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Research Article

In Vitro and *In Vivo* Biodegradation Study of Tamarind Kernel Powder (Xyloglucan)

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ABSTRACT

Purpose. The specific aim of present study was to investigate the biodegradation characteristics of xyloglucan, a natural film- forming polymer. *Methods.* Both *in vitro* as well as *in vivo* methods were used for assessment of the same. The *in vitro* degradation of xyloglucan film was followed in different fluids such as (simulated body fluid, simulated nasal fluid & simulated lung fluid) at different time intervals (0, 4, 12, 16, 20, 24, 28, 32 & 36) and *in vivo* by subdermal implantation in rats for up to 30 min. Rate and extent of degradation was followed in terms of film weight loss and swelling degree. *Results.* Although the rate of *in vitro* degradation was slow compared to the *in vivo*, xyloglucan films complete maximum degradation within 36min following subdermal implantation in rats within 30min. The films degraded following different rates, *in vitro* and *in vivo*, but the mechanism followed was primarily bulk degradation. *Conclusions.* Xyloglucan demonstrated highly *in vivo* biodegradable and in vitro degradation. The study provides valuable insight, which may lead to new application of xyloglucan in the field of drug delivery and challenge offers to formulator scientists.

INTRODUCTION

In the past 50–60 years synthetic polymeric food packaging materials are used widely due to various advantages such as high strength, elongation, low cost, lightness and water resistance [1]. These plastic materials are convenient, safe, strong and economical but not biodegradable. Polar biopolymers such as polysaccharides and proteins have been studied as potential alternatives in the field of novel drug delivery systems [2]. The interest in the study of biopolymer has witnessed a steady increase as they are environmentally friendly alternatives to synthetic, non-biodegradable films and have been used to coat different products. Polysaccharides are the most important source for a broad variety of advanced polymeric materials and have emerged as an immense renewable resource for biopolymers. These features have led to an outstanding increase in interest among scientists, research institutes and industrial companies [3-5].

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After cellulose, hemicelluloses constitute the second most abundant class of polysaccharides found in nature. Xyloglucan coming under the category of hemicellulose is biodegradable neutral polysaccharide with structure similar to cellulose. The backbone consists of α-1, 4-glucan residues about 80% of which are substituted by xylose. About 40% of xylose units are substituted with galactose. This substitution is the main reason for its solubility compared to cellulose [1, 6].

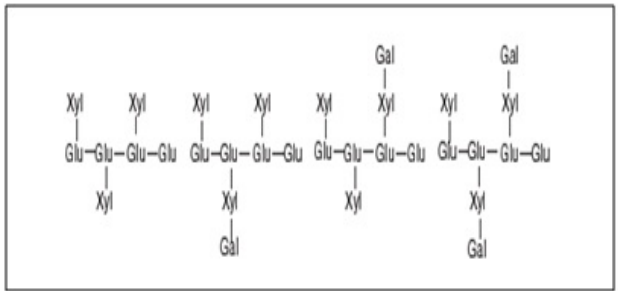

Figure 1: Three types of monomer units of TSP xyloglucan

Xyloglucan is the quantitatively predominant hemicellulosic polysaccharide in the primary walls of dicots and non-graminaceous monocots. Xyloglucan may account for up to 20% of the dry

weight of the primary wall. polysaccharide which is stored in the seed of *Tamarindus indica.* The primary structure of TSP consists of a (1-4)- b-D-glucan backbone chain which has (1-6)-a-D-xylose branches that are partially substituted by (1-2)- b-D-galactoxylose. The tamarind seed xyloglucan is composed of three units of xyloglucan oligomers with heptasaccharide, octasaccharide and nonasaccharide [7], which differ in the number of galactose side-chain which are shown in figure 1.

MATERIALS AND METHOD

Tamarind seed powder was received as a gift sample from DSP gokyo food & chemicals (Osaka, Japan), glycerol was obtained from Rankem (Mumbai, Maharashtra) and all other chemicals and reagents which are used for different fluids (simulated body fluid, simulated nasal fluid & simulated lung fluid) was obtained from Himedia (Mumbai, Maharashtra).

Preparation of Film

Films were obtained by the casting method. First, the solution was prepared by dissolving XYG (2-3%) in hot water with addition of 25%w/w of Glycerol as a plasticizer to total solid weight in a solution. The mixture was blended for 5 h with a mechanical stirrer (1000 rpm) at 95^{0}C to form a homogeneously gel-like solution. Bubbles were removed with an aspirator. The certain amount of gel-like solution thus prepared was poured on a glass plate. Water was evaporated from the molds at 25^{0}C for 15 hrs; the dried films were sealed in polyethylene bags and stored at 20^{0}C and at 50% RH for 1 week before performing the measurements.

Composition of Different Simulated Fluids for Degradation Study

The ion concentration of simulated body fluids (SBF) is nearly equal to those of the human body fluids plasma [8]. The composition of SBF was showed in Table 1. The SBF solution was buffered at pH 7.4 with tris(hydroxymethyl) aminomethane and HCl. The composing of simulated lung fluids (SLF) was showed in table 1. The solution described here was selected because it provides an environment that is relevant to extracellular fluid in the lung [9]. Simulated nasal fluid (SNF) is composing of ion concentration which was comparatively equal to SNF which was showed in table 1 [10].

In vitro Degradation in Different Simulated Fluids

The samples for weight loss and swelling degree (SD) test were squared with about 0.05–0.1 mm thickness and 2 cm length of side [11]. The degradation samples for tensile test were Dumb-bell shaped with 0.05–0.1 mm thickness and 4 mm width. The samples for tear test were 0.05–0.1 mm thickness. The samples were immersed in SBF, SLF and SNF which content in small vials.

Swelling Degree/ Swelling index

The SD was characterized at 37^{0}C. The experiments were carried out by measuring the weight gain as a function of immersion time in 20 ml solution [11, 12]. The SD was calculated according to equation given below-

$$Swelling\ degree\ (\%) = \frac{Wt - Wo}{Wo} \times 100$$

Where Wt is the wet weight and after degrading a predetermined time, Wo is the original weight of the sample.

Percentage weight loss

The weight loss was calculated by comparing the dry weight (Wd) of the remained sample after degradation for a predetermined time with the original dry weight (W0) of the sample as the equation [11, 12]. At pre-determined intervals of 0, 4, 8, 12, 16, 20, 24, 28, 32 & 36 min; samples were taken out, purged with distilled water and subsequently dried until absolute desiccation, then weighted.

$$Weight\ loss\ (\%) = \frac{Wo - Wd}{Wo} \times 100$$

In vivo Biodegradation Study

For the study of *in-vivo* study 3 male wistar rats are selected to monitor the in vivo degradation, films were subcutaneously implanted on the backs of male wistar rats (200-300 g). Anesthesia was induced by intra-peritoneal injection of a mixture of ketamine HCl (85 mg/kg body weight) and xylazine (12 mg/kg body weight) [13]. Tetracycline, 10mg/kg dose, was given at the time of surgery. An incision (2.5 cm) was inflicted laterally about the mid portion of the back. Subcutaneous pockets were formed around each incision, free film was inserted, and the wounds were closed by intermittent nylon sutures, 0.5 cm apart for 3 individual male wistar rats [14]. Films were explanted at 10, 20, and 30 minutes for analysis.

Table 1: Concentration of the components of SBF, SLF and SNF

Reagent For preparation of SBF	SBF pH7.5 (g/L)	SLF pH7.2-7.4 (g/L)	SNF (g/L)
Sodium Chloride	7.996	6.415	8.77
Sodium bicarbonate	0.350	2.703	-
Potassium chloride	0.224	-	2.98
Potassium hydrogen phosphate	0.228	-	-
Magnesium chloride	0.305	0.212	-
Calcium chloride	0.278	0.255	0.55
Sodium sulphate	0.071	0.079	-
Tris(hydroxylmethyl) aminomethane	6.057	-	-
Hydrogen chloride	Adjust	-	-
Sodium hydrogen phosphate	-	0.148	-
Sodium tartarate	-	0.199	-
Trisodium citrate dehydrate	-	0.180	-
Sodium pyruvate	-	0.172	-
Sodium lactate	-	0.175	-
Glycine	-	0.188	-
Distilled water	Up to 1000ml	Up to 1000ml	Up to 1000ml

Table 2: Time dependent swelling degree (%) of the XYG films in SBF, SLF and SNF

Swelling degree (%)										
Time (min)										
Sample Fluids	0	4	8	12	16	20	24	28	32	36
SBF	0	21.8	45.31	47.39	49.47	50.52	50.52	51.56	52.08	51.56
SLF	0	16.81	19.82	21.55	23.27	23.7	23.7	24.13	24.13	24.13
SNF	0	14.51	19.35	22.04	23.11	23.65	24.73	24.73	25.26	25.26

Table 3: Time dependent weight loss (%) of the XYG films in SBF, SLF and SNF

Weight loss (%)										
Time (min)										
Sample Fluids	0	4	8	12	16	20	24	28	32	36
SBF	0	10.6	14.61	21.9	25.31	28.5	34.65	36.47	36.93	37.15
SLF	0	14.34	22.17	31.15	38.29	44.02	48.42	50.49	50.95	51.18
SNF	0	16.16	22.88	31.44	36.31	41.87	47.66	53.91	57.61	58.08

RESULTS

1. Film formation

The prepared 2% XYG film using glycerol as a plasticizer is clear and transparent as shown in figure 2. The thickness of prepared XYG film was in the range of 0.05mm-0.1mm.

2. Swelling Degree

The results of swelling degree in simulated body fluids (SBF), simulated lung fluids (SLF) and simulated nasal fluids (SNF) were showed in table 2. For the samples with same concentration of films were prepared and placed it into the different fluids and studied at different time intervals. It can be noted from the plots that the degree of swelling for XYG films was higher in SBF than that of the SLF and SNF which is showed in figure 3(a) and 3(b). So, these findings suggest that the degradation rate of XYG films in SBF is slow than that of the SLF and SNF. Furthermore, swelling degree (SD) is also influenced by the solution viscosity of fluids, because the viscosity of SNF and SLF is more than that of the SBF. The viscosity is in sequence of SNF>SLF>SBF. So, it is clear that the higher the viscosity lower the swelling and as a result higher the degradation rate. The degradation rate and speed of XYG films in SNF and SLF is

nearly same and which is more rapidly degraded in SNF and SLF than that of SBF.

Figure 2: Transparent XYG films

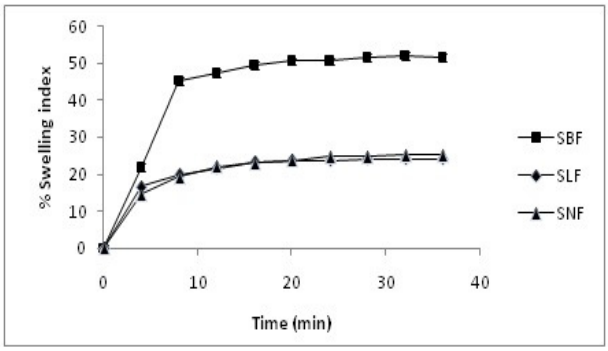

Figure 3(a): Percentage swelling index as a function of the degradation of XYG films.

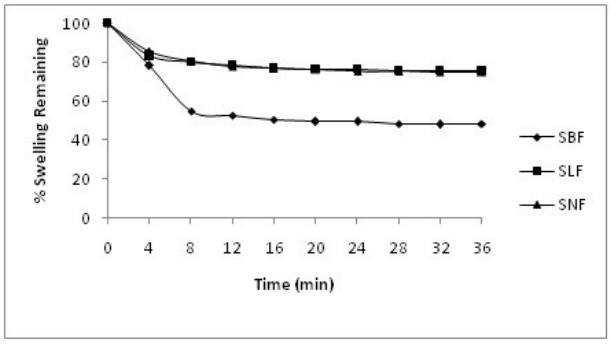

Figure 3(b): Percentage swelling remaining as a function of the degradation of XYG films.

3. % Weight loss

The weight loss of XYG films in SBF, SLF and SNF at different time intervals are shown in table 3 and the plots are shown in figure 4(a) and 4(b) and it was concluded weight losses of XYG films in SBF is slow as compared to the SLF and SNF. The XYG fims are quickly dissolved into the SLF and SNF and degradation velocity is more than SBF. The weight losses is nearly same for SLF and SNF.

4. *In vivo* degradation study

The *in-vivo* biodegradation study was carried out on 3 male wistar rats which are subcutaneously implanted with XYG films and study were carried out at 10, 20 and 30 minutes. The plot showed in figure 5 which results that the films are degraded quickly within 30 minutes.

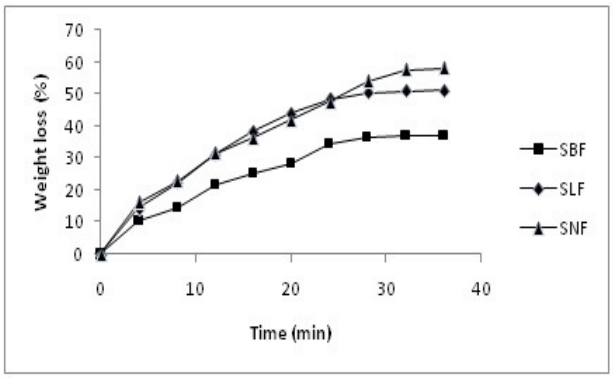

Figure 4(a): Percentage weight loss as a function of the degradation of XYG films.

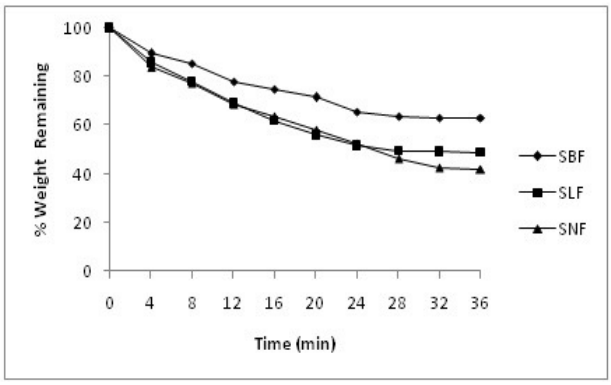

Figure 4(b): Percentage weight remaining as a function of the degradation of XYG films.

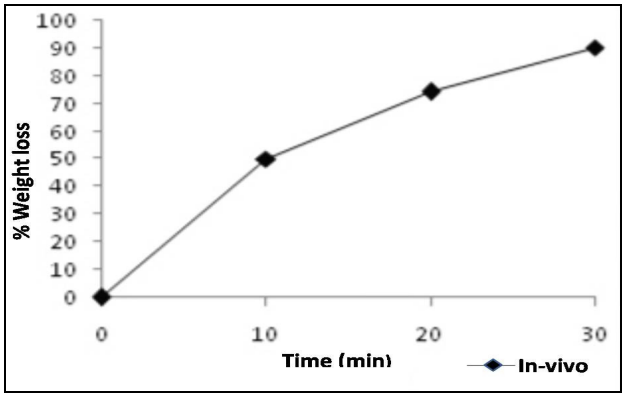

Figure 5: Percentage weight loss as a function of the biodegradation of XYG films *in vivo*.

5. *In vitro* and *in vivo* degradation comparison

The weight decline of the free films of XYG following *in-vitro* and *in-vivo* degradation indicates a faster decline when implanted into the rats (Fig. 6). The weight decline of films *in-vitro* I different fluids results into 37.15%, 51.18% and 58.08% as function to SBF, SLF and SNF respectively and weight decline *in-vivo* at the end of 30min was 90.23%.

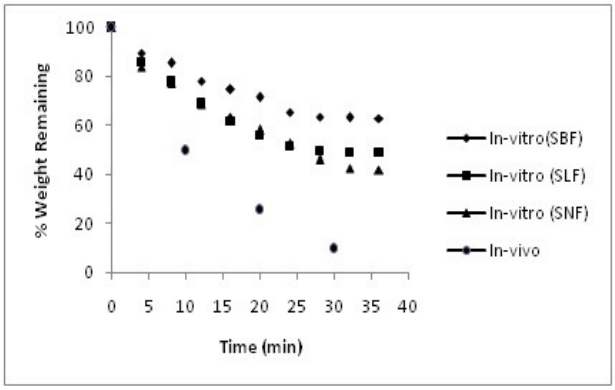

Figure 6: comparison of *in vitro* and *in vivo* percentage weight remaining as a function of the degradation of XYG films.

The weight remaining after degradation in-vitro was 62.85%, 48.82% and 41.92% of SBF, SLF and SNF respectively and *in-vivo* was 9.77% remained after 30min. It was concluded that the degradation rate of film in-vivo is quicker and faster than *in-vitro*.

DISCUSSIONS

This study investigates a natural polymer, xyloglucan, for its degradability in and with the different physiological environment. Xyloglucan shows faster degradation *in vivo* as compared twith *in vitro*. *In vitro* after placement in different physiological fluids such as SBF, SLF and SNF the xyloglucan shows faster degradation in SNF>SLF>SBF within 36 minutes, the weight loss is more quicker in SNF and SLF than SBF and also swelling behaviour of film more in SBF than SNF and SLF because of less viscosity of SBF it is not quickly degraded. After *in vivo* implantation in rats, the films showed weight loss of 90.23% within 30 minutes. The bulk degradation is evident both *in vitro* and *in vivo*. Although the xyloglucan is a highly biodegradable polymer which presumably will lead to new application of these polymer in the field of novel drug delivery system. In the future, this polymeric material may provide a relatively economical and readily available matrix for novel drug delivery.

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