Fabrication and Swelling Study of *Ocimum Basilicum* L. Starch Based Hydrogels

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Abstract

Starch-based hydrogels have gained significant interest in the field of biomedicine. In the present work, pH-sensitive hydrogels have been fabricated using starch (OBS) extracted from Ocimum basilicum L. seeds and their swelling capacities have been studied. To achieve this, three different grades of hydrogels (OBS-g-(PAM-co-PAA)-1 to OBS-g-(PAM-co-PAA)-3) have been fabricated by copolymerizing acrylamide and acrylic acid onto the backbone of OBS through solution free radical polymerization technique. N-(Hydroxymethyl)acrylamide has been used as the cross-linker while potassium persulphate served as the initiator. The synthesized hydrogels have been characterized for their structural determination using FT-IR spectroscopy. Their swelling behavior has been investigated under varying pH (from 2 to 10) conditions to evaluate pH sensitivity.

Keywords: Starch, Hydrogel, Swelling capacity, pH sensitive.

1. Introduction

Nowadays, due to their hydrophilic nature, high water absorption capacity, and biomedical properties, the three-dimensional polymeric network hydrogels are highly considerable in the field of drug delivery [1,2]. They exhibit key properties such as elasticity, transparency, and ease of chemical modification. Their high water-retention capacity imparts a tissue-like similarity to natural biological structures. Moreover, they are injectable and responsive to physiological stimuli such as pH, temperature, and metabolite concentrations, enabling the controlled and timely release of nutrients and therapeutic agents [3,4,5]. Among various stimuli-responsive hydrogels, pH-sensitive hydrogels are the most extensively studied and widely reported, owing to their ability to respond to the pH variations in different biological environments. The incorporation of specific monomers during the copolymerization process can impart stimulus-responsive behavior to starch-based hydrogels, enhancing their functionality in targeted applications [6,7,8]. Polyacrylic acid (PAA) has been widely reported in the literature as a suitable polymer for the development of pH-responsive drug delivery systems, particularly in the form of hydrogels [9,10,11]. On the other hand, polyacrylamide (PAM) based hydrogels possess several advantageous properties, including high water absorption capacity, and have high swelling capacity as their swelling behavior is highly sensitive to environmental conditions as the presence of amide moieties which are sensitive to temperature [12]. Due to the complementary properties of acrylamide (AM) and acrylic acid (AA), their copolymerization into a hydrogel matrix imparts a combination of desirable features, including high hydration polarity, strong hydrogen bonding capacity, pronounced hydrophilicity, tunable swelling behavior, and enhanced pH-responsiveness [13,14]. These smart hydrogels when fabricated from natural polymers, like starch, can achieve biocompatible and biodegradable properties [15,16]. Basil seeds which are tiny, black, ellipsoid, and used in traditional desserts, are scientifically known as *Ocimum basilicum* L. These seeds also considered in treating many diseases like diarrhoea, colic ulcer, dyspepsia etc., in traditional medicinal methods [17,18,19]. The exocarp of basil seeds contains a layer of polysaccharides, which would rapidly expand into a gum-like substance, or the basil seed gum (BSG), when the seeds are drowned in water [20]. Solution free radical polymerization is one of the ways to synthesize starch-based hydrogels [21,22]. Xiao and Yang reported the synthesis of physically cross-linked starch-g-PVA hydrogels where they studied a comparative performance between PVA based hydrogels and starch-g-PVA and the degradation rate of the starch-g-PVA hydrogel was higher than that of PVA hydrogel [23].

In the present work, three different hydrogel grades have been synthesized using *Ocimum basilicum* L. seeds starch, AM and AA monomers, N-(Hydroxymethyl) acrylamide (NHMA) cross-linker [24] and potassium persulphate (KPS) as an initiator. The effects of the chemical crosslinking and pH on the swelling capacity of hydrogels have been investigated.

2. Experimental Section

2.1. Materials

N-(hydroxymethyl) acrylamide (>98.0%), acrylamide (98.5%), acrylic acid (98%), potassium persulphate (KPS) (98%), sodium hydroxide pellets, hydrochloric acid were purchased from Loba chemie, Mumbai, India. Ethanol (99.9%) was purchased from MSB Chemical Limited, Mumbai, India. Sodium phosphate monobasic dihydrate was procured from

Rankem Avantor Performance Materials India Limited, India. Sodium dihydrogen phosphate monohydrate was purchased from Merck Specialities Private Limited, India. *Ocimum basilicum* L. seeds were purchased from a local market Lucknow, India. All chemicals were used as received.

2.2. Extraction and purification of starch

For the extraction and purification of starch, first *Ocimum basilicum* L. seeds were rinsed with distilled water and then soaked (water/seeds ratio = 20:1) at room temperature [25]. After one day of soaking, desirably swollen seeds were taken in small amounts on sieve having 40 mesh size (with diameter of sieve 6 inch) and rubbed through cotton cloth against the sieve [26]. The obtained mucilage slurry was scrapped with the help of scrappers and transferred the slurry immediately into 96% ethanol for the precipitation and purification. One volume of seed gum was taken in at least three volumes of absolute ethanol and kept for 30 minutes and then filtration and drying at 40 °C were followed.

2.3. Synthesis of *Ocimum basilicum* L. starch-based hydrogels [OBS-g-(PAM-co-PAA)]

Fabrication of three different grades of hydrogel i.e., OBS-g-(PAM-co-PAA)-1 to OBS-g-(PAM-co-PAA)-3 was carried out at 80 °C. Initially, 0.5 g of starch was dispersed in 40 mL of distilled water at 80 °C and continuously stirred at 500 rpm for 30 minutes. After obtaining a homogenous solution, addition of comonomers viz., 0.375 g of AM and 0.356 mL of AA was done. Cross-linker (NHMA) was added after the addition of comonomers, in varied amounts as mentioned in **Table 1**. Afterwards 20 mg of KPS was added to initiate the polymerization. To ensure an inert environment, nitrogen gas was continuously purged throughout the entire procedure. The reaction mixture was allowed to stir for one hour and then cooled down to room temperature while adjusting the pH to 8 by the addition of 1 N NaOH solution. Then the viscous mixture was poured into 200 mL of ethanol and kept for 24 hours. Thus, obtained hydrogel was vacuum filtered, dried at 70 °C in hot air vacuum oven and then stored in an air-tight container. The percentage of yield of these hydrogels was calculated by the following equation (1). and the results are reported in **Table 1**.

$$\text{Yield (\%)} = [W_h/(W_c)] \times 100. \qquad (1).$$

where, W_h denotes the weight of hydrogel, and W_c represents the total weight of starch and monomers employed in the hydrogel synthesis.

2.4. FT-IR spectral analysis

The FT-IR spectra of OBS and OBS-g-(PAM-co-PAA) were recorded by Perkin Elmer FT-IR spectrometer (model spectrum-2) in solid state using KBr pellet method. The IR spectra were recorded within the range of 4000 - 500 cm^{-1}.

2.5. Swelling capacity measurements

All three grades of hydrogels were weighed simultaneously (50 mg each) and placed separately in 3 mL of aqueous solutions of phosphate buffer solution (PBS) with pH 2, 3, 4, 5, 6, 7, 8, 9, and 10 for 24 hours. The pH was adjusted using 0.1 N HCl in distilled water for values below 6, and 0.1 N NaOH for values above 8. After 24 h of soaking and swelling, the samples were filtered and weighed. The changes occurred in hydrogels, at pH 2, after swelling are shown in **Figure 1**. The swelling percentages of hydrogels were determined by using equation (2) [27] and the results are tabulated in **Table 2**.

$$\text{Swelling percentage} = [(W_t - W_d)/W_d] \times 100 \qquad (2)$$

where, W_t is the mass of hydrogel at time t and W_d stands for dry gel mass.

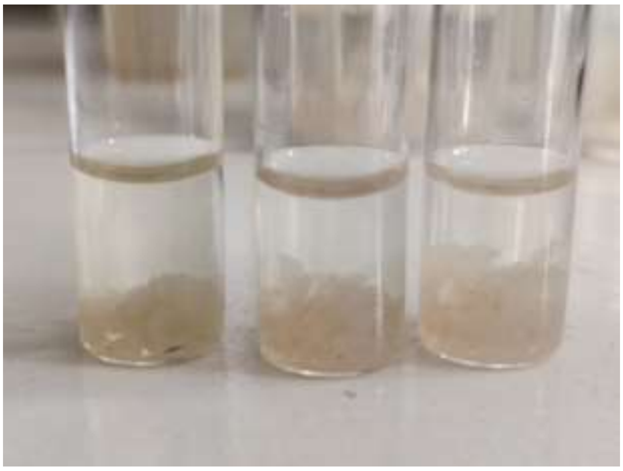

Figure 1. Swollen hydrogels at pH 2 while studying the effect of pH on swelling behavior.

3. Results and Discussion

3.1. Synthesis of OBS-g-(PAM-co-PAA) hydrogels

Three different grades of hydrogel were synthesized through graft copolymerization of AM and AA comonomers onto OBS starch and the synthesis details are reported in **Table 1**. The polymerization mechanism involves generation of anionic free radicals by the decomposition of KPS at 80 °C. The generated radicals abstract hydrogen from the hydroxyl groups of starch, thereby producing alkoxy radicals on its backbone. These reactive starch radical sites are surrounded by monomeric molecules of AM and AA leading to initiation followed by propagation where these become free radical donors to other molecules. NHMA, serving as the cross-linking agent, came into the role by crosslinking through its hydroxymethyl group onto free radical sites generated earlier. This occurred via two simultaneous process of vinyl polymerization and post-polymerization condensation, ultimately leading to formation of 3-D networked hydrogel. The mechanism of graft copolymerization is depicted in **Figure 2**.

Table 1. Synthesis details of different grades of OBS-g-(PAM-co-PAA) hydrogels

Starch (g)	Acrylamide (g)	Acrylic acid (mL)	NHMA (g)	KPS (mg)	Yield (%)
0.5	0.375	0.356	0.075	20	96
0.5	0.375	0.356	0.1	20	84
0.5	0.375	0.356	0.125	20	91.8

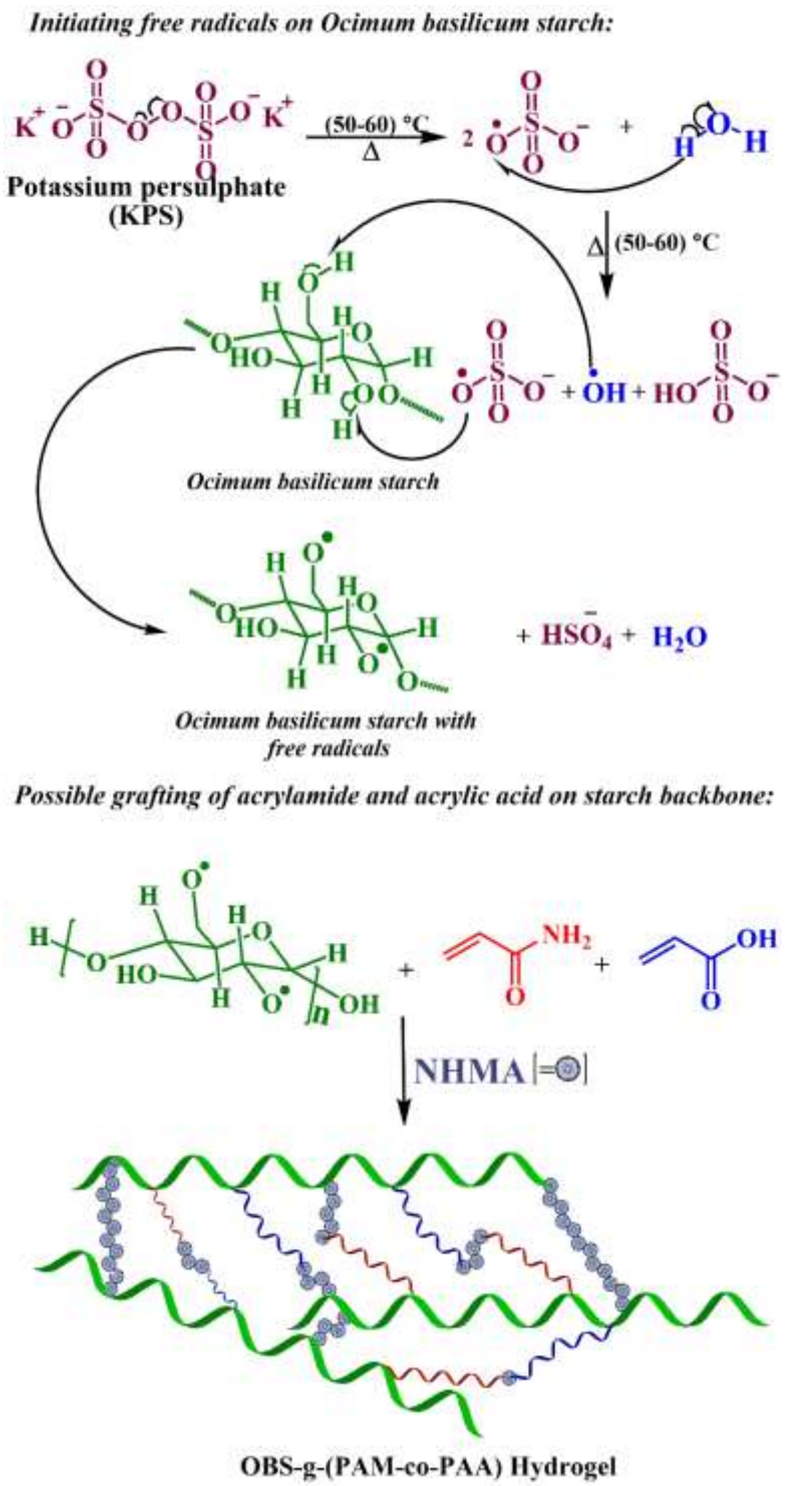

Figure 2. Mechanistic view of synthesized OBS-g-(PAM-co-PAA) hydrogel

3.2. FT-IR analysis

As depicted in **Figure 3,** the FT-IR spectrum of OBS showed a broad band at 3436 cm^{-1} which is a characteristic signal of –OH stretching vibration. Its other characteristic stretching signals of C−H and C−O were observed at 2926 and 1418 cm^{-1}, respectively. On the other hand, in case of the hydrogel OBS-g-(PAM-co-PAA), stretching bands of hydroxyl group of OBS and N−H of amide group of PAM overlapped with each other and a broad band was observed at 3347 cm^{-1}. The appearance of two signals at 1660 cm^{-1} and 1549 cm^{-1} were attributed to C=O stretching and N−H bending vibrations respectively. The absorption bands at 1718, 1589 and 1410 cm^{-1} are owing to the –COOH stretching, $–COO^{-}$ asymmetric stretching and $–COO^{-}$ symmetric stretching, respectively. Thus, the presence of the characteristic signals confirms the successful grafting of PAM chains and PAA chains onto OBS.

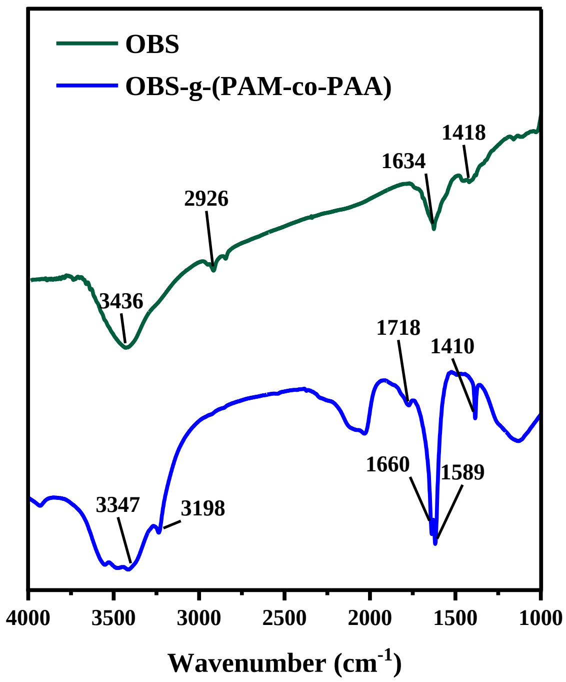

Figure 3. FT-IR spectra of OBS and OBS-g-(PAM-co-PAA).

3.3. Swelling behavior of the hydrogels

The fabricated polymeric hydrogels exhibit a favourable property of swelling. PAM based hydrogels are the networks of hydrophilic polymer chains that can absorb a significant amount of water, swelling to many times their original volume, and this swelling behavior is highly sensitive to environmental conditions. The pH-responsive behavior of all three grades of OBS-g-(PAM-co-PAA) hydrogels was evaluated across a pH range of 2 to 10. The variation in swelling percentage was determined by using equation (2). after immersing the hydrogel in aqueous solutions of PBS with pH 2, 3, 4, 5, 6, 7, 8, 9, and 10 for 24 hours and the results are tabulated in **Table 2**.

Table 2. The swelling percentage results of hydrogels after 24 hours.

Grade	Swelling percentage (%)								
	pH 2	**pH 3**	**pH 4**	**pH 5**	**pH 6**	**pH 7**	**pH 8**	**pH 9**	**pH 10**
OBS-g-(PAM-co-PAA)-1	886	890	882	898	1086	1118	1204	1062	1040
OBS-g-(PAM-co-PAA)-2	774	816	824	870	1030	988	1136	970	988
OBS-g-(PAM-co-PAA)-3	862	858	792	606	1024	812	852	870	818

The pH-responsive behavior of all three grades of OBS-g-(PAM-co-PAA) hydrogels was evaluated across a pH range of 2 to 10. Their swelling behavior indicated that all three grades were sensitive to the pH of the medium. The results show that OBS-g-(PAM-co-PAA)-1 and OBS-g-(PAM-co-PAA)-2 showed maximum swelling at pH 8, with swelling percentages of 1204% and 1136%, respectively. In these grades, in acrylic acid carboxylic acid (–COOH) groups are deprotonated ($–COO^{-}$) at higher pH and create electrostatic swelling repulsions [28] which led to more swelling at pH 8. But at pH 9 and 10, swelling saturation was attained and osmotic pressure gradient got flatten, Na^{+} ions increase and start shielding COO^{-} ions because of which electrostatic repulsion stops and swelling decreases. In OBS, structural rearrangement takes place in hydroxyl and carbonyl groups, due to which hydrogel network becomes less porous and reduces water uptake. Meanwhile there is no hydrolysis, or scission takes place in high alkaline medium due to which hydrogel network becomes rigid and swelling reduces [29,30]. On the other hand, at a lower pH, in acrylamide $–CONH_2$ groups are protonated to create $NH_3^{+}–NH_3^{+}$ electrostatic repulsion swelling, but its swelling will be decreased by the presence of chloride ions [4].

Further, as per the role of crosslinker is concerned, at pH 8 a maximum swelling percentage is seen in OBS-g-(PAM-co-PAA)-1 (1204%) when compared with OBS-g-(PAM-co-PAA)-2 (1136%) and OBS-g-(PAM-co-PAA)-3 (852%). This shows that the swelling capacity of chemically crosslinked hydrogels does have an effect of crosslinking on them. Generally, in hydrogels lower swelling capacity exhibits higher cross linking density, as increase in density leads to formation of shorter chain crosslinks. While swelling, these shorter chains exhibit high elasticity force which becomes equal to osmotic forces and cause lower relative swelling. It explains that OBS-g-(PAM-co-PAA)-1 have less shorter chain crosslinks, due to which it has higher swelling capacity as, cross-linker NHMA amount is low (0.075 g) as detailed in **Table 1**.

4. Conclusions

In this research, OBS starch based pH-responsive hydrogels were prepared by grafting PAM and PAA through the process of graft copolymerization by using NHMA as a cross-linker and KPS as an initiator. As both monomeric units and cross-linker are hydrophilic in nature, it showed a crucial influence on the swelling properties of the hydrogels. Swelling capacity was more affected by the different pH environments and crosslinking density as more swelling was observed in pH 8 of 1204% in OBS-g-(PAM-co-PAA)-1 but it was also observed that crosslinking density also affects swelling capacity as it increases swelling decreases and as it decreases swelling increases.

Acknowledgement

The authors gratefully acknowledge UGC, New Delhi, Government of India for the financial support through UGC-BSR Start-up Research Grant.

Conflicts of Interest

The authors declare no conflict of interest.

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