Submicron Particles of Double Network Alginate/Polyacrylamide Hydrogels for Drug Delivery of 5-Fluorouracil

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ABSTRACT: *The purpose of this study was to prepare a Double Network (DN) hydrogel made with sodium-alginate (Na-alg) natural polysaccharide and polyacrylamide (PAM) chains that target control release of anticancer drug. To this aim, the entitled submicron particles of double network hydrogels exhibit exceptional properties. A two-step strategy was used to obtain alginate/PAM hydrogels crosslinked by Ca^{2+} cations and* N, N`*-methylenebisacrylamide (MBA) as different types of crosslinker. Particles of DN alginate / PAM were employed for entrapping and releasing the model drug 5-fluorouracil (5-FU) as an anticancer drug. Scanning Electron Microscopy (SEM), Fourier Transform InfraRed (FT-IR) spectroscopy, ThermoGravimetric Analysis (TGA), Dynamic Light Scattering (DLS), swelling properties, drug entrapment efficiency, and drug release studies were also done. The particles showed porous structure, good swelling ability, and aqueous dispersibility with a size in the range of 280 nm. The resulting hydrogel was subsequently loaded with 5-FU and released patterns carried in 7.4 pH and 5.8 pH at a temperature of 37 ºC. The controlled drug release behavior was noticed and the finding of the study suggested that the alginate/PAM double network hydrogel is a promising carrier for 5-FU delivery.*

KEYWORDS: *Double network hydrogel, Drug delivery system, 5-fluorouracil, Sodium alginate polysaccharide, Polyacrylamide.*

INTRODUCTION

Despite the development of medical science in various fields, cancer treatment is one of the greatest challenges in the world. Numerous methods have been used to treat cancer such as chemotherapy, radiation, and surgical intervention, but cancer yet remains one of the deadliest diseases that affect about 10 million people each year [1]. With the complete destruction of healthy cells and systemic toxicity, the quality of life for cancer patients decreases sharply. Therapeutic moieties inefficacy to obtain selectivity of appropriate target tissues with the least damage has been studied by scientists [2]. The advancement of science and technology in cancer treatment fields has revealed an exquisite route in the growth of medical sciences like drug delivery systems, leading to the development of a novel drug carrier [3].

Hydrogels are wet, soft, and flexible materials. They are usually composed of individual hydrophilic polymer chains that are interconnected through chemical and

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1021-9986/2021/5/1386-1394 9/$/5.09

physical interactions. The ability to contain a large amount of water inside the network and three-dimensional structure are important features of this type of polymer. Hydrogels have potential applications in many fields such as implants, superabsorbents, microfluidics, soft contact lenses, and the materials science domain. Besides their wide variety of applicability, they have received much attention for being used in tissue engineering and drug delivery because of their stimuli-responsive property [4–6]. However, as one of the disadvantages of hydrogels, most of them suffer from a lack of mechanical strength. Many efforts have been made in recent years to solve this problem and several hydrogels with exceptional mechanical properties have been successfully developed [7,8]. As one of these types of hydrogels, double network hydrogels which are made by combining a soft and ductile polymer network with a rigid and brittle polymer network show excellent mechanical performance like high modulus and toughness [9,10]. Because of the characteristic properties of double network hydrogels such as favorable mechanical strength, high swelling ability, and porosity, they can act as a unique candidate material for use in drug delivery systems. In addition to the polymer network structure, the polymer type also plays an important role in the performance of the drug delivery system. Recently, natural polymers are mostly used in drug release systems due to their excellent biocompatibility, biodegradation, and nontoxicity upon in vivo administration. Among the natural polymers, chitosan, sodium alginate, cellulose derivatives, and guar gum are extensively employed in drug delivery systems. In general, hydrogels based on polysaccharides present the advantages of beings similar to the external extracellular matrix so there are many hydrogels based on polysaccharide, which have been used in the design of drug delivery systems for different anticancer drugs including 5-FU, by different routes of administration (injection or oral) [11–14]. In this work, sodium-alginate that is an anionic polysaccharide, crosslinked tightly with calcium chloride ($CaCl_2$) to form rigid and brittle polyelectrolyte structure, and Acrylamide Monomer (AM) is polymerized and crosslinked by using ammonium persulfate (APS) as an initiator and N, N -Methylenebisacrylamide (MBA) as a crosslinker to form a loosely cross-linked, flexible network inside the first brittle structure. The obtained hydrogel so-called double network hydrogel is immersed in 5-FU solution and stirred vigorously to achieve the anticancer drug carrier. 5-FU, a fluorinated pyrimidine, is one of the most commonly used drugs to treat cancer. It is most often used in combination with other cancer drugs to treat many types of cancer including colorectal cancer, breast cancer, skin cancer, and stomach cancer. 5-FU acts in several ways but principally works as a thymidylate synthase inhibitor. Interrupting the action of this enzyme blocks synthesis of the pyrimidine thymidine, which is a nucleoside required for DNA replication, hence, preventing the growth of abnormal cells [15–17]. In this study, parameters like %encapsulation efficiency and drug loading of 5-FU /alginate/PAM double network hydrogel have been also studied.

EXPERIMENTAL SECTION

Materials and methods

Calcium chloride, sodium alginate, ammonium persulfate, N, N'-methylenebisacrylamide, and acrylamide were purchased from commercial sources with the best quality.

FT-IR spectral measurements were performed using a Bruker spectrophotometer (Tensor 27, Germany) to confirm the crosslinking of the alginate/PAM matrix. The alginate/PAM particles were finely ground with KBr to prepare the pellets and spectra were scanned between 600 – 4000 cm^{-1}.

The particle size of the particles was measured by dynamic light scattering (DLS, HORIBA SZ-100, France). About 100 mg of the particles were immersed in 100 mL distilled water and sonicated to avoid agglomeration of particles during measurement.

ThermoGravimetric Analysis (TGA) of particles was recorded using SDT Q600, USA. A 5mg sample was placed in the aluminum sample holder and heated at a ramp of 10 ºC/min to 650 ºC under a nitrogen environment.

The SEM images of double network hydrogel were recorded using a JSM 6400 scanning electron microscope.

Synthesis of alginate-polyacrylamide double network hydrogel (Alginate/PAM)

Alginate/PAM double network hydrogel was synthesized in two steps. In brief, in the first step, all ingredients (AM, 0.35 mol, 25 g; MBA, 1.68 mmol, 0.26 g; APS, 4.76 mmol, 1.08 g; Na-alg, 1g) except $CaCl_2$ are dissolved in distilled water (100 mL) to obtain a homogeneous and transparent solution (Solution 1). Passing nitrogen gas for 10 min

degasses the Solution 1. This mixture is stirred for 3 h at 60 ºC. In the second step, for making fine hydrogel particles, an aqueous solution (solution 2) containing $CaCl_2$ (1 mmol, 0.15 g, 100 mL) is prepared and the Na-alg/PAM (Solution 1) is added dropwise and slowly to this solution and the mixture was stirred for 3 h. The hydrogel formed is centrifuged, washed repeatedly with water, ethanol, and dried. The dried hydrogels of known weights were immersed in distilled water as well as in different pH buffers (pH 5.8 – pH7.4) at room temperature. At selected time intervals (1h), the swollen hydrogels were removed, blotted quickly and carefully with absorbent paper, and then weighed. The following Equation (1) was used to determine water absorbency.

$$\text{Water absorbency}(\%) = \left[\frac{Ws - Wg}{Wg}\right] \quad (1)$$

Where Ws and Wg represent the weights of swollen gel and dry gel, respectively [18].

Estimation of drug loading and encapsulation efficiency

The specific amount of dried particles (30 mg) and drug (3 mg) were dissolved in distilled water (150 mL) and vigorously stirred for 24 h at room temperature. 5-FU loaded alginate/PAM particles were separated by centrifugation. The Drug Loading (DL) and entrapment efficiency (EE) of 5-FU in alginate/PAM double network hydrogel were determined using the following Eqs. (2, 3):

$$DL(\%) = \left(\frac{\text{Weight of drug in particles}}{\text{Weight of particles}}\right) \times 100 \quad (2)$$

$$EE(\%) = \left(\frac{\text{Weight of drug in particles}}{\text{Initial weight of drug}}\right) \times 100 \quad (3)$$

The weight of the drug in particles was obtained by subtracting the initial concentration of the drug and the supernatant [19].

Drug release

The drug released was performed by immersing a dialysis bag containing 5-FU /alginate/PAM particles (20 mg) in phosphate buffer solutions pH 5.8 and pH 7.4 (50 mL) at 37 ºC. At specific time intervals, 1 mL of the released buffer was aspirated and replaced by a fresh phosphate buffer solution. The concentration of drug was determined using a UV-Vis spectrophotometer (Perkin Elmer, Lambda 850, USA). The calibration curve was plotted using 4 standards of different drug concentrations at pH 5.8 and 7.4 (Fig. 1). The calibration curves show very good linearity (R^2 =0.9995 at pH 7.4 and R^2 =0.9960 at pH 5.8) in the range of drug concentrations (0-20 ppm).

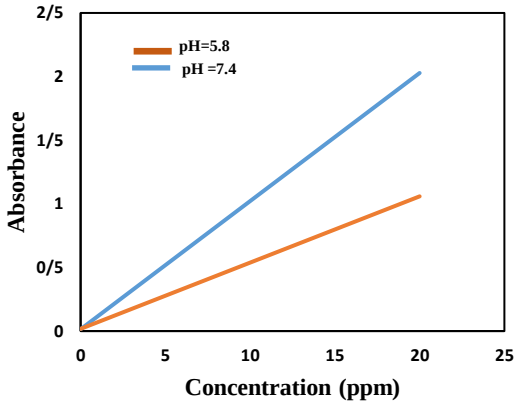

Fig. 1: Calbration curves of 5-FU at pH 5.8 and 7.4.

RESULTS AND DISCUSSION

Synthesis

In the first step, a Na-alg/PAM hydrogel is synthesized in which, for the first network, the PAM is crosslinked by covalent bonds in a diluted solution and Na-alginate is well-dispersed but not crosslinked. Alginate is a linear copolymer of α- L-guluronic acid (G unit) and β-D-mannuronic acid (M unit). The monovalent Na^+ cations do not cross-link alginate, whereas multivalent cations can crosslink alginate by simultaneously associating with carboxylic groups on different units of alginate chains [20]. In the second step, to make the second network, the Na-alg/PAM hydrogel is immersed in an aqueous solution of $CaCl_2$ resulting in a homogeneous and transparent alginate/PAM double network hydrogel crosslinked by Ca^{2+} cations (Fig. 2).

Characterization

Fig. 3 displays the FT-IR spectra of Na-alginate, acrylamide, alginate/PAM hydrogel, and drug-loaded alginate/PAM particles. The spectrum of Na-alginate shows a characteristic broad peak appearing at 3300 cm^{-1}, which corresponds to OH stretching vibrations of Na-alginate. The absorption bands at 1600 and 1410 cm^{-1} are assigned to asymmetric and symmetric stretching peaks of carboxylate salt groups respectively. The bands appearing

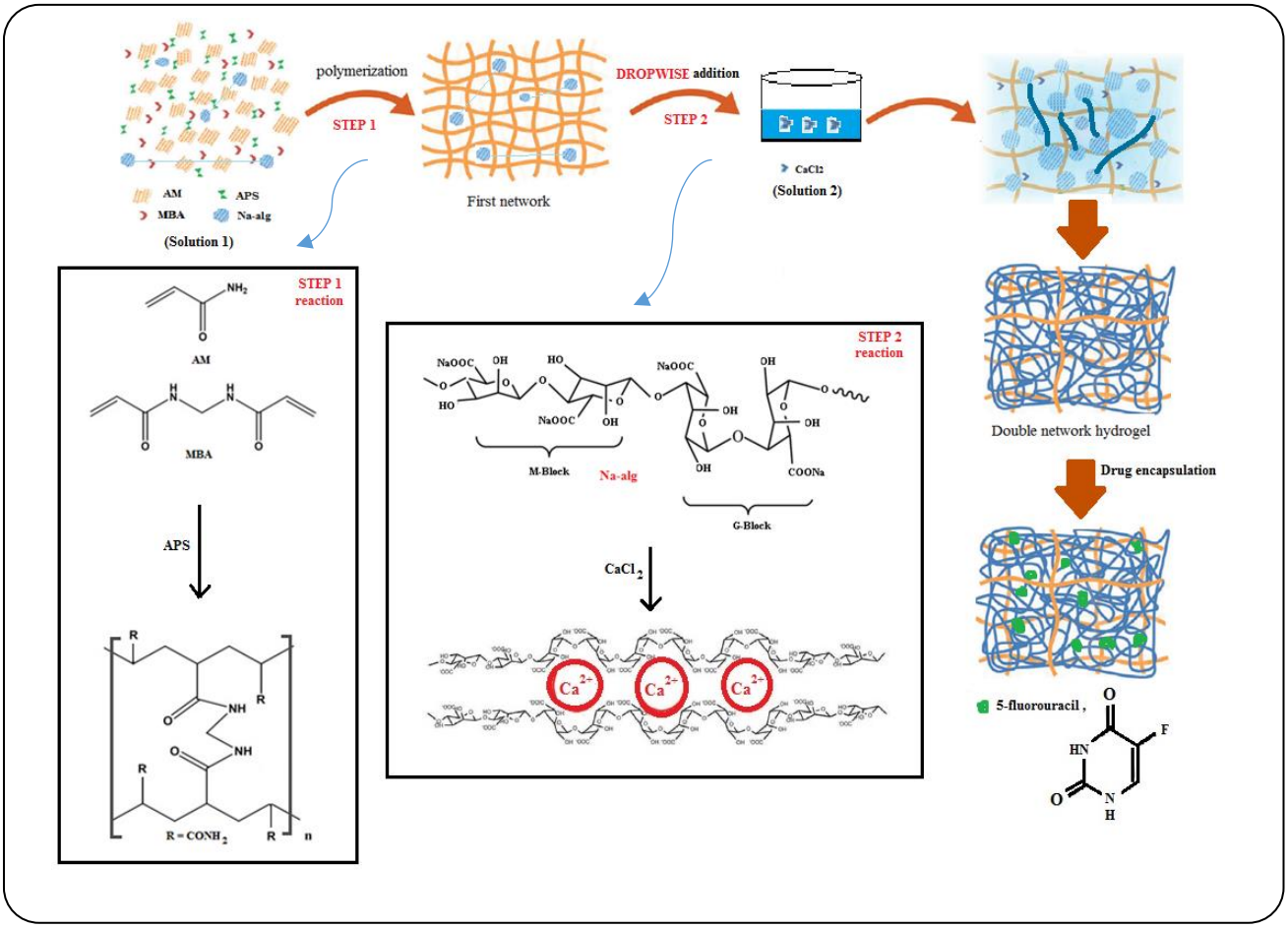

Fig. 2: Schematic illustration and mechanism of different steps for preparation of 5-FU /alginate/PAM double network hydrogel

at 1298 (C-O), 1125 (C-C), 1082 (C-O), 1027 (C-O-C), and 925 (C-O) cm^{-1} are attributed to saccharide structure. The FT-IR spectrum of acrylamide confirms the existence of carbonyl, and amide functionalities indicated by the absorption peaks at 1670, 3250, and 3320 cm^{-1}, respectively. Alginate/PAM double network hydrogel shows bands at 1675 and 1704 cm^{-1} consisting of carbonyl groups of PAM and carboxylate salt groups of alginate (Fig. 4c). When PAM was incorporated with the alginate network, COO^- stretching peak in alginate at 1410 and 1600 cm^{-1} shifted to 1598 and 1704 cm^{-1} in alginate/PAM. This result indicated the interaction between two polymer networks. The 5-FU peaks at 1550 and 1520 cm^{-1} are from the C=C stretches of the heterocyclic ring. 5-FU displays similar peaks in drug-loaded alginate/PAM double network hydrogel (Fig. 4d) which indicates successful loading of 5-FU on alginate/PAM double network hydrogel [21–24].

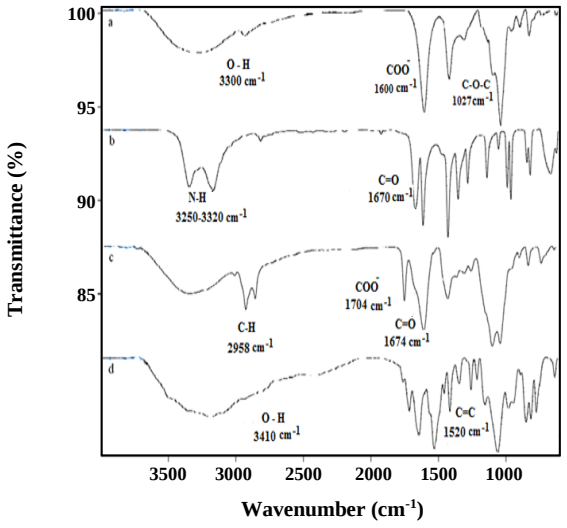

Fig. 3: FT-IR spectra of (a) Na-alginate, (b) acrylamide, (C) alginate/PAM double network hydrogel, and (d) drug-loaded alginate/PAM double network hydrogel.

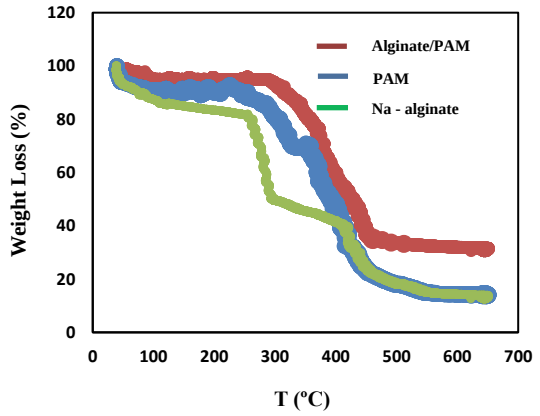

Fig. 4: TGA analysis of alginate/PAM double network hydrogel, PAM, Na - alginate.

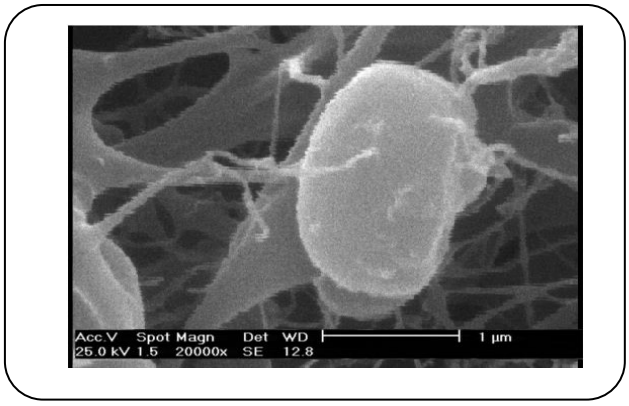

Fig. 5: SEM photograph of the alginate/PAM double network hydrogel.

ThermoGravimetric Analysis (TGA) of Na-alginate, PAM, and alginate/PAM double network hydrogel are displayed in Fig. 4. Na-alginate is a natural biocompatible polymer that contains sugar units. This polysaccharide lost moisture below 100 °C. Because of fragmentation and chains rupture, the most degradation of Na–alginate occurred between 220-380 °C. Na-alginate chain decomposition at 540 °C is 84.4%. Although PAM is a chemical origin polymer which produces by the chemical reaction of acrylamide monomer, MBA and APS but TGA analysis depicted the thermostability in increased order of PAM (540 °C, remained weight 15.24 %) < alginate/PAM hydrogel (550 °C, remained weight 32.29 %), verifying the existence of interaction between alginate, calcium ions and PAM chains in alginate/PAM double network hydrogel [25].

The surface morphology of the freeze-dried alginate/PAM double network gel is shown in Fig. 5. It can be seen that the gel has a highly porous network and spider's web structure which indicates its high affinity for water. By increasing the pore size and homogeneity of the prepared network, the hydrogel swelling was expected to increase.

The synthesized DN hydrogel did not show a particle settling after 20 days. Therefore, it was assumed that the particle size was micron/nanoscale. Particle size and size distribution have been analyzed by the dynamic light scattering technique. The result of the mean diameter of hydrogel produced by taking different amounts of crosslinking agent (MBA) are included in Table 1.

These results suggest that as the extent of crosslinking increases, the mean diameter decreased. This is attributed to the fact that with an increasing amount of crosslinking agent (MBA) in the matrix, shrinkage of particles occurred, thereby reducing their size [26,27]. On the other hand, with an increasing amount of AM, the size of the particles increased. This can be explained on the basis of the water-uptake capacity of PAM. For formulation code 3, the narrow size distribution of particles was observed with particles ranging in size from 190 to 330 nm (Fig. 6).

Water absorbency

Alginate/PAM double network hydrogel shows a pH-dependent swelling behavior. Therefore, the effect of pH 7.4 and pH 5.8 was investigated on water swelling, as shown in Table 2.

The result can be explained according to the following: The swelling behavior of alginate/PAM double network gel has a direct relationship with the composition of the hydrogel and both networks are definitely involved in the water absorption, especially when one of the constituent networks consists of ionizable groups. In this study, alginate is ionic and most of the carboxylate groups on its chain are converted to carboxylic acid groups at low pH of external solution, and the ratio of the nonionized (COOH) groups to the ionized ones (COO^-) along the polymer chain increases. This increases the possibility of formation of hydrogen bonds; subsequently, the hydrophilicity of the hydrogel decrease. At higher pH solution, the repulsion between the carboxylate groups, which are responsible for the higher swelling of the hydrogel, becomes prevalent and it loads to the higher swelling. The water absorption of the polyacrylamide will not be very affected by the change of pH, because this network does not have good ionizable chemical groups that can influence the swelling process [28].

Table 1: Result of mean particle size of different formulations.

Formulation code	AM		MBA		Particle size(nm)
	mol	g	mmol	g	
1	0.35	25	1.00	0.15	340
2	0.35	25	1.68	0.26	328
3	0.35	25	2.35	0.36	276
4	0.42	30	1.68	0.26	284
5	0.50	35	1.68	0.26	289

** Na-alg = 1 g; APS = 4.76 mmol, 1.08 g; $CaCl_2$ = 1 mmol, 0.15 g; distilled water = 100 mL*

Table 2: Water swelling and drug release profile of alginate/PAM hydrogel in different pH.

pH	Water absorbancy (g/g)	n	k	R
5.8	73	0.44	3.93	0.99
7.4	89	0.36	8.54	0.99

**water absorbancy of PAM: 31 g/g*

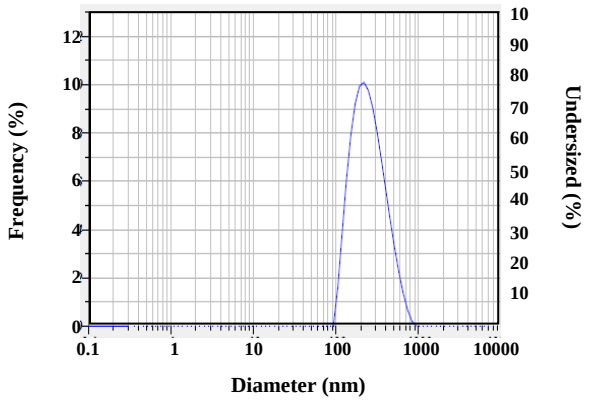

Fig. 6: Particle size distribution curve for alginate/PAM double network particles.

Encapsulation efficiency

The equilibrium swelling method was used for loading 5-FU into hydrogel networks. The hydrogels were allowed to swell and stir in the drug solution of known concentration for 24 h at room temperature. 5-FU molecules entrap inside hydrogel structure by hydrogen bonding, surface adsorption, and other types of interactions [29]. For 5-FU, encapsulation efficiency and drug-loading capacities were 21.5% and 2.1%, respectively.

Drug release

The release of 5-FU from hydrogel was assayed in phosphate-buffered saline pH 5.8 (pH tumor of the interstitial and endosome content) and 7.4 (pH of blood plasma) [30]. A sample of drug-loaded hydrogel was placed in a particular buffer and stirred vigorously for 120 h at 37 ºC to extract the drug from the hydrogel. Aliquot samples were taken after selected times. The solution was filtered and assayed by UV spectrophotometer at a fixed λ_{max} value of 267nm. The controlled release behaviors of 5-FU from alginate/PAM double network hydrogel at pH 5.8 and 7.4 are shown in Fig. 7, respectively. At pH 7.4, the release of 5-FU is higher than pH 5.8. This can be described based on a higher degree of water swelling as mentioned above due to carboxylic group's ionization in the network at pH 7.4.

The drug release kinetics data obtained from the hydrogel were analyzed by fitting to the following equation (4) proposed by Ritger and Peppas as follows:

$$\frac{M_t}{M_\infty} = k t^n \qquad (4)$$

Where M_t/M_∞ represents the fraction of drug released at time *t*, *k* is constant related to the properties of the drug delivery system and n is an empirical parameter characterizing the drug release mechanism. If the value of n is >0.5, the drug release process follows the non-Fickian or anomalous diffusion mechanism. When the value of n is <0.5, it indicates that the drug release process follows the Fickian diffusion mechanism. The values of n calculated according to the above method were found to be 0.44 and

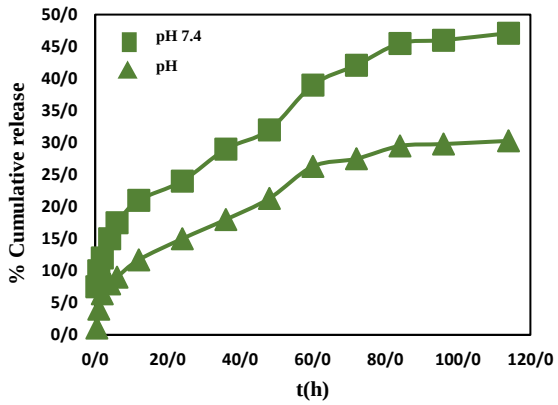

Fig. 7: Drug release of 5-FU in phosphate-buffered saline.

0.36 at pH 5.8 and 7.4, respectively (Table 2). Thus the alginate/PAM double network hydrogel follows the Fickian diffusion-controlled release mechanism at pH 5.8 and 7.4. In general, solute diffusion, polymeric matrix swelling, and material degradation are suggested to be the main forces for solute transport from drug-containing polymeric matrices. Specifically, Fick's law of diffusion provides the fundament for a description of solute transport from polymeric matrices. Fickian diffusion refers to the solute transport process in which the polymer relaxation time is much greater than the characteristic solvent diffusion time [31].

CONCLUSIONS

In This study, the novel double network hydrogel particles were successfully prepared using a facile two-step method to synthesize alginate/PAM double network hydrogel with excellent properties. FT-IR and TGA have suggested successful results for synthesized hydrogel. SEM of this type of hydrogel showed a highly porous structure. In this project, for obtaining submicron particles, we tried to add PAM containing alginate solution to Ca^{2+} ions, very slowly and dropwise. On the other hand, this way of adding calcium will make homogeneous hydrogel. By contrast, the fast addition of Ca^{2+} can agglomerate the alginate macromolecules, and subsequently, it causes the failure of making proper homogeneous hydrogel particles. DLS revealed a narrow size distribution of particles with a size range from 190 to 330 nm. In addition to the properties mentioned, alginate/PAM double network hydrogel showed good swelling ability, thus we decided to use it as a drug carrier in drug delivery. 5-FU, an anticancer drug was encapsulated with alginate/PAM double network hydrogel and release patterns carried in pH 5.8 and 7.4 at temperature 37 ºC.

Received : Dec. 25, 2019 ; Accepted : May 4, 2020

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