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Preparation and Characterization of Gellan Gum-Seaweed Beads as a Controlled Release Fertilizer: A Biodegradable and Environmentally Friendly Option

Adila Mohamad Jaafar[1,2*], Nurul Syafika Kamarudin[1] and Arina Shairah Abdul Sukor[3]
[1]Department of Chemistry, Faculty of Science
[2]Unit of Chemistry, Centre of Foundation Studies for Agriculture Science
[3]Department of Land Management, Faculty of Agriculture,
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
*Correspondence author (e-mail: adilamj@upm.edu.my)

The agricultural sector contributes significantly to the economy as it provides a source of food, as well as income and employment. Excessive usage of chemicals in this sector brings harm to both humans and the environment. For the purpose of formulating an environmentally friendly controlled release fertilizer, gellan gum hydrogel was selected as a host to be loaded with a species of seaweed, a natural fertilizer. The objective of this study was to develop a novel seaweed-based fertilizer hydrogel bead. GG-hydrogel beads (GG-B) loaded with seaweed (GG-SB) at various concentrations from 5% to 20% w/v were successfully prepared. FTIR spectra showed the presence of a combination of functional groups from both the host and the seaweed. Elemental analysis supported the FTIR results, as the carbon content increased in line with the concentration of seaweed loaded. The morphologies of GG-B and GG-SB showed distinct differences. The swelling study of GG-B and GG-SB at pH 7.0 showed increased swelling as the seaweed concentration decreased, however, the degradation study showed otherwise. It is hoped that the GG-SB material can be used in fertilizer production as it is safe for both people and the environment.

Key words: Hydrogel; gellan gum; seaweed; fertilizer

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The use of chemicals in the agrochemical industry is a common practice that has severely impacted the environment. For example, the application of excess chemical fertilizers can cause serious water and soil pollution. It is often necessary to add fertilizers to soil in order to supply nutrients that are needed for plant growth. However, the problem arises when these fertilizers are lost due to flooding, leaching, volatilization and degradation resulting from various factors including climatic changes and human activities [1]. Various studies have been conducted to find solutions to these problems. Among these, the use of hydrogels and controlled release formulations are interesting as they hold great potential for application in agriculture.

In general, hydrogels are hydrophilic polymer networks with three-dimensionally crosslinked structures that have the ability to swell to an equilibrium state in water or biological fluids [2]. Hydrogels are considered smart materials that are capable of altering their physiochemical properties in response to external chemical and physical stimuli [3]. These are named 'stimuli-responsive' hydrogels. Hydrogels are able to absorb and retain large amounts of water [4]. Due to this outstanding property, hydrogels have been used in agriculture activities as water reservoirs. A significant amount of research has been focused on the application of hydrogels in agriculture as they have excellent slow-release properties and good water retention capacity which can reduce fertilizer loss and enhance the availability of water in soil [3]. Additionally, hydrogels have been used as Super Absorbent Polymers (SAPs) for sustainable agricultural farming [1].

Hydrogels can be prepared from synthetic or natural polymers. Hydrogels based on natural polymers are nontoxic and possess good biocompatibility and biodegradability [5]. One of the hydrogel-forming natural polymers is gellan gum (GG). Gellan gum can be derived from *Pseudomonas elodae* bacteria. Two types of gellan gum can be obtained commercially, which have low and high acyl content. Gellan gum is an anionic polysaccharide chain that has the structure of a tetrasaccharide repeating unit of two β-D-glucose, one β-D-glucuronic acid and one α-L-rhamnose [3]. This type of polymer has the ability to form a hydrogel due to the presence of carboxylic groups in its glucoronic acid residues [6], and it can undergo the process of random coil to double helix transition upon cooling

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[7]. These natural hydrogel-forming polymers are advantageous in various applications such as drug release, wound healing and tissue engineering, as well as in agriculture. The use of natural polymers such as gellan gum have generated much interest in agricultural applications such as fertilizers, nutrients and herbicides due to their biocompatibility and biodegradability [8]. These biodegradable hydrogels are the most suitable materials to be used in agriculture because they are super absorbent materials that have water absorbency and water retention characteristics [1].

Seaweeds are a renewable natural marine resource that have a wide range of applications. Seaweed is used as the guest species in this study. Seaweed is utilized as a fertilizer because it contains highly effective nutrients and growth regulators that are important for the growth and development of plants [9]. Seaweed extracts can be in liquid form as a foliar spray, soil drench, or in powder and granular forms as soil conditioners in agriculture and horticulture. The ability of seaweed extracts to stimulate physiological and plant responses even at low concentrations have attracted interest in the world of agriculture [10]. They are known as 'plant biostimulants' [9]. Furthermore, seaweeds are biodegradable, non-toxic, nonpolluting and non-hazardous to living things. The advantage of using seaweed fertilizers in agriculture is that it contains polysaccharides such as alginates, fucoidans, laminarans, lichenan-like glucans and fucose containing glucans which have many functions in plants [11]. Alginates are used to promote plant growth and laminarans act as a plant's defence against fungal and bacterial pathogens [7] [12]. Seaweeds are also easy to handle, non-hazardous, low cost and very soluble in water, all characteristics that are advantageous for fertilizers.

In this study, gellan gum was used as the host, and seaweed as the guest species containing various nutrients and plant growth promoting hormones, to produce a controlled release formulation (CRF) fertilizer. In a CRF, the fertilizer is encapsulated inside a host, which is a hydrogel that slowly releases nutrients over the time. Besides providing the slow-release fertilizer to plants, CRF in the form of hydrogel beads may also increase the water holding capacity of soil. In agricultural applications, the slow release of CRF increases the probability that nutrients, for example nitrate (N), phosphorus (P) and potassium (K), will be taken up by plant roots [13]. This is because these systems are able to deliver the nutrients or active ingredient slowly and continuously for a longer duration to a specified target at a desired rate. It may also act as compost after the degradation process. In the formation of compost, the hydrogels may act as a soil conditioner to keep soil from becoming too acidic or too alkaline [3]. The combination of gellan gum hydrogel and seaweed as a controlled release fertilizer has become one of the best solutions to overcome the loss of fertilizer and to supply nutrients sustainably [14]. Therefore, this aim of this study was to investigate the effect of different concentrations of seaweed incorporated into gellan gum and to develop gellan gum hydrogel beads containing seaweed for use as a controlled release fertilizer for agriculture applications.

METHODOLOGY

1. Materials

Gellan gum (Gelzan™ CM, product number-G1910, lot number SLBM5458V), glycerin (product number-G2289, lot number SHBF8791V) and calcium chloride, $CaCl_2$ (product number-C5670, lot number SLBL5787V) were purchased from Sigma Aldrich, Malaysia. Seaweed powder was kindly provided by Green Bio Tech Trading Sdn.Bhd, a company based in Shah Alam, Selangor. All materials were used without further purification.

2. Preparation of Gellan Gum Bead and Gellan Gum–Seaweed Beads

Gellan gum beads (GG-B) were prepared by dissolving 0.5 g of gellan gum powder in 30 mL of deionized water (18 MΩ cm^{-1}) under constant magnetic stirring at 500 rpm at a temperature of 80 °C. To this solution, 2.5 mL of glycerin was added to establish and promote the flexibility of gellan gum solution. The reaction was maintained for about 15-20 minutes to obtain a homogenous solution.

To prepare the gellan gum–seaweed bead (GG-SB), a seaweed solution was added dropwise to the homogenous gellan gum solution. The mixture was stirred for 2 hours using a hot plate stirrer. GG-SB was incorporated with different concentrations of seaweed. The seaweed solutions were prepared by dissolving specific amounts of seaweed powder in deionized water at room temperature to form the concentrations in w/v% as follows: 5% (5 g in 100 ml H_2O), 10% (10 g in 100 ml H_2O), 15% (15 g in 100 ml H_2O) and 20% (20 g in 100 ml H_2O). The gellan gum-seaweed beads were formed by adding the mixed solution dropwise with a syringe into a cooled $CaCl_2$ solution for the crosslinking reaction to take place; this was done to strengthen the beads. The beads were then separated and washed with deionized water to remove excess $CaCl_2$. Next, the GG-SB were placed in petri dishes and dried in an oven for 48 hours at 35 °C. The GG-SB were then stored in a desiccator at room temperature for further characterization. The samples formed were labelled, e.g., 5% GG-SB (30:1) for a sample containing 30 ml of gellan gum solution with 1 ml of 5% w/v seaweed solution.

3. Characterization

The prepared GGB and GG-SB were characterized using various analytical techniques to study the

interaction of the gellan gum hydrogel and seaweed. The techniques involved in this study were Fourier Transform Infrared Spectroscopy (FTIR), Carbon, Hydrogen, Nitrogen and Sulphur Analysis (CHNS) and Variable Pressure Scanning Electron Microscopy (VPSEM).

3.1. Fourier Transform Infrared Spectroscopy

Fourier Transform Infrared Spectroscopy (FTIR) was used to detect the presence of the functional groups in seaweed as well as gellan gum hydrogel with and without the seaweed, using a Perkin Elmer FTIR model 1725X. All samples were prepared using the KBr pellet technique and analyzed in the region of 4000 to 400 cm^{-1}.

3.2. Elemental Analysis

CHNS analysis was performed to determine mass fractions or the amount of carbon (C), hydrogen (H), nitrogen (N) and sulfur (S) in the samples using a CE440 Elemental Analyzer-CHNS instrument. Each finely dried sample was weighed (5-10 mg) and mixed with an oxidizer, vanadium pentoxide, V_2O_5 in a tin capsule. This capsule was then combusted in a reactor at a temperature of 1000 °C. The products produced from the combustion were reported in weight percentages. The data from the combustion analysis of GGB and the different concentrations of GG-SB used were recorded.

3.3. Electron Microscopy

Variable Pressure Scanning Electron Microscopy (VPSEM) was used to study the morphology of the sample by scanning it with a focused electron beam over the surface and the cross section of the sample. The images produced by VPSEM showed a layered texture. To conduct this analysis, the dried beads were placed on aluminium stubs, dried and coated with platinum for about 2-3 minutes. For the cross section, the beads were cut with a blade before coating. Images of the surface and cross sections of the samples were observed and recorded with a LEO 1455 Variable Pressure Scanning Electron Microscope (VPSEM).

3.4. Swelling Study

To study the water absorption capacity of the GG-SB at various concentrations, a swelling test was conducted using a buffer solution with a pH of 7.0, resembling soil. A weighed mass of dried beads were washed and immersed in a petri dish containing 30 ml of the buffer solution at room temperature for 6 days. Then, the sample was gently dried using filter paper, and the weight of the swollen bead was measured and the swelling data for pure GG-B and different concentrations of GG-SB were recorded. The swelling percentage was calculated using Equation 1.

$$Swelling\ percentage\ (\%) = \frac{M_w - M_d}{M_d} \times 100\% \qquad \text{Equation 1}$$

where M_w is the weight of the swollen sample (g) and M_d is the weight of the dry sample (g).

3.5. Degradation Study

The ability of the sample to lose water was tested by the process of degradation. Known weights of dried samples of each concentration were placed in petri dishes and left at room temperature. The weights of these samples were measured every 24 hours until they became constant.The degradation data for GG-B and GG-SB at different concentrations were recorded. The calculation of the degradation percentage for each sample is shown in Equation 2 below:

$$Degradation\ percentage\ (\%) = \frac{M_f - M_i}{M_i} \times 100\% \qquad \text{Equation 2}$$

where M_f is the final weight of the sample (g) and M_i is the initial weight of the sample (g).

RESULTS AND DISCUSSION

1. Fourier Transform Infrared Spectroscopy

Figure 1 shows the FTIR spectra of seaweed, GG-B and GG-SB at different concentrations of seaweed, and the details are summarized in Table 1. The spectrum of seaweed shows the existence of a broad weak absorption band at 3313 cm^{-1}, due to the stretching frequency of the O-H and N-H groups of the amino acids which are a component of the proteins present in seaweed [15]. The peak at 1566 cm^{-1} corresponds to the N-H bending vibration of the amide structure and the peak at 1406 cm^{-1} is due to the C-N stretching of the amide group in proteins. A weak band appearing at 1354 cm^{-1} is due to S=O stretching, and is characteristic of the presence of lignin, which acts as a structural material in the support tissues of seaweed [16]

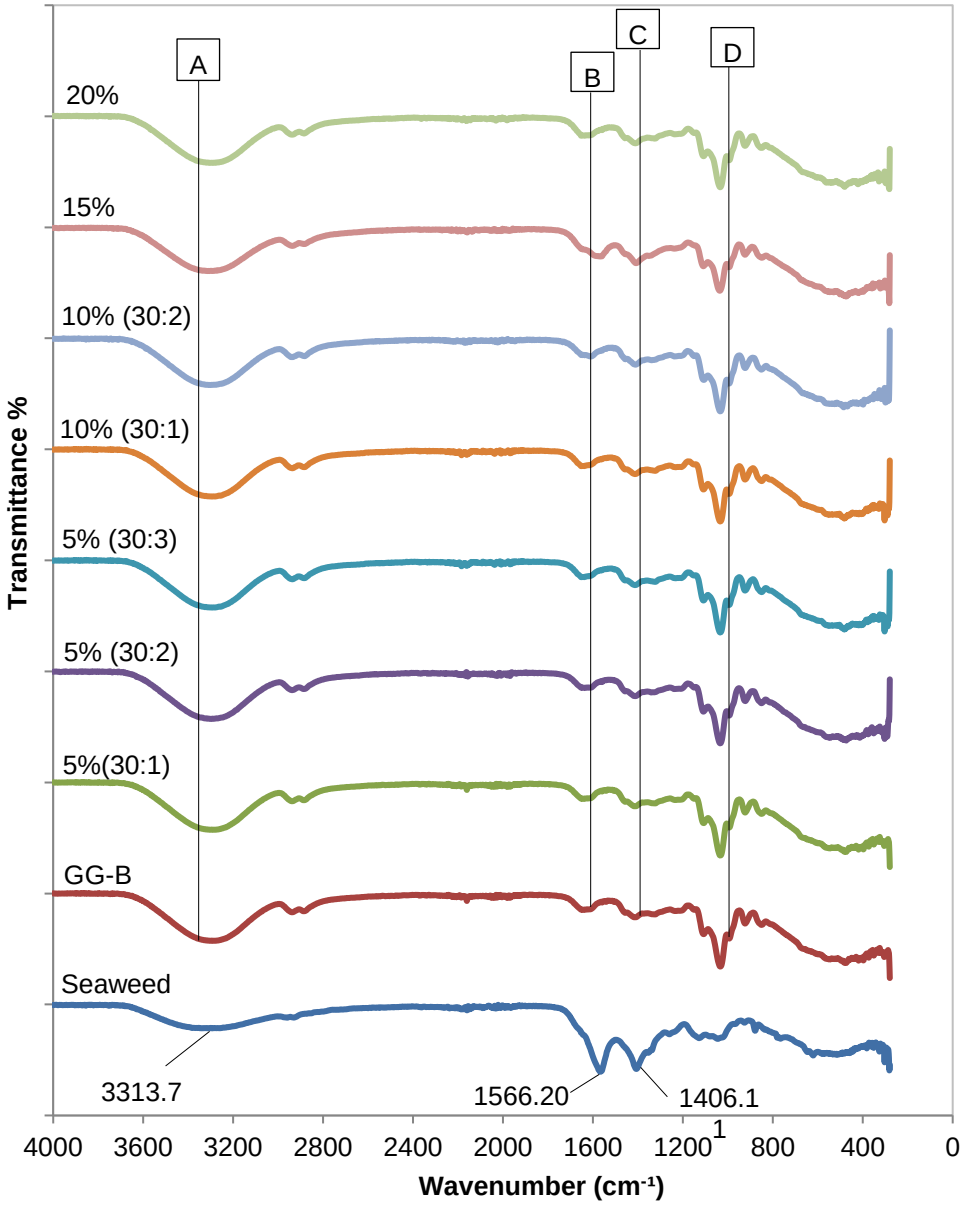

Figure 1. FTIR spectra of seaweed, gellan gum beads (GG-B), and GG-SB at different concentrations (5%, 10%, 15% and 20% w/v).

In the spectrum of gellan gum beads, the strong broad peak at 3304 cm^{-1} corresponds to the stretching frequency of hydroxyl groups, -OH, that are present in carboxylic acids and alcohols. For gellan gum beads loaded with seaweed, this peak had shifted to the range of 3298–3315 cm^{-1}. In Figure 1, the peak of the O-H group for GG-B had the same intensity as for GG-SB. Further, the weak absorption peak appearing at 1616 cm^{-1} is due to the C=O stretching from the glycosidic link present in gellan gum beads. The peaks in the range of 1570–1651 cm^{-1} are due to the shifting of the C=O stretching bands that were also present in the spectrum of gellan gum hydrogel loaded with seaweed. The band at 1413 cm^{-1} in the spectrum of gellan gum beads is attributed to the C=O shift due to the stretching of the carboxylate group. For GG-SB at different concentrations of seaweed, shifting occurred in the range of 1408–1415 cm^{-1}. The peak at 1031 cm^{-1} is due to the C–O stretching vibrations in carboxylic acids and alcohols. The peaks for each of the GG-SB had shifted to 1031–1035 cm^{-1}. From the FTIR spectrum of GG-SB, it can be concluded that the shifting of the functional group frequencies occurred due to interactions between the hydrogel matrix of gellan gum and seaweed. The main factor in the hydrogel's ability to swell is hydrophilicity, to which the presence of hydroxyl and carboxyl groups contribute significantly [17].

Table 1. FTIR data of GG-B and GG-SB at different concentrations of seaweed.

Samples	Type of vibration			
	A (O-H stretch)	B (C=O stretch)	C (C=O shift)	D (C-O stretch)
GG-B	3304	1616	1413	1031
5% w/v GG-SB (30:1)	3302	1649	1415	1031
5% w/v GG-SB (30:2)	3298	1651	1413	1031
5% w/v GG-SB (30:3)	3302	1645	1411	1031
10% w/v GG-SB (30:1)	3302	1651	1411	1033
10% w/v GG-SB (30:2)	3305	1610	1409	1033
15% w/v GG-SB	3315	1570	1408	1035
20% w/v GG-SB	3302	1651	1409	1031

2. Elemental analysis (CHNS)

Elemental analysis was used to determine the content of carbon, hydrogen, nitrogen and suphur in gellan gum, seaweed and gellan gum loaded with seaweed. As listed in Table 2, the C, H, N and S contents in seaweed were 22.18%, 3.54% 0.78% and 2.01% respectively. The high levels of C and H were due to the presence of carbohydrates and proteins in seaweed. Seaweed has different species that can exist in sulphated and non-sulphated forms.

In this study, the carbon content of the hydrogel incorporated with seaweed was expected to increase due to the addition of carbon from the seaweed. This is an indicator that the incorporation of seaweed into the gellan gum was successful. The results showed that the C and H content of the gellan gum bead, GG-B were high, at 28.77% and 7.24%, respectively. This is due to the presence of polysaccharides in the gellan gum hydrogel. The high content of hydrogen is also due to the hydroxyl groups in the hydrogel matrix. Nitrogen was present in much smaller amounts in GG-B, and this corresponds to the presence of proteins and the process of fermentation. GG-SB showed an increase in the carbon, hydrogen, nitrogen and sulphur content, consistent with the successful incorporation of seaweed at various concentrations in the gellan gum. The content of C increased from 30.05% for 5% GG-SB (30:1) to 31.67% for 20% w/v GG-SB and similarly, the H content went from 7.24% to 7.97%. The percentage of N also increased from 0.88% to 1.28% with the increase in seaweed concentration.

Table 2. The percentage content of C, H, N, and S in seaweed, GG-B and GG-SB.

Sample	Carbon %	Hydrogen %	Nitrogen %	Sulfur %
Seaweed	22.18	3.54	0.78	2.01
GG-B	28.77	7.24	0.13	0.00
5% w/v GG-SB (30:1)	30.05	7.66	0.88	1.09
5% w/v GG-SB (30:2)	30.69	7.78	0.95	1.38
5% w/v GG-SB (30:3)	30.91	7.76	1.03	1.19
10% w/v GG-SB (30:1)	31.03	7.90	1.12	1.02
10% w/v GG-SB (30:2)	31.17	7.87	1.16	1.41
15% w/v GG-SB	31.40	7.94	1.17	1.40
20% w/v GG-SB	31.67	7.97	1.28	1.98

3. Variable Pressure Scanning Electron Microscopy (VPSEM)

3.1. Surface morphology

The surface and cross-sectional morphology of the beads were observed through VPSEM. Figure 2 shows the surface morphology for GG beads and GG-SB at different concentrations under 1000× magnification. The VPSEM image showed that the GG-B had a smooth surface compared to the GG-SB. This is because GG-B without seaweed solution has a homogeneous polymeric network. The gellan gum loaded with seaweed solution, for example 5% w/v GG-SB with the ratio of 30 ml of gellan gum solution to 1 ml of seaweed as shown in Figure 2(b), showed an irregular surface. There were tiny round nodule-like structures that could be observed on the bead surface which could be due to the binding of the seaweed compound to the surface of gellan gum. Other than that, images of beads of gellan gum with high concentrations of seaweed solution, 10% to 20% w/v GG-SB, showed highly irregular surfaces which indicate that the seaweed is incorporated within the gellan gum polymer networks.

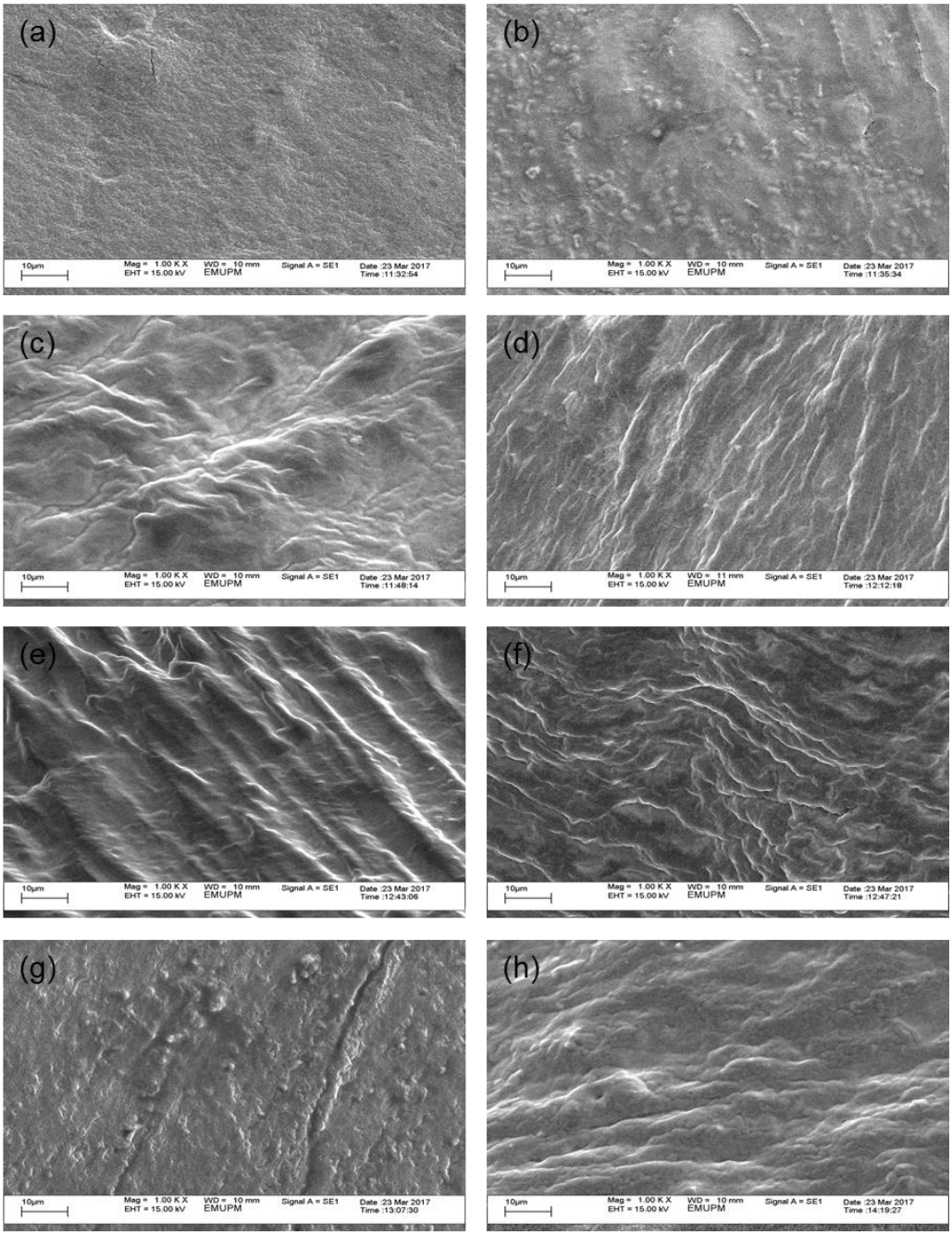

Figure 2. Surface morphology (1000x magnification) of (a) GG-B, (b) 5% GG-SB (30:1), (c) 5% GG-SB (30:2), (d) 5% GG-SB (30:3), (e) 10% GG-SB (30:1), (f) 10% GG-SB (30:2), (g) 15% GG-SB (30:1), (h) 20% GG-SB (30:1).

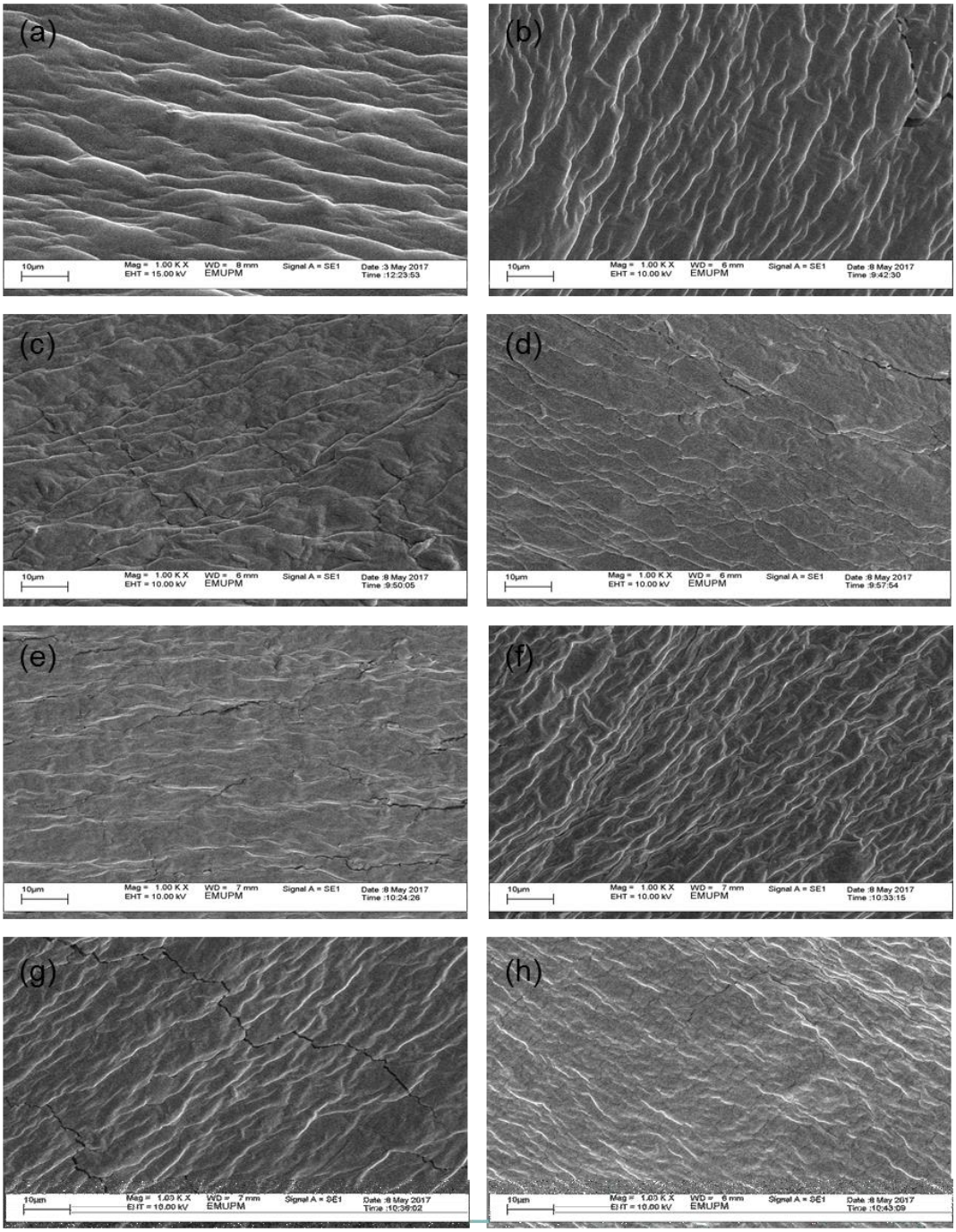

Figure 3. Cross-sectional morphology (1000x magnification) of (a) GG-B, (b) 5% GG-SB (30:1), (c) 5% GG-SB (30:2), (d) 5% GG-SB (30:3), (e) 10% GG-SB (30:1), (f) 10% GG-SB (30:2), (g) 15% GG-SB (30:1), (h) 20% GG-SB (30:1).

3.2. Cross–sectional morphology

The cross-sectional morphology of the beads was observed through VPSEM at 1000x magnification. Figure 3 presents images of the cross-sectional morphology of GG-B and GG-SB of different concentrations. More compact and dense layers were observed on the surface of the beads as the concentration of seaweed increased. The images also showed that the surface of the beads were highly irregular. The result obtained may be due to the binding of the seaweed in the layers of the gellan gum hydrogel.

4. Swelling Studies

GG-B and GG-SB at various concentrations were weighed and immersed in a solution with a pH of 7 resembling soil conditions over 6 days at room temperature, to study the swelling properties of the samples. Hydrogel is a hydrophilic polymer which can swell in aqueous solution due to its ability to absorb

and hold an amount of solvent [18]. Table 3 summarizes the swelling percentages of GG-B and GG-SB at various concentrations versus time (days).

From the graph in Figure 4, it can be observed that the swelling percentage increased with time. When the concentration of seaweed in the GG-SB was increased, the swelling percentage decreased compared to GG-B. GG-B showed a maximum swelling of 545.28% on the 6th day. 5% GG-SB with ratios of 30:1 and 30:2 also showed the same increases in swelling percentage as GG-B. For gellan gum loaded with seaweed, it was observed that the beads of 10% -20% GG-SB showed the maximum swelling in the first 4 or 5 days. After the 5th day, the results showed a decrease in swelling percentage as the beads de-swelled in solution. 20% GG-SB was found to have the lowest amount of swelling as the seaweed concentration increased. Solutions are able to enter the polymer network of hydrogels through diffusion and this causes the hydrogel to swell until it reaches the equilibrium state [19]. The diffusion rate of the solution depends on the volume or concentration of the guest species, the seaweed solution. High concentrations of seaweed cause the bead structure to become more rigid. This is because the seaweed covers the surface of the host, causing a decrease in the diffusion of solution into the beads [20].

Figure 5 shows the gellan gum bead, GG-B (a) before immersion, and (b) after immersion, in the pH 7 solution and the gellan gum loaded with seaweed, GG-SB (c) before immersion, and (d) after immersion in the pH 7 solution.

Table 3. Swelling percentages for GG-B and GG-SB of various concentrations versus time (days).

Time (Days)	Sample/Swelling percentage (%)							
	GG-B	5% (30:1)	5% (30:2)	5% (30:3)	10% (30:1)	10% (30:2)	15%	20%
1	389.86	286.84	272.06	241.38	191.94	160.71	142.63	121.94
2	450.11	289.47	283.82	244.83	200.00	182.14	180.84	130.56
3	508.70	380.39	388.24	300.00	280.11	264.12	191.58	144.44
4	511.59	444.23	415.59	362.07	350.23	296.43	210.23	200.00
5	515.94	440.39	407.35	365.52	314.52	300.00	289.47	225.00
6	545.28	490.11	405.26	362.07	300.00	292.86	250.68	159.72

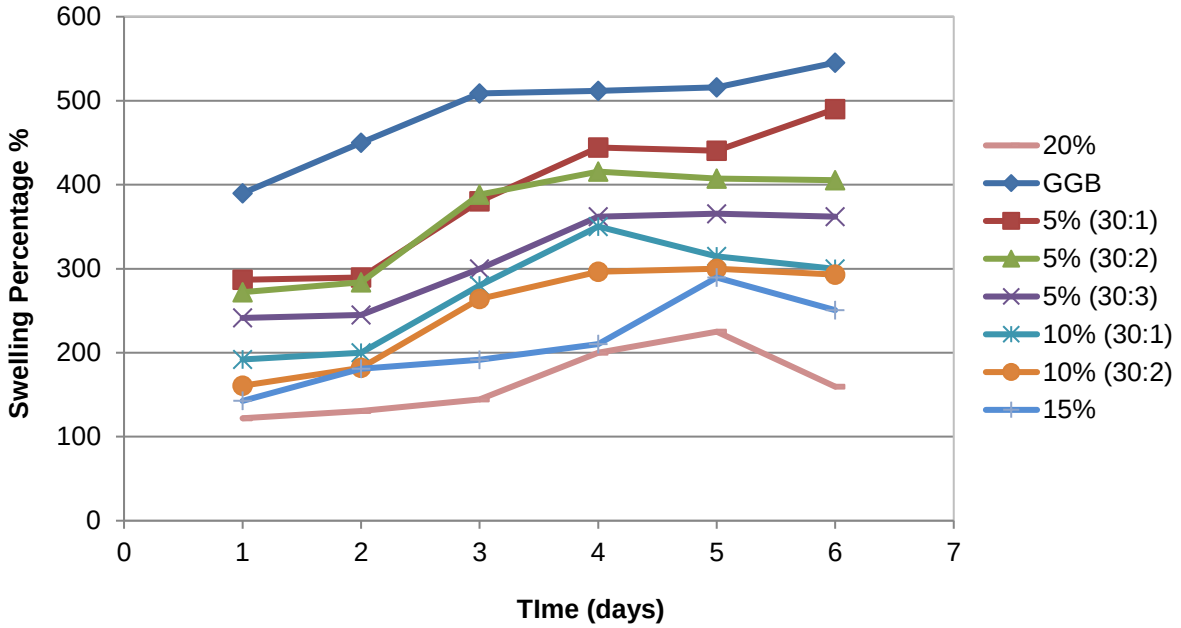

Figure 4. Variations of the swelling percentage against time (days).

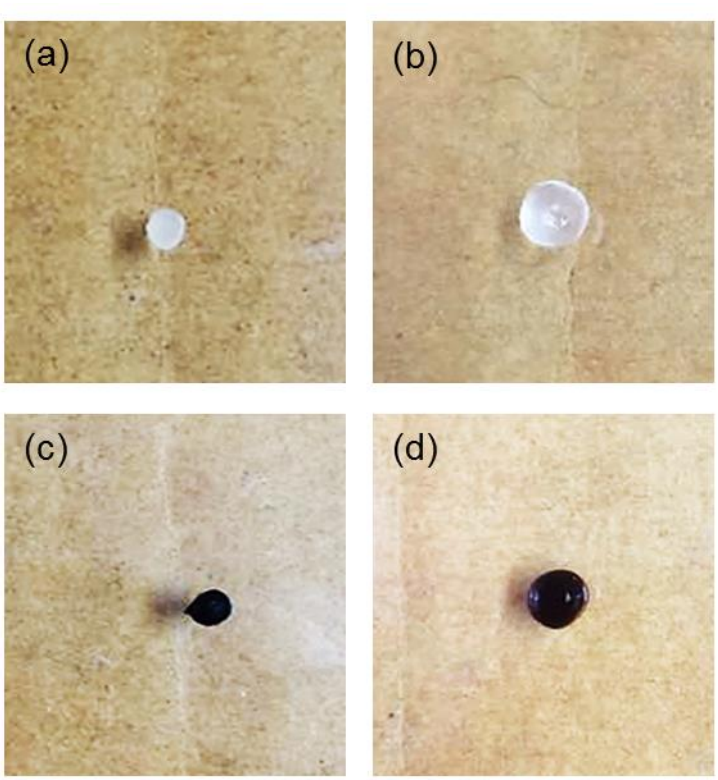

Figure 5. A gellan gum bead, GG-B (a) before immersion, (b) after immersion, and a gellan gum bead loaded with seaweed, GG-SB (c) before immersion, and (d) after immersion in the pH 7 solution.

Table 4. Degradation percentage of GG-B and GG-SB versus time (days).

Time (Days)	Sample/Swelling percentage (%)							
	GG-B	**5% (30:1)**	**5% (30:2)**	**5% (30:3)**	**10% (30:1)**	**10% (30:2)**	**15%**	**20%**
1	45.12	43.75	39.41	36.61	30.31	29.21	25.71	21.00
2	54.40	49.03	49.01	45.33	39.88	35.11	32.86	29.96
3	63.01	52.42	51.24	50.05	44.05	43.34	38.19	35.68
4	66.42	60.49	54.59	53.33	49.74	45.52	44.52	34.64
5	69.09	61.73	57.06	54.67	48.65	47.70	44.18	35.94
6	70.09	62.97	59.18	53.99	48.65	46.98	43.81	36.95
7	71.97	62.99	58.82	52.15	47.30	46.17	42.09	35.59
8	72.12	63.34	57.65	51.64	46.94	45.08	43.11	35.55
9	73.70	63.02	56.43	50.11	46.06	46.62	40.89	34.09
10	73.87	64.23	56.08	49.14	44.45	45.05	39.54	33.98
11	74.09	65.02	56.89	49.04	42.21	44.04	38.89	33.02
12	75.21	64.92	57.01	48.11	42.00	44.12	37.04	32.99

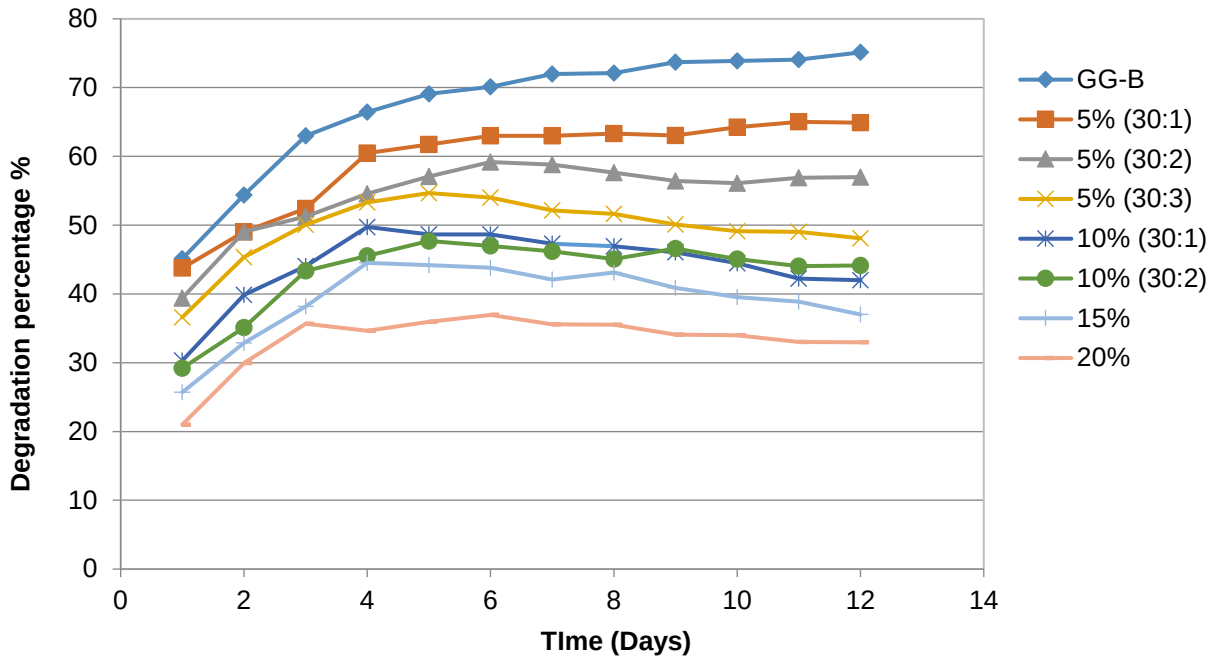

Figure 6. Graph of degradation percentage versus time.

5. Degradation Studies

Beads of each type were weighed and placed in sealed beakers at room temperature for 12 days to study their degradation properties. Table 4 shows the data for the degradation of the GG-B and GG-SB of different concentrations versus time. Based on the graph in Figure 6, the degradation of each type of bead showed a slight increase with time because the beads loaded with seaweed did not degrade as much as the gellan gum beads. The results indicate that GG-B had the highest degradation percentage (45.12% to 75.21%) compared to the other GG-SB. This is because the presence of hydroxyl groups in the gellan gum structure enables it to absorb moisture from the surroundings at room temperature, which leads to the increase in the weight of the GG-B [21]. However, the gellan gum beads with high concentrations of seaweed, 10% to 20% w/v GG-SB, showed a decrease in degradation percentage with time until they reached equilibrium state as the weight became constant. 20% GG-SB showed the lowest degradation percentage (21% to 36.95%), which slowly decreased to 32.99%. It was observed that high concentrations of seaweed limited the absorption of moisture into the bead, thus causing the GG-SB to degrade slowly. This result is caused by the formation of a rigid structure of GG-SB when the seaweed concentration is high. As both the GG-B and GG-SB underwent the degradation process, the size of the beads did not change much even though the weight of the beads increased.

CONCLUSION

In this study, gellan gum beads, GG-B, and gellan gum-seaweed beads, GG-SB, of various concentrations were successfully prepared. The gellan gum beads loaded with seaweed were prepared using different concentrations of seaweed solution in the range of 5% to 20% w/v. The study was performed to develop seaweed-based fertilizer hydrogel beads to create a controlled release formulation for fertilizer. FTIR spectra of the GG-B and GG-SB confirmed that seaweed of different concentrations were successfully loaded into the gellan gum hydrogel. This is because the FTIR spectra showed the presence of functional groups of both gellan gum hydrogel and seaweed. The CHNS analysis indicated the presence of both guest (seaweed) and host (gellan gum) species in the beads based on the content of C, H, N and S. From the VPSEM images, the surface and cross-sectional morphology of all the gellan gum-seaweed beads showed that the increasing seaweed concentrations resulted in highly irregular, dense and compact layers compared to the gellan gum hydrogel. From the swelling studies, it was clear that the swelling percentage decrease as the concentration of seaweed increased. The degradation test also showed a decrease in the degradation percentage with higher seaweed concentrations. These results indicate that high concentrations of seaweed solution could limit the absorption of water and slow the degradation process.

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