samples may be due to the presence of SY in the composite. The stability of the composites at higher temperature is an added advantage to biomaterials.

THERMO GRAVIMETRIC ANALYSIS OF of SG, SG-SY AND SG-SY-G

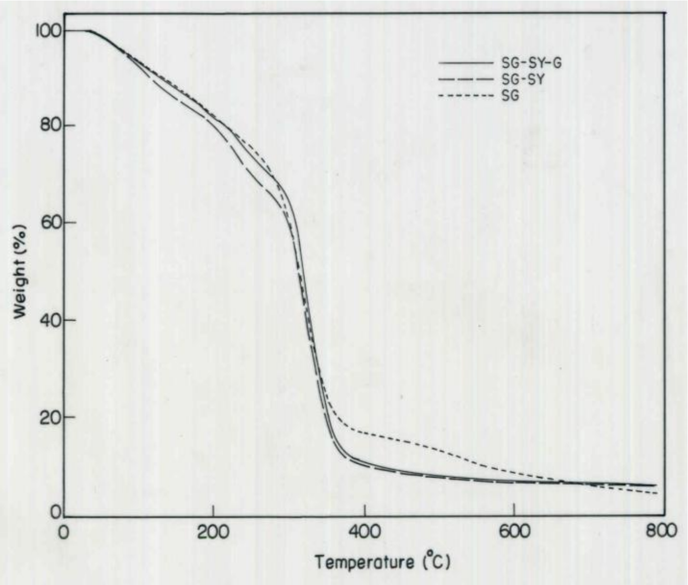

Figure 3.Thermogram of SG, SG-SY, SG-SY-G; A single step weight loss was observed in all the samples. However, the loss of weight was more for SG-SY-G sample at 372°C. This may be due to the presence of SY.

4.4 INFRA RED SPECTROSCOPY:

The IR spectrum of SG has shown (**Fig. 4a**) characteristic bands of in the finger print region (516 & 520 cm^{-1}), around 2900 cm^{-1} (2934 cm^{-1}) and in the region between 3200-3500 cm^{-1} (OH stretch region)[23]. The IR spectrum of SG-SY (**Fig. 4b**) exhibited absence of peak at 1077cm^{-1} and reduction in the broadness of the peak at OH stretch region. IR spectrum of SG-SY-G (**Fig. 4c**) has shown a sharp and broad peak at 1633 cm^{-1} indicating amide I band of soya protein. Broadening of the - OH peak from 3500-3000 cm^{-1} indicated the hydrogen bonding between functional groups of soya protein and sago starch.

Infrared spectra of SG, SG-SY and SG-SY-G

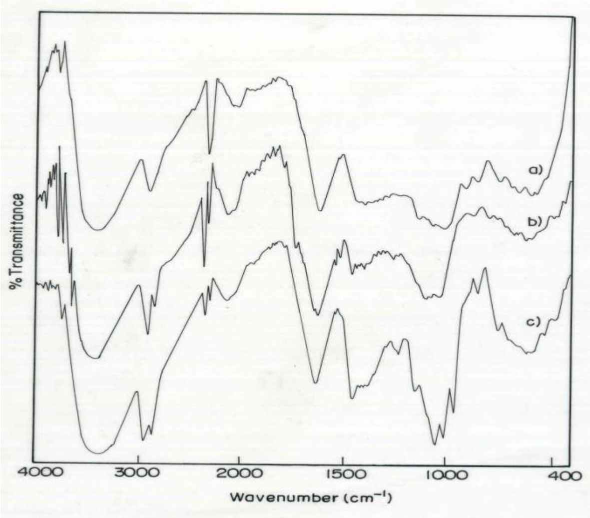

Fig.4 a, b and c .IR spectrum of SG (a), SG-SY(b), SG-SY-G(c); In all the spectra characteristic bands of carbohydrate are observed in the finger print region i.e 516 and 520cm^{-1} ; SG-SY-G sample has shown broadening of –OH peaks from 3500 to 3000cm,$^{-1}$ indicating hydrogen bonds between SG and SY.

5. CONCLUSION

In this study, optimal levels of soya protein, sago starch and gluteraldehyde were found out to prepare a composite film that exhibits better mechanical properties. The scanning electron microscope studies have shown a smooth surface of the SG-SY-G film which is a prerequisite for a wound dressing material

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