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Radiation Synthesis of Polyampholytic and Reversible pH-Responsive Hydrogel and Its Application as Drug Delivery System

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Abstract

Graft copolymerization of acrylic acid (AA) and acrylamide (AAm) onto chitosan (CS) was carried out using gamma irradiation. Their swelling behavior was investigated. The hydrogels before and after alkaline hydrolysis were confirmed by FTIR spectroscopic studies. The hydrogels show ampholytic and reversible pH-responsiveness characteristics. The swelling variations were explained according to swelling theory based on the hydrogel chemical structure. The ability of the prepared copolymer to be used as gastric antibiotic delivery system was estimated using amoxicillin trihydrate as a model drug. Release of amoxicillin trihydrate from these investigated hydrogels was studied. For non-ionized drugs, such as amoxicilin trihydrate, the electrostatic polymer/ polymer interactions take place between the cationic groups from CS and the anionic ones from PAA resulting in entrapping the drug into the mesh space of the hydrogel. The non-ionized amoxicillin release was controlled by the swelling/eroding ratio.

Keywords: Chitosan, Polyampholytic hydrogel; pH-responsive; Drug release

Introduction

Gastric Helicobacter pylori infection plays a crucial role in gastroduodenal diseases [1]. The antimicrobials used in the therapeutic regimes of these diseases are amoxicillin, clarithromycin and metronidazole and a proton pump inhibitor as omeprazole [2]. In vitro, H. pylori is susceptible to amoxicillin with MIC90 of 0.12 mg/l [3]. Therefore, if amoxicillin could be delivered to local sites/ areas very close to the mucus layer, pharmaceutical formulations, with lower antibiotic doses, might be employed.

Recently, several authors have pointed out that pH sensitive swelling covalently and non-covalently crosslinked hydrogels seem to be useful for localized antibiotic delivery in the acidic environment of the gastric fluid [4,5]. One of the most important advantages of these hydrogels is that formulations remain during more time than conventional ones on the targeted site. Several covalently cross-linked chitosan-based hydrogels have been developed for site specific therapy by different authors [6, 7]. Chitosan has been used in

many studies of hydrogels because of its good properties such as non-toxicity and biodegradability [8–13]. This polymer possesses reactive functionalities represented by the amino groups, it is easily degraded by enzymes, and the degradation products are not toxic [9,11,]. However, heterogeneous polymer mixtures may also be used to form hydrogels without the need for covalent cross-linking [8,11,].

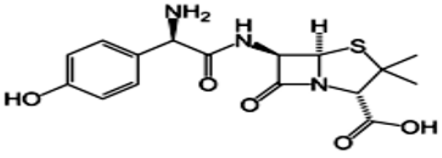
Polymeric hydrogels are suitable to serve as drug delivery systems. They are non-toxic and high swellable three dimensional network structures. On the forefront of controlled drug delivery, hydrogels, as enviro-intelligent and stimuli-sensitive gel systems, can modulate drug release in response to pH [14], temperature, ionic strength, electric field, or biological trigger [15]. pH-responsive hydrogels are prepared by including weak polybase or polyacid within the polymeric hydrogel. The presence of such groups causes the conformational transition from collapsed to swollen states at certain pH value. pH responsive hydrogels could be designed to control and target the drug to a specific site such as colon depending on the range of pHs domains in the body. Polyelectrolytes, which are very special class of pH sensitive polymers, are polymers with covalently bound ionic groups. Corresponding to the nature of the ionic groups, they are classified as polyanions, polycations or polyampholytes.

Radiation-induced copolymerization and crosslinking of copolymers has been increasingly used for creation of novel biomaterials. Radiation initiation of chemical reactions offers several advantages over chemical methods: it is simple, additive free and sterilization of the product takes place simultaneously, i.e. the produced polymer will be sterilized and free of carcinogenic materials (initiators and/or crosslinkers). In this work, γ-ray as source of initiation and crosslinking was used to graft copolymerization of mixture of acrylic acid as polyanion and acrylamide onto chitosan to produce ampholytic CS-g-poly(AA-co-AAm) hydrogels to be used as a drug carrier for antibiotic drug delivery will be estimated using amoxicillin trihydrate. Amoxicillin (α-amino-hydroxybenzylpenicillin) was a semisynthetic, orally absorbed, broad-spectrum antibiotic. It is now widely used in a standard eradication treatment of gastric Helicobacter pylori infection combined with a second antibiotic and an acid-suppressing agent [16–18].

Experimental

Materials

Chitosan (CS) was kindly by pronova Biopolymer, Inc (USA). The degree of deacetylation and molecular weight were determined as 85 % and 50,000, respectively. Acrylic acid (AA) and acrylamide (AAm) of purity 99% (Merck, Germany) were used as received. Other chemicals, such as citrate, phosphate buffer salts of analytical reagents, were purchased from El-Nasr Co. for chemical Industries, Egypt . Amoxicillin (GlaxoSmithKline Co., British)



Amoxicillin

Preparation of hydrogel

CS-g-(AA-co-AAm) copolymer hydrogels were obtained by gamma irradiation-induced copolymerization of aqueous solutions of CS (0.5 g of CS was dissolved in 30 ml of 1 wt.% acetic acid solution), AA and AAm mixtures with different volume ratio (Table 1) in small glass tubes using ^{60}Co gamma rays, 10, 15 and 20 kGy, at a dose rate 2.86 Gy/s. After copolymerization, the tubes were broken, the formed polymeric cylinder were removed and cut into discs of 2mm thickness. All samples were washed in excess water to remove the unreacted component then air dried at room temperature. All the samples used in this study possess almost 100% gelation, i.e., no extractable monomers and/or polymers.
Saponification of CS-g-poly(AA/AAm) was carried out by heating with stirring in 1 N NaOH solution according to Mahdavinia et al. [19] IR was carried out to confirm the hydrolysis process as shown in figure 1.

Table 1: Composition of the feed mixture

Samples	CS (v%)	AA (v%)	AAm (v%)
H_1	10	0	10
H_2	10	2	8
H_3	10	3	7
H_4	10	5	5
H_5	10	7	3
H_6	10	8	2
H_7	10	10	0

Preparation of buffer solution of different pH's

0.2 M (citric acid/trisodium citrate) and 0.2 M (sodium dihydrogen phosphate/ disodium hydrogen phosphate) were used to prepare buffer solution ranged from 3 to 5 and 6 to 8, respectively. 0.2 M HCl was used to prepare solutions of pH 1and 2.

Swelling studies

The prepared copolymer hydrogels were soaked in buffer solution of different pH values ranged from 1 to 8 at 37°C. The swelling (S) was determined from the following equation:

$$S = \frac{W_s - W_d}{W_d}$$

Where w_d and w_s represent the weights of dry and wet hydrogels, respectively.

Preparation of Amoxicillin trihydrate -loaded hydrogel

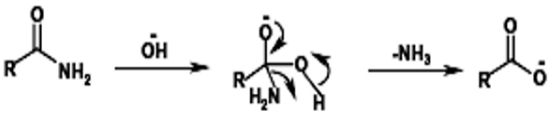
Polymer-drug conjugate was carried out by swelling equilibrium method. The dry hydrogel (0.1 g) was allowed to swell in the drug solution of known concentration for 24 h at 37°C until the complete absorption and than dried. The concentration of the rejected solution was measured to calculate percent entrapment of the drug in the polymer matrix. The experiment was performed for three hydrogels of each composition to take an average value of the delivery result.

Release of Amoxicillin trihydrate

Release experiments were performed by placing (0.1g) the CS-g-poly(AA-co-AAm) hydrogels loaded with amoxicillin trihydrate were put in 25 ml of 0.1 M HCl (pH 1.4) at 37ºC. At predetermined time one millilitre sample was withdrawn to follow the release process. The concentration of amoxicillin trihydrate was measured by UV spectroscopy (UNICAM UV/Vis Spectrometer. 1000 Model) at λ= 228 nm. After the complete release; the hydrogels were immersed in pH 3.0 buffer solutions and then, 0.1mol/l, HCl for 2 days to remove remaining drug may be loaded in the gel system.
The total uncertainly for all experiments ranged from 3 to 5%

Results and Discussion

PAA and PAAm were simultaneously grafted onto chitosan (CS) in homogenous medium using γ-rays as initiator and crosslinking agent In previous communication, which described a detailed study of the swelling behavior of CS-g-poly(AA-co- AAm) hydrogel copolymer [19]. However, in a preliminary study for the present work, a number of hydrogels with different monomer compositions of AA and AAm were synthesized by using γ-rays and their equilibrium swelling were determined at different pH. To obtain a hydrogel with high swelling capacity, the CS-g-poly(AA-co-AAm) hydrogel copolymer was hydrolyzed with NaOH solution. During the saponification, ammonia evolves and amide groups are converted to carboxylate salt. This reaction can be shown as below:



FTIR analysis of hydrogels

FTIR spectrum (Fig. 1) showed (a) before and (b) after hydrolysis of CS-g-poly(AA-co-AAm) hydrogel. It showed the presence of the very intense characteristic band at 1563 cm-1 is due to C=O asymmetric stretching in carboxylate anion that is reconfirmed by another sharp peak at 1401 cm-1 which is related to the symmetric stretching mode of the carboxylate anion. Combination of absorption of the carboxylate and alcoholic O–H stretching bands are appeared in the wide range of 2550-3500 cm-1 [20] as shown in Fig. 1(a,b). As shown in this Fig. 1(b), the intensity of carboxylate groups (1563 cm-1) is increased after hydrolyzing the hydrogel. This is attributed to conversion of amide groups to carboxylate salt.

Effect of monomer composition on swelling capacity

The influence of equilibrium swelling of the hydrogels prepared with various ratios of monomers is shown in Fig. 2. The equilibrium swelling of CS-g-poly(AA-co-AAm) hydrogel is due to the effect of both functional groups of ionic carboxylate (from AA) and non-ionic amide (from AAm). As can be seen from this figure,

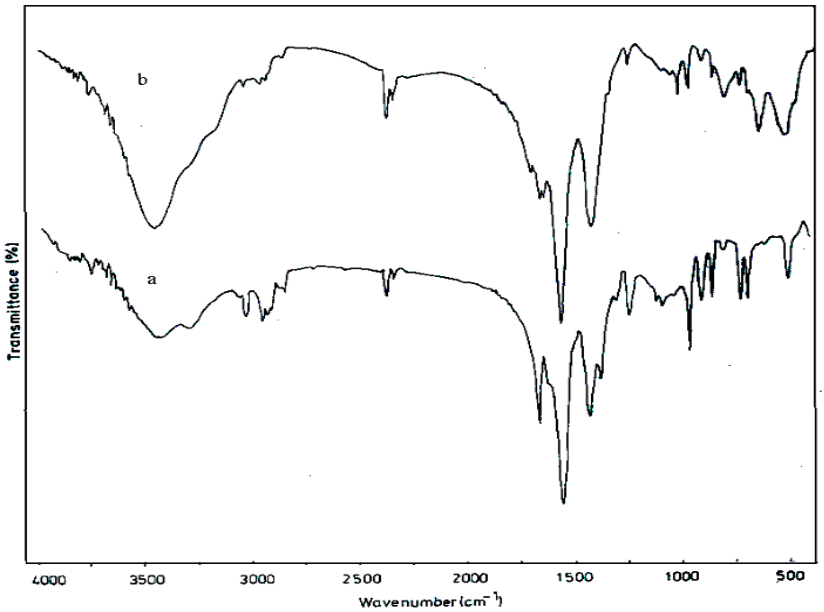


Fig. 1: FTIR spectroscopic analysis of CS-g-p(AA-co-AAm) before (a) and after hydrolysis (b).

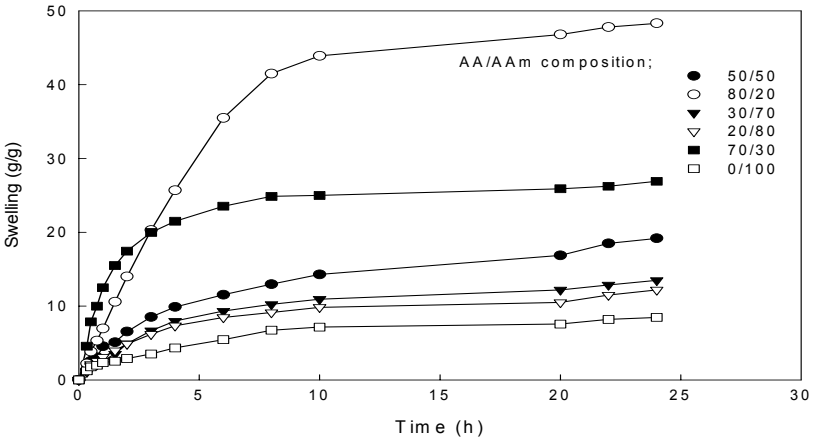


Fig. 2: Effect of time (h) on the equilibrium swelling for different CS-g-p(AA-co-AAm) compositions at pH 7.

swelling capabilities for all copolymer compositions are increased by increasing in the AA content in the initial comonomer feed solution. The increase observed in the swelling could be attributed to the increase in hydrophilic character of each AA monomeric unit and so, the number of hydrogen bonds formed with water.

The alkaline hydrolysis of CS-g-poly(AA-co-AAm) values, copolymer resulted in a very pronounced increase in its swelling almost 10 times higher than that for the unhydrolysis one (Fig. 3). This was reasonably due to the amide groups are converted

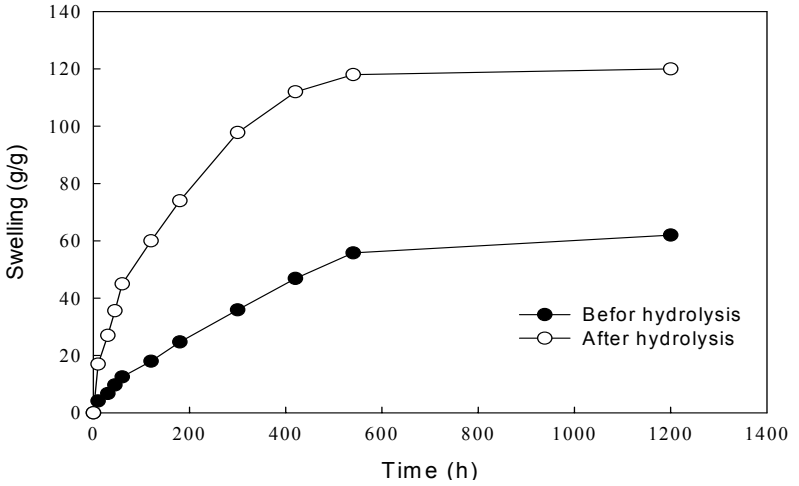


Fig. 3: Effect of swelling time on the equilibrium swelling (g/g) for before and after hydrolysis CS-g-p(AA-co-AAm) hydrogels; (AA/AAm) (50/50) at pH 3, dose 10 kGy.

to carboxylate salt, which possesses more easily ionizable and electrolytic groups having much higher hydrophilic properties.

The influence of irradiation dose on the equilibrium swelling for CS-g-poly(AA-co-AAm) hydrogels is shown in Table 2. From the results it may be seen that, the equilibrium swelling decreases with increasing irradiation in all prepared hydrogel. This is due to the enhancement of crosslinking process at higher doses, and as a consequence the diffusion and swelling properties are hindered by the formation of network structure.

Table 2: Effect of irradiation doses (kGy) and temperature (°C) on the equilibrium swelling for different CS-g –p(AA-co-AAm) hydrogel at pH 7

Composition	Doses (kGy)			Temperature (°C)			
	10	15	20	25	35	45	55
50/50	42	30	23	35	49	55	65
80/20	60	46	37	45	66	78	95

Effect of pH on the equilibrium swelling

The effect of copolymer composition on the swelling of the prepared CS-g-poly(AA-co-AAm) hydrogels at different pH's was studied and the results are presented in Fig. 4. It can be seen that the swelling behavior of the produced copolymer is greatly influenced by its composition. In this system, a combination of attractive or repulsive electrostatic interactions and hydrogen bonding are the main reasons for existence of several phases observed in various environmental conditions. CS-g-poly(AA-co- AAm) hydrogel has both amine (chitosan backbone) and carboxylate (PAA chains) as functional groups. Chitosan is a weak base with an intrinsic pKa of 6.5. PAA contains carboxylic groups that become ionized at pH values above its pKa of 4.7. Since hydrogel swells differently in media with different pHs, we have investigated its pH-dependent swelling reversibility. The species

involved are NH^+_3 and COOH (at pH 1-3), NH_2 and COO^- (at pH 7-11) and NH^+_3 and COO^- or NH_2 and COOH (at pH 4-7). Under acidic conditions, the swelling is controlled mainly by the amino group (NH_2) on the C-2 carbon of the chitosan component. It is a weak base with an intrinsic p*Ka* of about 6.5[21] and so it gets protonated and the increased charge density on the polymer should enhance the osmotic pressure inside the gel particles because of the NH^+_3 -NH^+_3 electrostatic repulsion. This osmotic pressure difference between the internal and external solution of the network is balanced by the swelling of the gel. At pH > 4.7, the carboxylic acid component comes into action as well. Since the p*Ka* of the weak polyacid is about ~ 4.7, its ionization occurring above this value may favor enhanced absorbency. However, under pH 6.4, or in a certain pH range, 4-7, the majority of the base and acid groups are as NH^+_3 and COO^- or NH_2 and COOH forms, and therefore ionic interaction of NH^+_3 and COO^- species (ionic crosslinking) or hydrogen bonding between amine and carboxylic acid (and probably carboxamide groups) may lead to a kind of crosslinking followed by decreased swelling. At pH ≤ 8, the carboxylic acid groups become ionized and the electrostatic repulsive force between the charged sites (COO^-) causes an increase in swelling. Either protonated (NH^+_3) or deprotonated (COO^-) groups increase charge density on the polymer causing an enhancement of the osmotic pressure inside the gel particles because of the NH^+_3– NH^+_3 or COO^--COO^- electrostatic repulsion. This osmotic pressure difference between the internal and external solution of the network is balanced by the swelling of the gel.

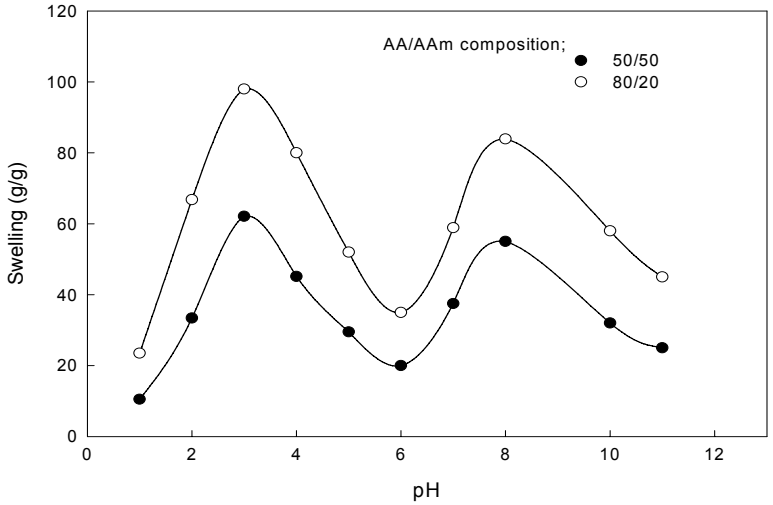


Fig. 4: Effect of pH on the equilibrium swelling for different CS-g-p(AA-co-AAm) compositions at dose; 10 kGy.

Effect of Temperature on swelling

The temperature dependent equilibrium swelling behavior of the hydrogels in deionised water (pH 7) at a temperature range 25- 55°C is shown in Table 2. As the temperature of the hydrogels in the swelling state increased, the swelling ratio of the hydrogel samples increased. All hydrogels exhibited a temperature responsive

swelling behavior due to the association/dissociation of inter/intra molecular hydrogen bond within the matrix. [22]

Amoxicillin trihydrate loading

For the investigation of cationic drug adsorption behavior of CS-g-poly(AA-co-AAm) hydrogels prepared in this study, hydrogels were initially swollen in amoxicillin solution in a concentration range of 0.25 – 1.0 mg/mL

The total amount of amoxicillin trihydrate adsorbed into 0.1g of dry gel at different initial drug concentrations is given in Fig. (5). As can be seen from this figure, the amount adsorption of total amoxicillin trihydrate increased with increasing AA content in the gel system and initial drug concentration. The reason for this increase was attributed to the higher protic acid content of the gel system and specific interactions between ionized polymer and drug molecules and also, to the higher free volume available for diffusion. With non-ionized drugs, such as amoxicilina trihydrate, the electrostatic polymer/ polymer interactions take place between the cationic groups from CS and the anionic ones from PAA and the drug is entrapped into the mesh space of the hydrogel[23].

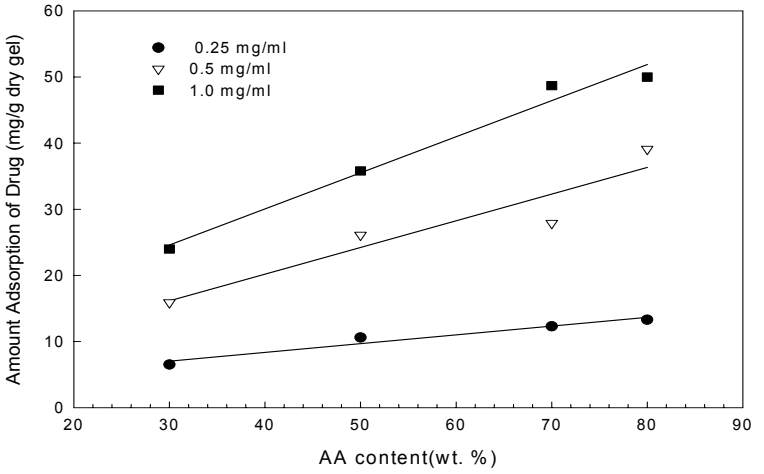


Fig. 5: Effect of AA content and drug concentration on the adsorption capacities of CS-g-p(AA-co-AAm) hydrogels at pH 7.

Release of amoxicillin

Most of the hydrogels are glassy in their dehydrated state, and drug release generally involves simultaneous absorption of water and desorption of drug via a swelling controlled mechanism[24].Some authors[23, 25] have reported that the use of pure chitosan formulations in oral administration is limited due to their fast dissolution in the stomach and their limited capacity for controlling the release of drugs.

Fig. 6 shows the cumulative release rates of different composition of CS-g-poly(AA-co-AAm) with varying amounts of PAA and AAm. All compositions present an initial release effect may be attributed to the diffusion of the drug caused by

rapid gel swelling and also the release of drug adsorbed towards the surface of the gel matrix [26]. As AA content increase in the copolymer, the release rate and total released drug increases. These results could be attributed to the swelling behavior of the copolymer hydrogel. The composition with the ratio of AA/AAm (80/20) presented a suitable controlled release profile, about 57.55% and a 75.98% of amoxicillin trihydrate was released after 1 and 2 h, respectively. These release results will ensure maximum availability of the drug in the stomach.

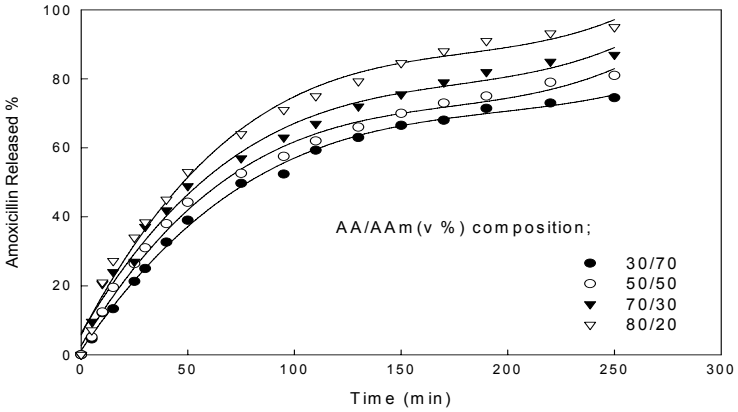


Fig. 6: Amoxicillin trihydrate released from CS-g-p (AA-co-AAm) hydrogel as a function of time with varying composition, at pH 1.4, initial drug Conc.; 0.5 mg/ml, wt. 0.1 g.

To investigate more precisely the effect of polyampholytic formation on the release of amoxicillin, the results were analyzed according to the following equation:

$$M_t/M = kt^n$$

where M_t/M is the amount of amoxicillin (%) released at time (h); *n* is a diffusional exponent and k is the apparent release rate (%/h).

Table 3 summarizes the kinetic constants (K), release exponents (n), correlation coefficients (r^2) following linear regression of release data of amoxicillin trihydrate from CS-g-poly(AA-co-AAm) polyampholytic hydrogels. These results are reported in Table 3, amoxicillin trihydrate hydrogels presented a non Fickian release with n values between 0.34–0.46. The existence of a slow macromolecular relaxation process in the swollen region is believed to be responsible for the observed non-Fickian behavior [27].

Table 3: kinetic constants (K), release exponents (n), correlation coefficients (r^2) following linear regression of release data of amoxicillin trihydrate from CS-g-p(AA-co-AAm) polyampholytic hydrogels

CS-g-p(AA-co-AAm) hydrogels	*n*	*K*	r^2
H_3	0.34	1.78	0.992
H_4	0.43	2.08	0.9852
H_5	0.46	2.51	0.9982
H_6	0.42	2.37	0.9995

The kinetic parameters obtained here may suggest that release of non-ionized amoxicillin (trihydrate) was controlled by a combination of diffusion/ dissolution of the polyionic complex. The results, thus obtained, show good agreement with the literature[23].

Figure 7 describes the amount of amoxicillin trihydrate released from CS-g-poly(AA-co-AAm) (80/20) hydrogels as a function of time for different amounts of the loaded amoxicillin trihydrate (0.25, and 0.5 mg/ml). The results, thus obtained, show good agreement with the literature[28]. The amount released depends upon the initial amount of the drug present in the polymer matrix. Thus, the amount of amoxicillin released is in the following order:

H_6 loading 0.5 mg/ml > H_6 loading 0.25 mg/ml

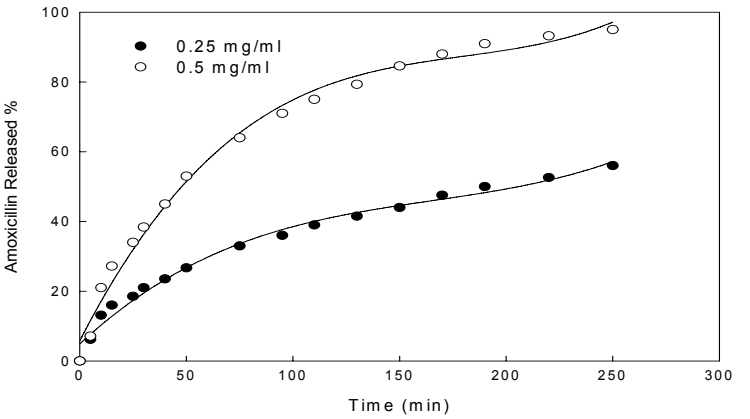


Fig. 7: Amoxicillin trihydrate released from CS-g-p(AA-co-AAm) (80/20) hydrogels as a function of time for different loaded amounts of amoxicillin trihydrate at 37ºC, wt. 0.1 g.

The study of the release of amoxicillin trihydrate from hydrogels also depends on the pH value of the release medium. Compared with the release % at pH 1.4, 3.0, 6.0 and 10.0 is 92, 75, 25 and 45%, respectively from hydrogels loaded 0.5 mg/ml. Amoxicillin trihydrate was released more rapidly at pH 1.4 and this results, thus obtained, show good agreement with the literature [29-31]. It suggests that the drug release profiles of hydrogels are pH sensitive. This result is also in good agreement with the effect of the pH values on swelling of hydrogels as mentioned earlier. Namely, at pH 3.0, the relatively high swelling degrees of hydrogels result in higher release rates.

Conclusion

Polyampholytic and Reversible pH-Responsive hydrogels, CS-g-poly (AA-co-AAm) synthesized by γ-radiation induced polymerization and crosslinking. The swelling of hydrogel exhibited high sensitivity to pH. Study effect of H^+/OH^- concentration carried out at various pHs shows that the swelling of hydrogel causes several large volume changes. Ionic repulsion between charges groups incorporated in the gel matrix by an external pH modulation could be assumed as the main driving force

responsible for such swelling changes. Investigating the ability of the prepared polyampholytic network to be used as a carrier for drug deliver system showed a promising result not only in the field of drug targeting but it also shows the possibility of controlling the released amount and release rate. Polyampholytic and pH-Responsive Hydrogel by radiation process with suitable amoxicillin release profiles for site-specific antibiotic delivery in the stomach.

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