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Water Soluble Cationic Xylan-Alginate Polymer as Packaging Retardant-Release Peptide Drug Delivery

Saber Ibrahim[1], Maha Sultan*[1], Zenat Adeeb[2]

[1]Packaging Materials Department, [2]Paper and Cellulose Department, National Research Centre, Dokki, 12622, Egypt.

Abstract: To overcome the problem of peptide drugs degradation in acidic gastric juice of stomach upon oral administration, peptide drugs can be entrapped into pH-responsive hydrogel. Cationic xylan was prepared and characterized by FT-IR, X-ray diffraction, degree of substitution, and charge density. Cationic xylan/alginate hydrogel was prepared and characterized by FT-IR and SEM. The effect of cationic xylan and alginates components on drug loading, encapsulation efficiency percentages was investigated. The results revealed that the formulae 1:2 exhibited the better loading and encapsulation efficiency 42% and 85% respectively. The swelling behavior and release characteristics in different pH were studied. The swelling and release properties were pH - dependent. The formulation 1:2 resulted into the better swelling and release at pH 8.0. The kinetics of BSA release was determined using the Korsmeyer-Peppas equation. The BSA release suggested Fickian diffusion. The effect of cationic xylan and alginates contents on thermal stabilities was studied. Thermal stabilities increase with increasing alginates contents. Generally, the cationic xylan/alginate could be the most satisfactory protein delivery to the intestine.
Key words: Packaging, Drug delivery, Cationic, Hyrogel, pH-responsive.

Introduction:

The problem of peptide and protein drugs degradation under the acidic pH of the gastric medium in the stomach upon oral administration pay more attention to drug designer to make continuous attempts to attain enhanced oral administration of protein drugs. In order to improve the oral administration, protein drugs could be easily protected from the acidic gastric juice by entrapment into pH-responsive hydrogel. The polymers carrying basic or acidic groups can be developed to fabricate pH-responsive polymers. These polymers may include synthetic or natural[1].

Because of biocompatibility, biodegradability, and non-toxicity of natural polymer, they have been used in pharmaceutical applications. Chitosan as naturally occurring polycationic polymer has been widely used in for gastrointestinal drug delivery. However, chitosan does not dissolve in water but can easy dissolve in low pH solution medium limiting its application as a stomach delivery target.

Chen and his partners prepared a chitosan-alginate complex as a pH-sensitive hydrogel for oral peptide delivery[2]. But, this complex showed unsatisfactory release properties at low pH (1.2). In addition to the acidic medium required for chitosan dissolution, acidic environment may denature protein drugs[1,2]. So chitosan have been modified to overcome this problem by introducing functional groups enable it to dissolve in water[3].

Xylan is one of the most abundant biopolymers found in agricultural residues such as hard wood and furthermost rice straw and rice husk. Xylan is composed of (1→4)-linked anhydroxylose units. The chemical derivatization of xylan is a promising path to create new biopolymer with certain functional groups and degree of substitution[4]. To add water soluble group to xylan backbone, it is necessary to impart xylan with cationic groups by using the most commonly derivatizing agent, 2, 3-epoxyproplytrimethylammonium chloride[5]. Thus, the scientific target of this study was to fabricate and characterize cationic xylan-alginate coacervate polymer as drug release-retardant material and to analyze the influence of different coacervate polymer components ratios on variable properties. The coacervate was strengthened by the use of glutaraldehyde as a cross-linking agent. Thus, the primary objective of this study was synthesis of water soluble cationic xylan using 2, 3-epoxyproplytrimethylammonium chloride agent. The cationic xylan was characterized by FT-IR, degree of substitution, charge density, and X-ray diffraction. The cationic xylan/alginate polymer was characterized by FT-IR, SEM, swelling characteristics as a function of pH, drug encapsulation efficiency, loading, and mode of drug release. Thermal analysis of different polymer formulations was investigated.

Materials and Methods

Materials

Xylan from beech wood, M_{wt} $(132)_n$ g/mol was purchased from Carl Roth Gmblt. 2,3-Epoxypropyltrimethylammonium chloride (M_{wt} 151.63 g/mol, assay ≥ 90 %) was delivered from Aldrich. Sodium alginate, Bovine serum albumin protein was purchased from Sigma-Aldrich.

Cationization of xylan in organic solvent – NaOH water mixture

To 50 ml of mixture of organic solvent (tetrahydrofuran) and sodium hydroxide solution 1:1 (v/v), xylan (5g) was suspended and stirred for one hour at 60 ^{o}C. Cationizing agent, 2, 3-epoxypropyltrimethylammonium chloride (EPTA) and NaOH molar ratio was 1:0.5 and the molar ratio of cationizing agent and anhydroxylose units was 2:1. Subsequently, the cationizing agent (EPTA) was added drop wise to the suspension, and the mixture was stirred for 6 hours at 60 ^{o}C. The sample was neutralized, precipitated, purified, and freeze-dried[6].

Preparation of alginate-cationic xylan polymer

Alginate-cationic xylan coacervate polymer was prepared as described. Briefly, alginate was dissolved in water with constant stirring, and then bovine serum albumin protein as a model protein drug was suspended and stirred well. Appropriate amounts of cationic xylan were suspended into the alginate-protein drug mixture and stirred to obtain homogeneous solution. The drug entrapped polymer was taken in 500 ml beaker and the blend was sonicated for 5 minutes. Then (0.5 ml) glutaraldehyde crosslinker at pH 2.0 was added to the mixture, and the polymer blend was further sonicated for another 5 minutes. Five formulations were prepared and the assigned formulation as cationic xylan/alginate polymer weight ratios as 1:1, 1:2, 2:1, 3:0, and 0:3 to keep 3Wt % polymer concentrations.

Characterization

FT-IR Spectroscopy

The xylan, cationic xylan, alginate, and cationic xylan/alginate hydrogel samples were ground into small particles and dried in vacuum at 50 ^{o}C for 24 hours. The samples were analyzed by FT-IR (JASCO FT/IR-4100, Japan).

X-ray diffraction:

X-ray diffraction of xylan and cationic xylan were carried out by PANalytical XPERTPR O Super X-ray diffractometer.

Elemental analysis

The degree of substitution of cationic xylan and xylan was determined by estimation of nitrogen content. Nitrogen content was measured with Elemental analyzer (Vario El Elementary, Germany). Total degree of substitution (DSs) was calculated according to the equation (Schwikal et al., 2011).

DS = (60×w (N))/ (14× w(C) - 72×w (N)

1.1.1. The charge density of the cationic xylan

The Millikan test is a typical method for measuring the electric charge carried by oil droplets. Nevertheless, it can also be used to measure the electric charge carried by particles from eq. 1 and 2[7].

$r = (9\eta\upsilon/2g\rho)^{1/2}$ (eq. 1)
r: radius of particles, η: Viscosity of air ($1.8X10^{-5}$), υ: velocity of particles, υ=l/t,
g: specific gravity (9.8 m/s^2), ρ: density of xylan (0.86 g/cm^3).
$q = 4/3 (\Pi r^3 g\rho/E)$ (eq. 2)
q: Charge density
E: Electric field,
E=V/d
V: applied volt and d: distance between two plates (5 mm).

A Millikan oil droplet tester (Type MOD-5, made by Pai Zhong Co., Nanjing, China) was used for the experiment with testing condition: Temperature 25 ^oC, humidity 72 – 75%. The dropping time t (no voltage is applied, l is kept as constant distance) and voltage V across the two parallel sheets (particle is kept stationary) were measured, and 10 sets of data were collected. By means of equation 1 and 2, the radius and electric charge of each particle divided was the unit charge of electron ($1.6X10^{-18}$) was thus calculated and the mean value tabulated in table1.

Determination of polymer surface characteristics by SEM

The surface characteristic of cationic xylan/alginate polymer was examined by the help of SEM. The Scanning electron microscope using SEM Model Quanta 250 FEG (Field Emission Gun) attached with EDX Unit (Energy Dispersive X-ray Analyses), with accelerating voltage 30 K.V., magnification14x up to 1000000 and resolution for Gun.1n).

Swelling characteristics of cationic xylan-alginate polymer

Cationic xylan-alginate polymers (0.5g) were suspended in 50 ml of acid buffer (pH1.5) and phosphate buffer (pH 8.0) for 5 hours. At the equilibrium, the samples were re-weighed. Swelling equilibrium ratios were calculated as the following equation:

$Q_s = [(W_c - W_o) / W_o] \times 100$

Where Q_s is the swelling ratios at equilibrium, W_c, W_o are the weight of swelling samples at the equilibrium, and the initial weight of samples respectively[8].

Estimation of drug loading and encapsulation efficiency

The cationic xylan-alginate polymer entrapped with protein drug samples (0.5g) were pulverized and incubated in 10 ml 0.02 M phosphate buffer (pH 7.0) at room temperature for 24 hours. The suspension was then centrifuged at 4000 rpm for 30 minutes. The supernatant was assayed spectrophotometrically for bovine serum albumin protein content at wave length 280 nm[9]. The results of drug loading and encapsulation efficiency were calculated as following:

% Drug loading = (amount of drug in hydrogel/ amount of hydrogel) × 100

% Encapsulation efficiency = (Actual loading/ Theoretical loading) × 100

In vitro protein release

The release behavior of BSA protein as a polypeptide drug model is carried out by immersing (0.5 g) of loaded sample in buffer (pH 1.5 and 8.0) as a release medium as the low pH of the gastric medium in the stomach and high pH of intestine. At fixed intervals (each 2 hours); release medium solution (2ml) was withdrawn to detect BSA protein content and continued to 12 hours. BSA protein content was estimated spectrophotometrically. Absorbance/ concentration were measured at 280 nm. To find out the release of BSA protein mechanism, protein release data was fitted against release time according to Korsmeyer-Peppas release model:

$M_t/M_\alpha = kt^n$

Where, $M_t/M_{\alpha\ is}$ the fraction of protein released at time (t), k is the rate constant, and (n) is the release exponent[10].

Thermal analysis

The cationic xylan/alginate polymers of different formulations were tested using Perkin-Elmer Thermo gravimetric Analyzer Model TGA 7. The temperature was programmed from 50- 650 ^oC, at a heating rate of 10 ^oC/ min in nitrogen atmosphere with flow rate of 50 cm^3. The reference material was α- alumina**.**

Results and Discussion

FT-IR

FT-IR spectra of xylan and its cationic derivative were shown in Figure 1. A strong board band at 3423 cm^{-1} due to hydrogen bonded hydroxyl groups.

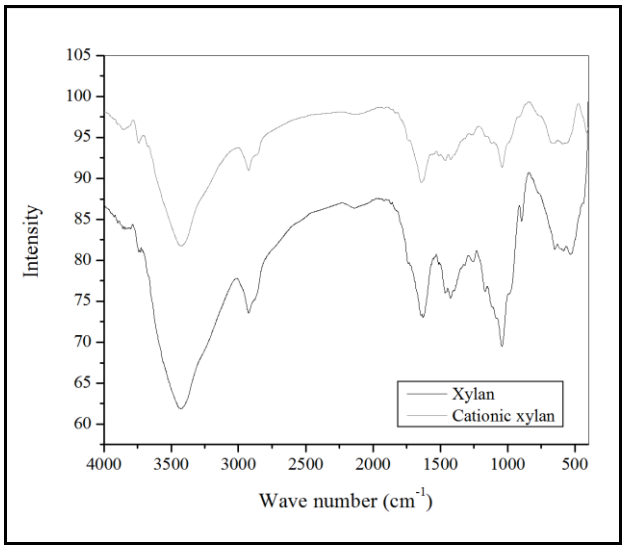

Figure 1: FT-IR spectra of xylan and its cationic derivative

The band at 2920 cm^{-1} is due to symmetric C-H vibration of xylan of intensity 75.57 that increases to 90.95 in cationic derivative indicating introducing $-CH_3$ in the xylan backbone and subsequently the existence of cationic moieties into xylan skeleton. The band at 1042 cm^{-1} (intensity 69.48 in xylan) increases to 91.35 that is owing to C-O-C skeletal vibration as an evidence of etherification by epoxypropyltrimethylammonium chloride. An intense peak at 1639 cm^{-1} is due to absorbed water in the isolated beech wood xylan. A sharp band at 895 cm^{-1} is attributed to β-glycosidic linkages between the sugars units[11].

X-ray diffraction of cationic xylan

As could be seen in Figure (2), the XRD pattern of xylan and its cationic derivative respectively, XRD patterns showed similar profile. Xylan has crystalline peak at 19.19^o. However, in cationic xylan that peak

exhibited less crystallinity. The crystallinity destruction of xylan due to the quaternization under alkaline conditions reduces the amount of the crystalline part and their structure could be considered as close to amorphous[12].

The characteristics of prepared cationic xylan

Table 1: Elemental analysis, degree of substitution (DS %), and charge density of cationic xylan compared with xylan

	Elemental analysis				DS%	Charge density
Xylan	H%	C%	N%	S%	-	354
	8.06	82.15	-	-		
Cationic xylan	H%	C%	N%	S%	0.29	711
	11.4	78.18	4.63	-		

Elemental analysis of cationic xylan was shown in Table (1). The cationization reaction of xylan is grafting a cationic group on the xylan backbone, by etherification of the hydroxyl functions. The reaction efficiency is characterized by the degree of substitution (DS) which corresponds to the number of cationic group grafted per sugar unit. The maximal degree of substitution (DS_{max}), achieved when all hydroxyl groups are etherified. From the results of elemental analysis and based on nitrogen content, the degree of substitution of cationic xylan is 0.29.

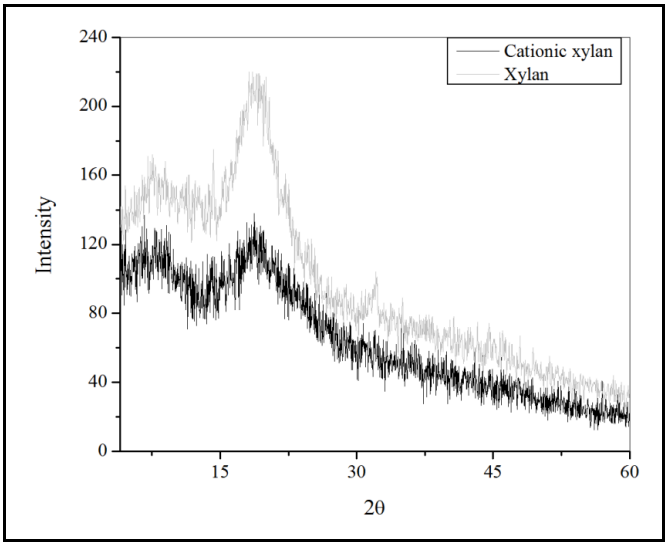

Figure 2: X-ray diffraction of xylan and cationic derivative

The numbers of electric charge per particles of xylan and cationic xylan were shown in Table (1).The mean value of determined electric charge was recorded for xylan and cationic xylan as presence in table 1. The particles were directed to negative plate during the experiment. The tabulated value can be pointed to increase the charge density over the particles after the modification of xylan. The number of charge over the particles were nearly duplicated which confirm the results of degree of substitution.

FT-IR spectrum of alginate-cationic xylan

FT-IR spectra of alginate, cationic xylan, and alginate-cationic xylan hydrogel are presented in Figure 3. The bands at 3423 cm^{-1} is due to hydrogen bonded hydroxyl groups. Strong peaks appear at 2926 and 2856 cm^{-1} due to symmetrical and asymmetrical -C-H. Intense peak appears at 1744 cm^{-1} may be due to carbonyl group of 1-4 linked α-L- guluronic and β-D-mannuronic acid residue of alginate.

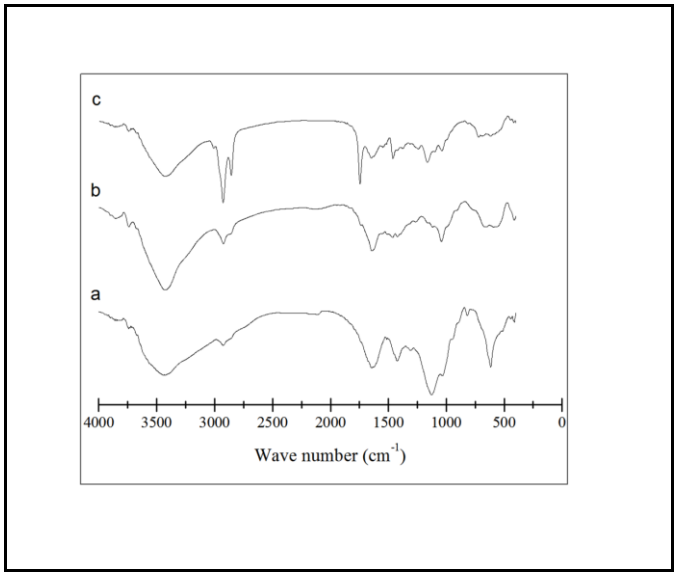

Figure 3: FT-IR of alginate (a), cationic xylan (b), and alginate-cationic xylan hydrogel (c)

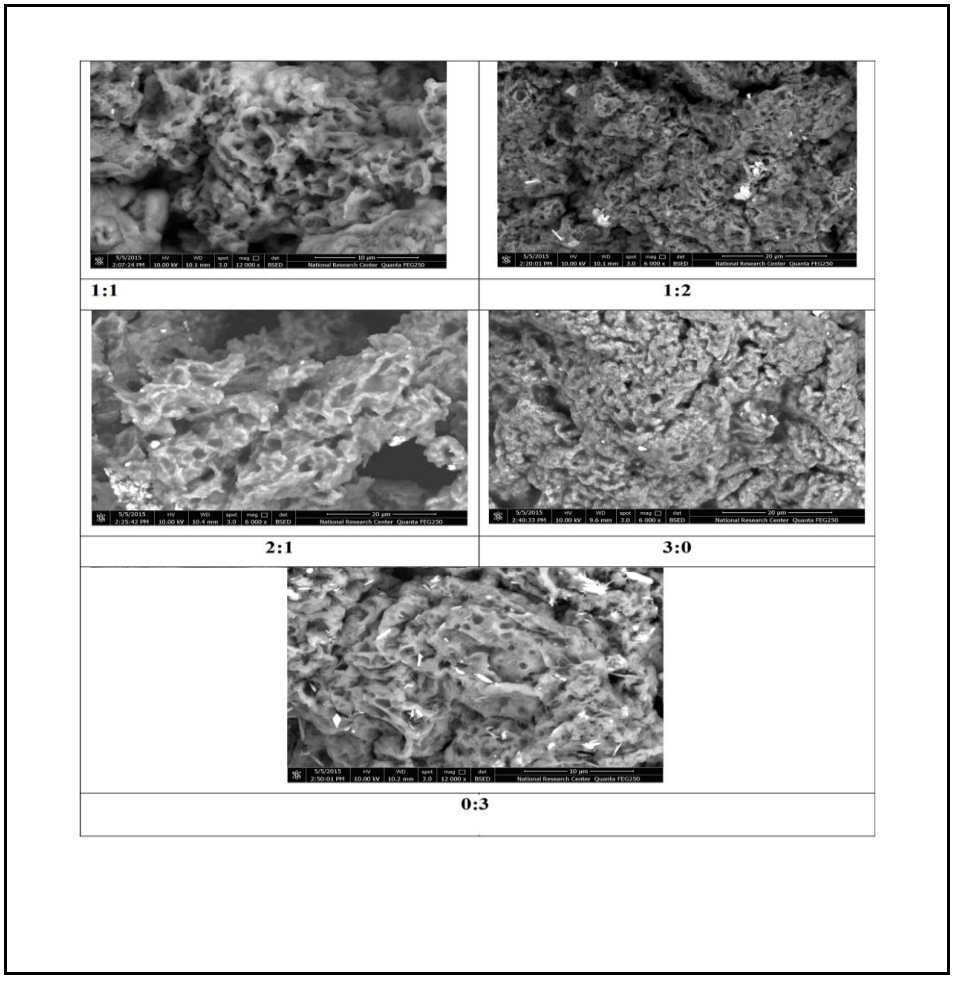

Figure 4: SEM of cationic xylan/alginate polymers

Surface topographic (SEM)

As could be seen, the surface morphologies of cationic xylan-alginate hydrogel polymers of different formulations are captured and explained in Figure (4).

The SEM images of low alginate contents formulations (cationic xylan / alginate: 3:0, 1:1, 2:1) do not exhibit porous nature and their surfaces are dense. However, the formulations of high alginate contents (cationic xylan/alginate: 1:2 and 0:3) showed almost porous nature, exhibited numerous open channels like structures and network like structures. SEM image shows the entrapment of BSA that appears as needle crystals within the polymer network after drying and the excess of BSA particles adhere to the surface.

Estimation of drug loading and encapsulation efficiency

Drug loading and encapsulation efficiency percentages of different formulations of cationic xylan and anionic alginates were presented in the above Table (2). The cationic xylan: anionic alginate (1:2) combination was found to be the most suitable combination as it has comparatively better drug entrapment and encapsulation efficiency in the preliminary studies. This results reported as the drugs can retain in the porous structure of cationic xylan/alginate polymer[1].

Table (2): Formulation variables, drug loading, and drug encapsulation efficiency percentages

Formula code Cationic xylan/alginates	Cationic xylan (g)	Alginate (g)	Glutaraldehyde (ml)	BSA content (mg)	Drug loading %	Drug encapsulation efficiency %
3:0	3	0	0.5	90	35.11	78.10
1:1	1.5	1.5	0.5	90	36.14	80.39
1:2	1	2	0.5	90	42.2	85.22
2:1	2	1	0.5	90	40.42	81.01
0:3	0	3	0.5	90	44.03	88.17

Swelling characteristics

The pH variation has an important role in swelling behavior of hydrogel polymer of polyelectrolyte nature like cationic xylan/alginate polymer.

The swelling properties of cationic xylan/alginate polymer could be seen as a function of pH (1.5) in Figure 5 (left side). At low pH, the increase in alginate content in hydrogel formulation contributes to reduce swelling ratios. As reported, alginates shrink at low pH and the buffer solution cannot diffuse easily.

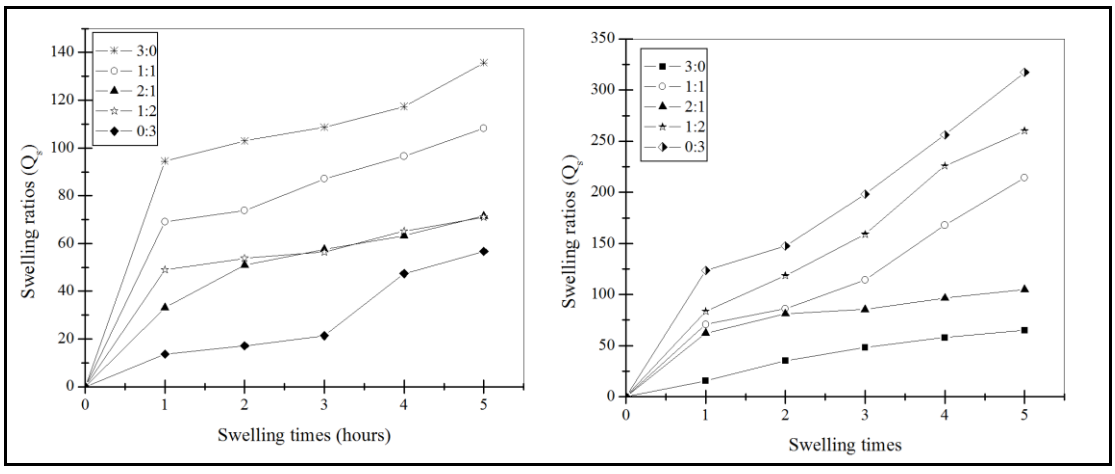

Figure 5: Swelling ratios of different formulation of cationic xylan-alginate polymer (the left side pH 1.5 and the right side pH 8.0)

Also, the effect of pH medium on swelling properties in slightly alkaline medium was studied as seen in Figure 5 (right side). At a slightly alkaline pH (8.0), the swelling ratio increases with increasing alginate content in the polymer formulation. This observation may be explained that at that slightly alkaline pH, the ionization extent of carboxylic acid increases, producing more carboxylate anions along the alginate backbone thus creating more perfect anion-anion repulsion, creating more relaxation and thus more solvent uptake[13].

Generally, the formulation 1:2 was assigned as the most suitable protein protection formulae. It is the most satisfactory protein delivery to the intestine.

In vitro release and kinetic analysis of BSA

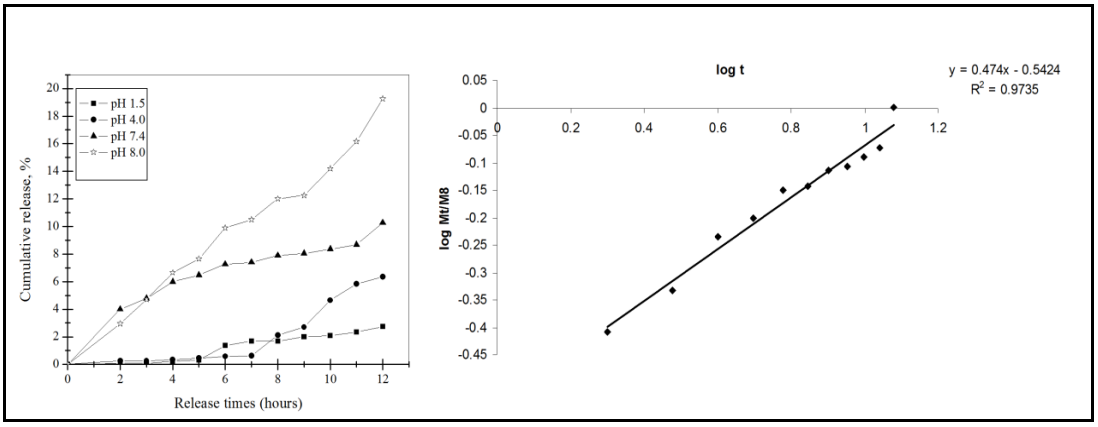

Figure 6: Release and kinetic behavior of cationic xylan/ alginate polymer loaded with BSA protein

The in vitro BSA protein release was done in different pH medium (1.5 and 8.0) as the low pH of the gastric medium in the stomach and high pH of intestine. The cumulative BSA protein release pattern was shown in Figure (6), it is noted that the cumulative release behavior showed positively relationship with progress of release times. As could be seen in Figure (6), cationic xylan /alginate hydrogel (1:2) was chosen among the other different formulations because of its better drug encapsulation efficiency percentages.

It is well seen that BSA exhibited initial rapid release from cationic xylan/alginate polymer based on the phenomenon known as a burst effect "rapid dissolution of the BSA molecules attached to the polymer surface". A subsequent slow release pattern is observed during which drug molecule embedded inside holes and spaces of hydrogel diffuse out.

At low pH (1.5) the hydrogel exhibited lesser release than that at high pH (8.0) this may be owing to that alginate shrinks at low pH. Cumulative release increases with increasing pH. At low pH, cationic moieties ($-NH_3^+$) groups and anionic alginate results into more electrostatic attraction along the polymer backbone thus strengthening the intermolecular hydrogen bonding interaction, as a result the buffer solution cannot diffuse into network easily. However, at slightly alkaline pH, further anion-anion repulsion forces of carboxylate anions along alginate polymer skeleton, more further relaxation, and more protein release.

The kinetics of BSA release was determined using the Korsmeyer-Peppas equation to calculate the value of release exponent "n". The test was performed on formulation 1:2 at pH (8.0). The "n" value is determined to investigate different release mechanism. When "n" = 0.45, Fickian diffusion, 0.45< n <0.89 anomalous (non-Fickian diffusion), n = 0.89 case II – transport, and n > 0.89 super case II – transport. From the results illustrated in Figure (6), correlation coefficient (0.9735) and n (0.474), the BSA release suggested Fickian diffusion, i.e, and relaxation rate of polymer network is faster than diffusion rate, diffusion through swelling is the main factor controlling BSA release[14].

Thermal analysis

TGA thermograms of cationic xylan-alginate polymers of different cationic xylan/alginate ratios were shown in Figure 7. The thermograms of cationic xylan/alginate formulations of 1:1, 1:2, and 2:1 showed five distinct degradation stages. The first stages range between 35.3 ^oC-126.2 ^oC, 35.3 ^oC-263.8 ^oC, and 35.4 ^oC-169.3 ^oC with weight loss percentages of 24.7, 33.6, and 7.0 respectively. This may correspond to the loss of adsorbed and bound water. The four degradation stages correspond to degradation of cationic xylan and alginates. The cationic xylan degrades in the range 120 ^oC-250 ^oC and alginates degrade in the temperature range 250 ^oC-400 ^oC. The start degradation temperature increases with increasing alginate contents indicating that thermal stabilities of cationic xylan/alginates increase with increasing alginate contents.

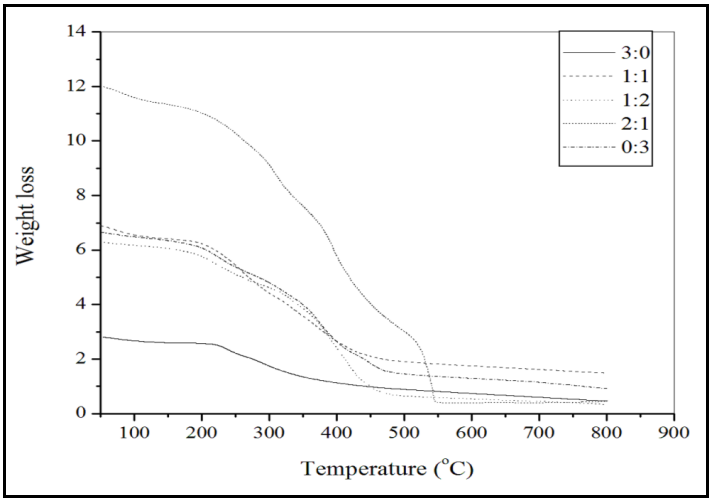

Figure 7: TGA thermograms of cationic/alginates hydrogel polymer of different formulations

Conclusion

Oral administration of peptide drugs was improved by entrapment into pH-responsive hydrogel composed of cationic xylan and alginate. Cationic xylan was prepared and its structure was confirmed by FT-IR, X-ray diffraction, degree of substitution, and charge density. The effect of cationic xylan and alginates components on drug loading, encapsulation efficiency percentages, swelling, and release profile at different pH was investigated. The results revealed that the formulae 1:2 exhibited the better loading, encapsulation efficiency, swelling, and release at pH 8.0 indicating that this formulation is suitable for intestine delivery. The formulae 1:2 release suggested Fickian diffusion. Thermal properties of cationic xylan/alginates polymers were studied. Thermal stabilities increase with increasing alginates contents

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