Preparation of starch-poly-glutamic acid graft copolymers by microwave irradiation and the characterization of their properties

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1 **ABSTRACT**

2 Graft copolymers of waxy maize starch and poly- γ -glutamic acid (PGA) were produced in an
3 aqueous solution using microwave irradiation. The microwave reaction conditions were
4 optimized with regard to temperature and pH. The temperature of 180°C and pH7.0 were the
5 best reaction conditions resulting in a PGA graft of 0.45% based on nitrogen analysis. The
6 average graft content and graft efficiency for the starch-PGA graft copolymer prepared at 180°C
7 and pH7.0 were 4.20% and 2.73% respectively. The starch-PGA graft copolymer produced at
8 180°C and pH7.0 could absorb more than 20 times its own weight amount of water and form a
9 gel. The preliminary rheology study revealed that the starch-PGA graft copolymer gel exhibited
10 viscoelastic solid behavior while the control sample of waxy starch showed viscoelastic liquid
11 behavior.

1. Introduction

Starch is a low cost, multipurpose, biodegradable biopolymer. Depending on the reagent and parameters used, modifying starch by grafting a monomer onto it can create a graft copolymer with increased water absorbent capabilities, thermal stability, and increased viscosity. Starch graft copolymers can be applied in many ways including waste water treatment, in cosmetics, as flocculants (Pal, Sen, Ghosh, & Singh, 2012), and as super absorbers; and therefore greatly attract scientists' interests (Jyothi, 2010). Various modified starch-based copolymer preparations have been reported and described (Fanta, 1996). One of the processing methods to prepare starch graft copolymers is microwave irradiation or heating. Microwave assisted synthesis is a useful tool for a wide range of chemical reactions. This is due, in part, to its precisely controlled temperature and pressure environment. Processes performed under microwave heating offer several advantages over conventional heating, such as reduced reaction times, larger yields, more eco-friendly, and less auxiliary reactions. This creates an end product that is more pure and not contaminated by other reagents (Hoogenboom & Ulrich, 2007; Petit, Reynaud, & Desbrieres 2015). Microwave heating has been used more and more in polymer synthesis during recent years (Kempe, Remzi Becer, & Schubert, 2011; Singh, Kumar, & Sanghi, 2012). Zhou et al. (2006) reported a method by which starch-based foams were prepared by microwave heating. Other starch derivatives have also been prepared utilizing microwaves (Shogren & Biswas, 2006). Additional methods of producing starch-based graft copolymers using microwave heating have been reported (Singh, Tiwari, Pandey, & Singh, 2007; Mishra, Mukul, Sen, & Jha, 2011; Singh, Tiwari, Pandey, & Singh, 2006; Kumar, Setia, & Mahadevan, 2012; Chang et al., 2009). However, the method used in this study is a narrower, more refined procedure for graft copolymer polymerization. Poly- γ -glutamic acid (PGA) is a water soluble

poly amino acid. It is not only a biodegradable, but also an edible biopolymer. The unique properties of the PGA allow it to have many potential applications in drug delivery, food, water treatment, cosmetics and other fields (Bajaj & Singhal, 2011).

The purpose of this work is to develop a starch-based biopolymer that is biodegradable, edible, and absorbs plenty of water while still retaining its gel properties; this new starch-based biomaterial could have a variety of useful applications. For example, starch grafted PGA could be used as stable hydrogel particles for controlled or targeted release of lipophilic and hydrophilic bioactive agents in the GI tract (Zhang, Zhang, Chen, Tong, & McClements, 2015).

Waxy starch, which is composed of highly branched amylopectin, was used in this study rather than normal, less expensive starch because the amylopectin forms a more stable paste or gel-like material (Doublier & Llamas, 1993; Zhang et al., 2015). PGA was adopted for this study because it is water soluble; and thus it should be miscible with starch. Microwave irradiation has been used to produce starch-PGA copolymers (Pal et al., 2012); however, the reaction first grafted glutamic acid onto amylose chains and then polymerized the monomer. This new procedure uses microwaves to graft PGA onto amylopectin.

Properties including water absorption, water solubility, graft content, graft efficiency, and rheological behavior are reported.

2. Materials and methods

2.1. Materials

Unless otherwise stated, all materials were obtained from commercial sources and used as supplied. Waxy maize corn starch (7350) was obtained from Tate & Lyle (Decatur, IL, USA). The ambient moisture content of the starch was 11.2% by weight, using gravimetric measurements (AACC 44.01.01). Acetanilide Calibration Standard was purchased from Perkin Elmer (PN 0240-1121 - C 71.09% H 6.71% N 10.36%). Enzymes (Alpha-amylase and amyloglucosidase) were obtained from Megazyme (Wicklow, Ireland).

2.2. Poly- γ -glutamic acid (PGA) preparation

2.2.1. Strain Information and Preservation:

Bacterial cultures were cultivated to produce PGA. *Bacillus licheniformis* ATCC 9945a was purchased as a lyophilized powder from the American Type Culture Collection (Manassas VA) and was reconstituted in sterile ATCC Medium 21 (Bacillus Medium) according to ATCC instructions (<http://www.atcc.org/Products/All/9945a.aspx#generalinformation>). The medium contained the following in g/L: K₂HPO₄, 0.5; Ferric Ammonium Citrate, 0.5; MgSO₄, 0.5; Glycerol, 20.0; Citric Acid, 2.0; L-Glutamic Acid, 4.0 (all chemicals were at least reagent grade and were obtained from Sigma-Aldrich (St. Louis, MO, USA)). The pH of the medium was adjusted to 7.4±0.2. Muroid colonies were aseptically selected from Bacillus Medium plates solidified with 1.5% agar and streaked onto fresh Bacillus plates and stored at 4°C for use as inocula for the PGA production. All plates were re-streaked on a monthly basis to maintain muroid phenotype. In addition, *B. licheniformis* ATCC 9945a was maintained in a cryogenic state in Bacillus Medium containing 10% (v/v) glycerol at -80°C.

2.2.2. PGA Production:

1 PGA production was achieved at the bench-top scale in a 10-L volume using a New
2 Brunswick Scientific BioFlo 3000 (now part of Eppendorf Inc., Enfield, CT). The medium
3 (Medium E) used for PGA synthesis was previously described by Birrer et al. (1994). The
4 inoculum was prepared by aseptically transferring a single mucoid colony from the refrigerated
5 stock plates (described in the previous section) into 10 ml of Media E (pH 7.4) contained in a
6 sterile test tube and incubated at 37°C, 250 rpm for 24 h in a shaker incubator. The entire 10 ml
7 sample was then aseptically transferred into 50 ml of Media E and incubated again as described
8 above. After an additional 24-hour the 50 ml culture was aseptically transferred to 1 L of Media
9 E and the culture then allowed to grow under identical conditions noted above for another 24 h
10 after which the entire 1 L inoculum was aseptically centrifuged and the supernatant discarded.
11 The pelleted cells were resuspended in 150 ml of fresh Media E from the fermenter itself and
12 then aseptically poured back into the 10-L fermenter as inoculum for PGA production. The
13 fermentation conditions were set at 37°C. The media was sparged with sterile air at 1
14 liter/minute and stirred with an impeller operating at 600 rpm. The pH of the media, at the start
15 of culturing, was 7.4. The fermentations were conducted for 96 h after which the entire culture
16 was centrifuged ($10,000 \times g$, 4°C, 20 minutes) to separate the cells from the PGA-containing
17 supernatant. The cell pellets were washed twice with deionized water to achieve maximum PGA
18 recovery. The water washings were added to the supernatant and the entire aqueous phase
19 (supernatant) was lyophilized to dryness. The residual PGA polymers were resuspended in one-
20 quarter volume deionized water and then precipitated into excess cold ethanol. The PGA was
21 then recovered and dried under vacuum to a constant weight. In order to confirm that the PGA
22 was pure, amino acid analyses were done as described in the method below.

2.3. *Amino acid test to confirm the purity of PGA:*

Dehydrated samples were hydrolyzed in 6N HCl containing a small amount of phenol. The hydrolysis flasks were extensively purged of oxygen using a PicoTag workstation (Waters Corp., Milford, MA, USA), and then incubated at 110°C for 20 hours. Hydrolyzed samples were filtered, dried under vacuum, and derivatized with AccQFluor reagent (Waters) following the manufacturer's directions. Chromatography was performed using procedures described as 'system 1' by Cohen and De Antonis, with α -aminobutyric acid as an internal standard. Separation was achieved using an AccQTag C18 reverse-phase column (Waters); detection by fluorescence used excitation with 250 nm light and measured emission at 395 nm. Hydrolysis, derivatization, and analysis of each sample were performed in triplicate (Cohen & De Antonis, 1994).

2.4. *Preparation of Starch-PGA graft copolymer*

A 2% (wt. %) suspension of waxy maize starch (dry weight) using nanopure water (Millipore 18.2 M Ω ·cm) was placed in a pressure vessel and gelatinized by microwave radiation which heated the suspension from 23°C to 140°C in 5 minutes. The gelatinized paste was then freeze dried and stored for further use. Attempting to graft PGA without pregelatinising the waxy starch will not yield measurable grafting of the two materials. A 2% (wt.%) solution of PGA using nanopure water was dissolved and freeze dried. The freeze dried, microwaved starch and freeze dried PGA were the stock material for the preparations.

A 2% solution of the stock freeze-dried waxy maize starch was then combined with a 2% solution of the stock freeze-dried PGA (adjusted to pH 6.0) at a 1:1 ratio. Combined samples

were then heated in open vessels for 2 hours at 120°C, 140°C, 160°C, 180°C and 240°C respectively. Microwave reactions were carried out using a Initiator Exp Microwave (Biotage Inc., Charlotte, NC), and an Ethos MicroSYNTH 1600 microwave labstation (Milestone Inc., Shelton, CT, USA). Reactions were performed in a 20 ml pressure vessel (354833) in the Biotage microwave oven and in a 270ml and 500 ml Teflon pressure vessel (45161T and HPR3600 respectively) in the Milestone microwave oven. There were no differences between the products made using either of the microwave systems. The two samples that had the highest water absorption qualities were repeated, this time with polyglutamic acid adjusted to a pH of 4.0, 5.0, 7.0, and 9.0 with 0.5 M NaOH and 0.5M HCl where needed. The native pH of the sample was approximately 6.5. Optimum temperature was determined by the sample that had the highest water absorption traits. All results are then compared, and the sample with the highest water absorption properties and highest nitrogen content were chosen. Un-reacted or soluble starch and PGA were removed from the sample pellet by washing 2× with nanopure water, centrifuging, and decanting the supernatant, leaving behind the gel-like starch-PGA copolymer. During the washing of the sample (after the WSI/WAI assay described in 2.5.), the physical appearance of the sample was examined. Sample was dried in a vacuum oven at 60°C overnight and nitrogen analysis was performed.

2.5. Characterizations

The water absorption index (WAI) is the weight of gel obtained per gram of dry sample. A 0.1 - 0.2 g sample was suspended in 30 ml of water at room temperature in a 50-ml tared centrifuge tube, stirred for 8-10 hours, and centrifuged at 6,000 rpm for 60 min. The supernatant liquid was poured carefully into a tared evaporating dish. The remaining gel/pellet was weighed

and the WAI calculated from its weight as $WAI = (\text{wet weight of pellet} / \text{dry weight of sample})$.
The water solubility or the water solubility index (WSI) was calculated as $WSI = (\text{dry weight of supernatant} / \text{dry weight of sample}) \times 100$. The pellet was then washed by adding 40 ml of nanopure water into the 50 ml centrifuged tube, stirred for one hour, then centrifuging at 6,000 rpms for 60 minutes. The supernatant was discarded, and the process was repeated. The remaining pellet was then used for Carbon, Hydrogen, and Nitrogen (CHN) analysis.

Nitrogen composition of the graft copolymer was determined by CHN analysis on the Perkin Elmer 2400 series II CHNO/S Dumas-type elemental analyzer (Waltham, MA). Approximately 2 - 4 mg of material was used for each nitrogen analysis, and the instrument was calibrated with an acetanilide standard. Nitrogen analyses were carried out in triplicate, and the results were averaged. Existence of nitrogen in the sample indicates the formation of starch-PGA copolymer.

Graft content, graft efficiency, and graft frequencies were calculated using the following equations:

Graft Content (GC) (%): also known as % add-on, i.e., percentage of g-PGA in st-g-PGA

$$GC = 100 \times (N_{\text{st-g-PGA}} / N_{\text{aGA}}) \quad (1)$$

Where $N_{\text{st-g-PGA}}$ represents the measured nitrogen % in st-g-PGA (insoluble precipitate);

$N_{\text{aGA}} = 10.85$ (percent Nitrogen in anhydrous (H_2O 18.02 g/mol) poly-glutamic acid).

$$W_{\text{g-PGA}} = (N_{\text{st-g-PGA}} / N_{\text{aGA}}) \times W_{\text{st-g-PGA}} \quad (2)$$

1 Where W_{g-PGA} is the weight of grafted PGA; $W_{st-g-PGA}$ is the weight of starch-graft-copolymer
2 (insoluble precipitate).

3 Grafting Efficiency (GE)(%): amount of PGA grafted onto starch (ratio of g-PGA to total PGA)

$$4 \quad GE = 100 \times ((N_{st-g-PGA} / N_{aGA}) \times (W_{st-g-PGA} / W_{PGA}^i)) \quad (3)$$

5 Where W_{PGA}^i is the weight of initial poly-glutamic acid.

6 Graft Frequency (N_{AGU}) (AGUs per g-PGA): number of AGUs between grafts i.e. high #s =
7 lower frequency

$$8 \quad N_{AGU} = (Mn_{g-PGA} / 2M_{AGU}) \times ((100-GC)/GC) \quad (4)$$

9 Where N_{AGU} is the graft frequency; Mn_{g-PGA} is the number average molecular weight (from
10 HPLC-SEC) of g-PGA; $M_{AGU}=162$ (molecular weight of the anhydrous glucose unit (starch)).

11 2.6. *Rheological characterizations*

12 A strain-controlled Rheometric ARES rheometer (TA Instruments, New Castle, DE) was
13 used to perform the rheology studies (Xu, Bietz, Felker, Carriere, & Wirtz, 2001). The 50-mm
14 diameter cone and plate geometries were adopted. The cone angle was 0.04 radians. The
15 temperature was controlled at $25 \pm 0.1^\circ\text{C}$ by a water circulation system. Linear viscoelastic
16 measurements were conducted for 5% (wt%) starch-PGA graft copolymer in nanopure water
17 (Millipore 18.2 M Ω ·cm). When the starch-PGA mixed with water (5%) at room temperature, a
18 gel-like material was formed. The control material was a 5% (wt.%) waxy starch produced with
19 the same method of starch-PGA preparation but only using starch without PGA. To ensure that
20 all the measurements for the materials were made within the linear range for the linear

viscoelastic properties studies, the strain-sweep experiments were conducted initially between 0.1% and 300% of strain. An applied shear strain valued in the linear range obtained from previous strain-sweep experiment was adopted for the dynamic linear viscoelastic property measurements for the same material; new samples were used for each measurement. Linear viscoelasticity indicates that the measured parameters are independent of applied shear strain. Small-amplitude oscillatory shear ($<1\%$) experiments were conducted over a frequency (ω) range of 0.1-500 rad/s to measure the shear storage (G') and loss (G'') moduli. The storage modulus represents the non-dissipative component of mechanical properties. The elastic or “rubber-like” behavior is suggested if the G' spectrum is independent of frequency and greater than the loss modulus over a certain range of frequency. The loss modulus represents the dissipative component of the mechanical properties and is characteristic of viscous flow. The phase shift or phase angle (δ) is defined by $\delta = \tan^{-1}(G''/G')$, and indicates whether a material is solid with perfect elasticity ($\delta=0$), or liquid with pure viscosity ($\delta=90^\circ$), or something in between ($0 < \delta < 90^\circ$).

3. Results and Discussions

3.1. WSI, WAI, and nitrogen analysis

First, the optimum conditions for preparing starch-PGA graft copolymers were determined. After trying pH 4.0, 5.0, 6.0, 7.0, and 9.0, as well as the temperatures 120°C, 140°C, 160°C, 180°C, 200°C and 240°C; pH 6.0-7.0 and 160°C-200°C were chosen as the better conditions due to the water absorption qualities of the graft copolymers produced under these

conditions. Table 1 displays the final experimental design. Samples 3, 6, 9, 12, 15, and 18 were controls, which did not use PGA. The product of starch-PGA was a semi-clear gel-like material; while the control one was a viscous fluid or liquid-like material (Table 1). The water solubility index, water absorption index, and nitrogen content are shown in Table 2. PGA is a polypeptide that contains nitrogen, and starch is a carbohydrate without nitrogen. The products of samples 1, 2, 4, 5, 7, 8, 10, 11, 13, 14, 16, and 17 were starch-PGA graft copolymers evidenced by a different amount of nitrogen; but the control samples 3, 6, 9, 12, 15, and 18 did not have nitrogen detected (Table 2). At a temperature of 200°C, the product samples' color became brown indicating that there was slight overheating (Table 1). The percentage of nitrogen for the starch-PGA graft copolymers was in a range of 0.27% - 0.59% (Table 2). It was noticed that, in the product samples, the higher the reaction temperature, the greater the percentage of nitrogen, indicating an increase in PGA content. The percentage of nitrogen for the starch-PGA samples heated at 180°C and 200°C, did not exhibited much difference, which suggested that the yield would be similar at these two temperatures. The average water absorption index (WAI) for the starch-PGA graft copolymers produced at pH6.0 and 180°C (samples 4 and 5) was 14.74; while the average WAI for the starch-PGA graft copolymers produced at pH7.0 and 180°C (samples 13 and 14) was 21.80, which was the best water absorption among all product samples. For comparison, WAI for the starch controls were 0.71 at pH 6.0, 180°C and 0.97 at pH 7.0, 180°C respectively. The graft content increased with the higher reaction temperature (Table 2), which indicated that more PGA was grafted onto the starch at higher temperature. The graft efficiency, which is the amount of grafted PGA relative to the total formed PGA amount, also increased with the increasing reaction temperature.

1 The graft efficiencies for the samples produced at 160°C were much lower than those for the
2 samples produced at 180°C and 200°C (Table 2). This is consistent with the soluble data in
3 Table 2; the more soluble, the larger amount of PGA in the soluble fraction. The average graft
4 content and graft efficiency for the starch-PGA graft copolymer prepared at 180°C and pH7.0
5 were 4.20% and 2.73% respectively. These values are similar to the efficiencies reported for
6 starch nanoparticles, grafted with PGA, using a click reaction (Zhang, et al., 2013). The overall
7 graft content and graft efficiency were relatively small, implying that the PGA is difficult to graft
8 onto the starch. The possible reason is that the PGA is already an existing polymer. In most
9 cases, starch graft copolymers are synthesized by forming graft sites on the starch, and then
10 polymerizing a monomer (such as glutamic acid) onto the starch.

11

1 **Table 1.** Summary of reaction conditions.

Sample #	Starch (g)	PGA (g)	pH	Temp (°C)	Time (hour)	Physical Appearance
1	0.30	0.30	6.0	160	2.0	Semi-clear gel
2	0.30	0.30	6.0	160	2.0	Semi-clear gel
3	0.60	0.00	6.0	160	2.0	Tiny liquid-like material
4	0.30	0.30	6.0	180	2.0	Semi-clear gel
5	0.30	0.30	6.0	180	2.0	Semi-clear gel
6	0.60	0.00	6.0	180	2.0	Tiny liquid-like material
7	0.30	0.30	6.0	200	2.0	Brown gel
8	0.30	0.30	6.0	200	2.0	Brown gel
9	0.60	0.00	6.0	200	2.0	Tiny liquid-like material
10	0.30	0.30	7.0	160	2.0	Semi-clear gel
11	0.30	0.30	7.0	160	2.0	Semi-clear gel
12	0.60	0.00	7.0	160	2.0	Tiny liquid-like material
13	0.30	0.30	7.0	180	2.0	Semi-clear gel
14	0.30	0.30	7.0	180	2.0	Semi-clear gel
15	0.60	0.00	7.0	180	2.0	Tiny liquid-like material
16	0.30	0.30	7.0	200	2.0	Brown gel
17	0.30	0.30	7.0	200	2.0	Brown gel
18	0.60	0.00	7.0	200	2.0	Tiny liquid-like material

2 Nano pure water was adjusted to the desired pH using 1M NaOH or 1M HCl and then was added
3 to the starch and PGA mixture.

4

1 **Table 2.** Starch-PGA properties

Sample	WSI	WAI	Average % Nitrogen	Weight fraction of St-g- PGA	Weight fraction of solubles	Weight of grafted pGA	Graft content (%)	Grafting efficiency (%)	Grafting frequency
1	44.83	13.70	0.40	0.20	0.80	0.0044	3.69	1.48	1613
2	53.94	9.72	0.27	0.20	0.80	0.0030	2.49	1.00	2419
3	89.91	0.82	0	0	1.00	0	0	0	0
4	56.46	14.05	0.49	0.27	0.73	0.0073	4.52	2.44	1305
5	52.82	15.42	0.44	0.29	0.71	0.0071	4.06	2.37	1460
6	92.50	0.71	0	0.00	1.00	0	0	0	0
7	45.37	16.18	0.59	0.26	0.74	0.0085	5.44	2.82	1073
8	41.26	15.66	0.53	0.35	0.65	0.0103	4.88	3.42	1202
9	93.00	0.73	0	0	1.00	0	0	0	0
10	70.33	1.74	0.30	0.01	0.99	0.0002	2.76	0.07	2171
11	68.32	2.54	0.27	0.005	0.995	0.0001	2.49	0.02	2419
12	89.51	0.84	0	0	1.00	0	0	0	0
13	46.99	20.55	0.45	0.32	0.68	0.0079	4.15	2.64	1427
14	47.07	23.05	0.46	0.33	0.67	0.0084	4.24	2.81	1394
15	89.74	0.97	0	0	1.00	0	0	0	0
16	41.19	18.28	0.52	0.36	0.64	0.0105	4.79	3.49	1226
17	41.48	19.12	0.59	0.36	0.64	0.0118	5.44	3.95	1073
18	88.20	2.65	0	0	1.00	0	0	0	0

2

3.2. *Rheological properties*

The starch-PGA graft copolymers and starch samples used in rheology measurements were prepared at 180°C and pH7.0. The linear rheological properties of 5% (wt.%) control sample of starch and starch-PGA graft copolymer are shown in Fig. 1. The curves of both storage (G') and loss moduli (G'') for the starch control sample were highly frequency dependent; and loss moduli were higher than storage moduli at all measured frequencies. The storage and loss moduli for the 5% control starch sample were in the range of 0.005 Pa – 4.94 Pa and 0.01 Pa – 10.61 Pa respectively (Fig. 1). The phase shifts for the 5% starch sample were from 63.8° to 73.8°. These results indicated that the control starch material exhibited viscoelastic fluid or liquid behavior (Ferry 1980). However, the linear rheological properties for the 5% (wt.%) starch-PGA graft copolymer showed distinguish behaviors from those for the control starch sample (Fig. 1). The curves of both storage (G') and loss moduli (G'') for the starch-PGA graft copolymer were nearly frequency independent; and storage moduli were higher than loss moduli at all measured frequencies. Both storage (G') and loss moduli (G'') for the 5% starch-PGA graft copolymer were much greater than those for the 5% control starch sample. The plateau storage and loss modulus for the 5% starch-PGA graft copolymer were about 370 Pa and 27 Pa respectively (Fig. 1). The phase shifts for the 5% starch-PGA graft copolymer were in the range of 4.3° to 9.2°, which were much lower than those for the control starch sample. These results suggested that the starch-PGA graft copolymer was a gel-like viscoelastic solid material and a cross-linking network (Ferry 1980).

Fig. 1. Linear viscoelastic properties of frequency sweep experiment for the 5% (wt.%) starch control sample and the 5% (wt.%) starch-PGA graft copolymer gel. Storage modulus (G') or loss modulus (G'') as function of frequency at 25°C with 1% strain. Filled symbols: G' , opened symbols: G'' . ($\bullet$, $\circ$): 5% (wt.%) control sample starch-starch, ($\blacksquare$, $\square$): 5% (wt.%) starch-PGA graft copolymer.

4. Conclusion

1 The starch was successfully grafted with poly(glutamic acid) using a microwave-assisted
2 reaction. PGA graft content of less than 5% was sufficient to significantly change the
3 rheological properties of starch at room temperature and increase the water absorption at least
4 twenty fold. In most work, starch graft copolymers are synthesized by forming graft sites on the
5 starch and then polymerizing a monomer (such as glutamic acid) onto the starch. This study
6 shows that two polymers – starch and PGA – can be covalently linked using microwave
7 irradiation. Some previous applications for starch graft copolymers already noted are many,
8 including waste water treatment, cosmetics, as flocculants (Pal et al., 2012), and as super
9 absorbers. The unique property of the starch-PGA copolymer allows it to have many potential
10 applications in drug delivery, food, water treatment, cosmetics and other fields (Singh et al.,
11 2007). More detailed rheological property studies of starch-PGA graft copolymers are needed to
12 explore their potential applications.

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1 **Figure captions**

2 **Fig. 1.** Linear viscoelastic properties of frequency sweep experiment for the 5% (wt.%) starch
3 control sample and the 5% (wt.%) starch-PGA graft copolymer gel. Storage modulus (G') or
4 loss modulus (G'') as function of frequency at 25°C with 1% strain. Filled symbols: G' , opened
5 symbols: G'' . (●, ○): 5% (wt.%) control sample starch-starch, (■, □): 5% (wt.%) starch-PGA
6 graft copolymer.