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Hydrophilic amylose-based graft copolymers for controlled protein release

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Abstract

Graft copolymerization of 2-hydroxyethylmethacrylate (HEMA) onto high-amylose starch using ceric ammonium nitrate (CAN) as an initiator was carried out in an aqueous solution. Our attention has been focused on efficient and well controlled grafted copolymerization methods in order to obtain a biocompatible copolymer for controlled-release of drugs and proteins. Here, we report on the synthesis and physico-chemical characterization as well as other experiments to test the enzymatic resistance and the capacity of fermentation by colonic bacteria of this product. It can be observed that this copolymer gives a good controlled-release of drugs (Theophylline and Procaine hydrochloride), as well as proteins (BSA). The in vitro experiments let us know that quantities lower than 10% of BSA are released at pH 1.5 (stomach), keeping the protein in the matrix to be released at higher pHs (intestine), where the matrix suffers anaerobic degradation.

Keywords: Starch copolymers; Enzymatic degradation; Protein delivery; Hydrogels; Controlled-release

1. Introduction

Starch is a potentially useful carbohydrate for biodegradable materials because of its natural abundance and low cost. Starch is a polysaccharide, which consists of amylose and amylopectin as constituents. Amylose is known to be degradable in the colon tract (Milojevic et al., 1996), which is an interesting quality for drug delivery in this area.

However, the starch-based materials, such as the thermoplastic starch, have some drawbacks including the poor long-term stability caused by the water absorption, the poor mechanical properties, and the process ability. In order to solve some of these problems, various physical or chemical modifications of the starch have been considered. In recent years, particular attention has been attached to the synthesis of starch graft copolymer due to an increasing interest in the synthesis of new cost-effective polymers.

The grafting copolymers of many hydrophilic monomers such as acrylamide (Hebeish, El-Rafie, Higazy, & Ramadan, 1996), acrylic acid (Trimnell & Stout, 1980) and acrylonitrile (Pourjavadi & Zohuriaan-Mehr, 2002) onto starch have been utilized to prepare biomaterials with different applications. The use of 2-hydroxyethylmethacrylate (HEMA) as a grafting monomer onto a range of polymeric substrates to produce new biomaterials has been increasingly successful and previous studies showed that the presence of HEMA in copolymers improves the biocompatibility of the materials (Benson, 2002; Casimiro, Leal, & Gil, 2005; Ferreira, Rocha, Andrade, & Gil, 1998).

Controlled delivery of bioactive agents has been a major field of research of the last years. A variety of methods have been used to target biologically active molecules to specific sites and extend their therapeutic lifetimes once inside the body. Polymeric drug carrier

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systems have several advantages in optimizing patient treatment regimes. Biomaterials, specially based on the polysaccharides, have attracted considerable attention to act as a smart candidate for the controlled release of therapeutic agents to the specific sites in the GI tract. In particular, starch graft copolymers are capable of delivering drugs at constant rates over an extended period of time. Also in previous works developed by our group, different types of starches were grafted with acrylic monomers offering good quality for controlled drug delivery (Alias, Goñi, & Gurruchaga, 2007).

The aim of this investigation was the modification of inexpensive, naturally occurring and abundantly available carbohydrates for effective colon-targeted drug delivery. To this end, we have prepared and characterized high-amylose starch (A)-based copolymers in which HEMA was grafted onto a carbohydrate backbone due to its biocompatibility and hydrophilic character. Copolymer synthesis and characterization, as well as other experiments to test enzymatic resistance and the capacity of fermentation by colonic bacteria, were carried out. Finally, tablets developed with our copolymers were tested to observe the dissolution behavior of the drug and model protein.

2. Experimental

2.1. Materials

Amylose (70% of amylose content starch from maize, Tg = 110 °C, Viscosity = 28–43 [Specific viscosity], pH 4.0–7.0 [20% aqueous suspension]).

2 hydroxyethyl methacrylate (HEMA) (Merck, Germany) and Ceric ammonium nitrate (CAN) (Fluka, Germany).

Anhydrous theophylline (Th) ($M_w = 180.20$) (Sigma–Aldrich), albumin from bovine serum (SBA) (Sigma).

Sodium hydrogen phosphate heptahydrate (Sigma–Aldrich), sodium phosphate monohydrate (Sigma–Aldrich), sodium chloride (Panreac, Spain), potassium chloride (Sigma–Aldrich), hydrochloride acid (Panreac, Spain) and citric acid monohydrate (Sigma–Aldrich).

Pancreatin (Sigma), α-amilase (Sigma) from hog pancreas. Boehringer enzymatic kit (Roche, Germany).

Valeric acid (Sigma–Aldrich), isopropyl alcohol (Sharlab).

2.2. Methods

2.2.1. Synthesis of A-HEMA copolymers

The synthesis of starch-based graft copolymers by Ce (IV) ion method has been studied extensively by our research group (Alias et al., 2007; Echeverria, Silva, Goñi, & Gurruchaga, 2005; Goni, Gurruchaga, Vazquez, Valero, & Guzman, 1992), obtaining very good results for a lot of monomers, HEMA among them.

The polysaccharide A (4 g), that was dispersed in the reactor containing 290 ml of bidistilled water previously (Echeverria et al., 2005) and the assembly was placed in a thermostatic bath at 30 °C under a constant light source. Thus, later, 0.094 mol (11.43 ml) of HEMA distilled monomer were added. After 15 min, 10 ml of the initiator solution (CAN) (0.1 M ceric ammonium nitrate in 1 N nitric acid) were added quickly.

After 4 h, the reaction was stopped using a hydroquinone aqueous solution (1%). Bidistilled water was used to remove the nitric acid from the product. The obtained product was frozen and dried by lyophilization (Cryodos-80 TELSTAR) obtaining a white powder.

Once the product was dried, it was forming agglomerates, reason because it was milled until homogeneous powder was obtained.

The obtained solid is composed of the grafted copolymer, unreacted carbohydrate and PHEMA homopolymer. This would be the composition of a so-called "crude copolymer".

2.2.2. Grafting yields

To characterize the graft copolymer we followed various steps. For greater accuracy, the ungrafted carbohydrate should be removed; however, we saw that the amount of the remaining carbohydrate was less than 3%, so this step was ruled out. As described previously (Goni et al., 1992), different characterization parameters of graft copolymers were calculated:

- Percent grafting efficiency (% GE = percentage weight of graft copolymer with respect to total product) to determine the quantity of homopolymer formed in the reaction.

$$\%\ \mathrm{GE} = \frac{\text{Graft copolymer (g)}}{\text{Total product (g)}} \times 100$$

- Percentage grafting (% G = percentage weight of grafted acrylic polymer with respect to grafted carbohydrate) to determine the ratio between the acrylic and carbohydrate components.

$$\%\ \mathrm{G} = \frac{\text{Grafted acrylic polymer (g)}}{\text{Grafted carbohydrate (g)}} \times 100$$

- Percentage total conversion (% TC = percentage weight of total acrylic polymer with respect to initial monomer).

$$\%\ \mathrm{TC} = \frac{\text{total acrylic polymer (g)}}{\text{Initial monomer (g)}} \times 100$$

- Weight increase (% WI = percentage weight increase of the final product with respect to the weight of initial carbohydrate).

$$\%\ \mathrm{WI} = \frac{\text{Total product (g)}}{\text{Initial carbohydrate (g)}} \times 100$$

- Total weight: weight of the total product after copolymerization reaction.

2.2.3. Particle size distribution

Formulators of controlled-release matrices should take into account the particle size of the polymer incorporated into the matrix, since particle size can be one of the factors that affects hydration and, thus, the rate of gel formation and drug release (Alderman, 1984).

However, with respect to its influence on drug release, the polymeric matrix matters much more than the particle size distribution when considered alone (Echeverria et al., 2005).

Owing to the complexity and heterogeneity of particles, the particle size distribution was measured by laser ray scattering using a Malvern Mastersizer 2000 analyzer equipped with an accessory Hydro 2000 SM and an ultrasonic probe in order to avoid clusters of the particles. The sample was thoroughly dispersed in ethyl acetate and a continuous particle suspension under ultrasounds was exposed to a laser ray. An average of three tests was performed for each sample.

2.2.4. Scanning electron microscopy (SEM)

The surface and morphology of the particles were studied by SEM (SEM-Hitachi-S-2700) with and accelerating voltage of 15 kV. Previously, the surface of the powders was coated with gold.

2.2.5. Rheological study

Drugs are released in the body by a leaching process through the polymeric matrix, which swells with physiological fluids. Thus, the hydrophilicity, the swelling capacity, and even the capacity of forming gel are desirable and indeed necessary and the release process depends closely on these polymer characteristics.

Polymers of HEMA have been studied previously, demonstrating its hydrophilicity (Hill, O'Donnell, Pomery, & Whittaker, 1995). In previous works of our group the Percentage of Equilibrium Water Content (%EWC) was measured for A-HEMA copolymer giving these values:

$$\%\mathrm{EWC} = \frac{100(\mathrm{Ws} - \mathrm{Wd})}{\mathrm{Ws}}$$

	pH 1.5	pH 5	pH 8
%EWC	53.29 ± 3.29	55.24 ± 3.03	57.18 ± 3.47

%EWC Equilibrium Water Content.
Ws Weight of the swollen matrix.
Wd Weight of the dried matrix.

To determine the viscosity of the different swollen graft copolymers, cone-plate mode was used (C60/2) with a 6.0 cm of diameter and 2° of angle. Every sample was tested at 37 °C. Deformation amplitude sweeps were carried out prior to the frequency sweeps (between 10^{-2} Hz and 50 Hz) to determine the zone of lineal viscoelasticity. In this zone, storage modulus G′ and dynamic viscosity η' remain constant and the shift equivalent to deformation, $\gamma°$, increases lineally when deformation amplitude grows (Zárraga, 2001).

The following dynamic functions: storage modulus G′, loss modulus G″ and the complex dynamic viscosity η^* help us to understand the rheological behavior of our system.

2.2.6. Enzymatic degradation

Native starch may not be suitable in some controlled drug delivery systems due to its substantial swelling and rapid enzymatic degradation in biological fluids (Tuovinen et al., 2004). However, this characteristic enzymatic degradation could be considerably decreased by graft copolymerization of starch. α-amylase is the main enzyme involved in the hydrolysis of 1,4-α-D-glucosidic linkages in starch, so a solution of these enzymes was used in the enzymatic hydrolysis. To carry it out, (Fredriksson et al., 2000) 50 mg of carbohydrate or graft copolymer were dispersed in a capped bottle with 100 ml of 0.022 M K–Na–Phosphate buffer (pH 6.9) containing sodium chloride (0.4 M) and tempered at 37 °C for 10 min under constant magnetic stirring (300 rpm). Porcine pancreatic α-amylase (500 U) was immediately added and samples (1 ml) taken after 1, 2, 5, 8 and 24 h of incubation. The samples were rapidly transferred to small centrifugation tubes. Samples were centrifuged at 3000 rpm for 5 min to remove the remaining starch or copolymer in the sample, preventing the continuation of the enzymatic hydrolysis.

The extent of hydrolysis was calculated as the proportion of starch degraded to maltose at different incubation times. Maltose concentration was calculated from the sample supernatant using a Boehringer enzymatic kit for the determination of maltose. 100 mg of starch was considered 0% degradation and 100 mg of maltose was considered 100% degradation (in the case using a dispersion of 100 mg of starch).

2.2.7. In vitro dissolution tests

In vitro dissolution testing is an established method to control the release kinetics of drugs from solid matrices. The dissolution testing of theophylline, procaine hydrochloride, and BSA, was performed in a USP apparatus 1 (basket method) at 60 rpm. In the paddle assembly the tablets were introduced in a basket to prevent the floating (sink conditions). Theophylline and procaine hydrochloride were used as low and high-water solubility drug, respectively. It is known that BSA is not a therapeutic protein but, as in many research groups of controlled release, BSA was used as model protein due to its reasonable cost.

Previous to the dissolution tests, tablets of theophylline, procaine hydrochloride, BSA and graft copolymer were prepared by mixing and compressing components in a hydraulic press until a crushing strength of 80 N. The tablet composition was 25/75 in weight: model drug/A-HEMA copolymer (tablet weight: 500 mg). Three tablets of each composition were tested individually in 900 ml of buffer solution at 37 °C. Different dissolution mediums

were used depending on the pH required: pH 1.5 (gastric fluid), pH 5, pH 6.8 and pH 8 (intestinal fluids).

Pancreatin was added in some cases to check possible enzyme influence in the release profiles. Pancreatin (1750 U) was added when using pH 6.8 as is recommended by US Pharmacopoeia. Aliquots of 2 ml were manually taken from the dissolution vessels. The concentration of the drug delivered was determined by UV–VIS spectrophotometry at their maximum absorbance: 271 nm for the anhydrous theophylline, 291 nm for procaine hydrochloride and 277 nm for the BSA. Each data point is the average of three individual measurements.

The different tests that were carried out are shown in Table 2.

2.2.8. In vitro colonic fermentation by anaerobic bacteria

It has been demonstrated that high-amylose starch is partially resistant to the enzymes present in the small intestine but totally degradable by anaerobic colonic bacteria present in the colonic area. Short-chain fatty acids (SCFAs) and other substances (hydrogen, methane and carbon dioxide) are obtained as fermentation products (Nordgaard, Mortensen, & Langkilde, 1995). So, "in vitro" fermentation assays were carried out simulating the colonic environment to check if our high-amylose starch as well as its copolymers was susceptible to degradation.

Fresh stool specimens were obtained <30 min after defecation from five healthy Wistar rats, free to eat without restrictions and without record. Faeces (4.2 g) were pooled and homogenized under anaerobic conditions (N_2) in 30.8 g of isotonic sodium potassium chloride (NaKCl 100 mM, NaCl and 50 mM KCl) obtaining a 12% final dispersion fecal medium. The carbohydrate samples (200 mg) were administered as dry powder to small Erlenmeyer flasks closed by septum. Five milliliters of fecal dispersion were added to each flask obtaining 40 mg sample/ml of fecal dispersion. Samples were homogenized under anaerobic conditions (N_2) and placed in a thermostatic bath at 37 °C. Samples were incubated for 1, 2, 4, 6, 24 and 48 h of fermentation and fecal suspensions without additions served as control incubations. Freezing terminated the bacterial fermentation and specimens were stored at −18 °C until analysis. When analysis was carried out, samples were defrosted and passed by syringe filters to remove solid particles.

As explained in previous works, SCFAs are the main products of carbohydrate anaerobic fermentation. For this reason Acetic acid, Propionic acid and Butiric acid were determined by gas–liquid chromatographic analysis after filtration (Nordgaard et al., 1995). 0.5 μl of filtered sample were injected into a Shimazu GC-14 B gas chromatograph equipped with a 0.53 mm megabore, 30 m long DB-WAXTER cross-linked Polyethilenglycol of high-polarity column with a film thickness of 1 μl. The carrier and make up gas was nitrogen with flow rates of 120 kPa of pressure. Injection and flame ionization detector temperatures were 200 °C. The oven temperature was 125 °C (5 min)/ 15 °C × min/ 180 °C (10 min). SFCA concentrations were calculated from areas of gas–liquid chromatography peaks (the internal standard was valeric acid).

3. Results and discussion

3.1. Synthesis of A-HEMA copolymers

After filtering and washing, over 13.5 g of A-HEMA were obtained. This quantity is referred to "crude copolymer".

The choice of the polymeric excipient is of obvious importance to get the desired release profile. To this aim, a good characterization of powders is necessary. Some of the physico-chemical properties of powders and other characterisations were studied in this work, so Grafting yields, Particle size distribution, Scanning electron microscopy (SEM), Rheological study, Enzymatic degradation, In vitro dissolution tests and In vitro colonic fermentation by anaerobic bacteria studies were carried out.

3.2. Grafting yields

The different grafting yields are shown in the Table 1. In general all the yields are quite high.

The value of %WI shows that the weight of the final product after the reaction was more than three times the weight of the initial carbohydrate.

Focusing on % GE, it can be observed that almost all the HEMA have been grafted onto the amylose chain and almost none of the PHEMA homopolymer was formed.

The percentage grafting % G was also high indicating the strong tendency of the polymer to graft onto the carbohydrate backbone.

%TC reveals the quantity of monomer (EMA) that polymerized forming either grafts or homopolymer, and the values showed that almost all of it polymerized in every case.

3.3. Particle size distribution

The obtained particle size distributions are shown in Fig. 1 (a) belongs to amylose and (b) to A-HEMA copolymer. Looking at the figure, it is possible to see the particle size distribution that the virgin amylose has and the distribution of the A-HEMA copolymer after milling. The knowledge of this parameter is very important to formulate a controlled drug tablet because the size distribution could determine the applicability of these copolymers like the excipient to develop matrices for direct compression.

So, increasing particle size, flux properties are improved, but powder compaction decreases due to the decrease of the contact surface. The particle size can also affect to the hydration capacity and to the gel formation speed, which will determine the drug release profiles.

Table 1
Yields of A-HEMA graft copolymerization

Graft copolymer	Total weight(g)	% WI	% GE	% G	% TC
A-HEMA	13.54 ± 0.63	342.30 ± 4.31	98.98 ± 0.60	249.09 ± 5.53	80.35 ± 0.48

Total Weight: total weight of the copolymer obtained in the reaction.
% WI: Weight increase (percentage weight increase of the final product with respect to the weight of initial carbohydrate).
% GE: Percentage grafting efficiency (percentage weight of graft copolymer with respect to total product) to determine the quantity of homopolymer formed in the reaction.
% G: Percentage grafting (percentage weight of grafted acrylic polymer with respect to grafted carbohydrate) to determine the ratio between the acrylic and carbohydrate components.
% TC: Percentage total conversion (percentage weight of total acrylic polymer with respect to initial monomer). This parameter refers to the polymerized mononer, either as the homopolymer or copolymer.

Table 2
Characteristics of the different dissolution tests

Excipient (375 mg)	Model drug (125 mg)	pH	Enzyme presence	Figure
A-HEMA	Theophylline	1.5	No	5
		5.0	No	5
		6.8	No	5
		8.0	No	5
A-HEMA	Hydrochlorine procaine	1.5	No	6
		5.0	No	6
		6.8	No	6
		8.0	No	6
A-HEMA	BSA	1.5	No	7
		5.0	No	7
		6.8	No	7
		8.0	No	7
A-HEMA	BSA	6.8	Yes	8
		6.8	No	8

After the A-HEMA graft copolymer synthesis, milling is required to obtain a fine powder to make the correspondent tablets. With the milling, we can control particle size according to our interests.

3.4. Scanning electron microscopy (SEM)

SEM allows much high-magnification of the grafted copolymer particles and is possible to see the shape and the surface aspect of them. Fig. 2 shows that grafting introduces big changes on the surface and in the size of the carbohydrate particles. The grafting also causes an irregular morphology with high-heterogeneity creating lobule aggregates. The wrinkled or porous topography should enhance the hydrophilia and water absorption capacity.

3.5. Rheological study

To obtain an adequate rheological characterization, apart from the viscosity, it is necessary to know the storage and loss modulus (G′ and G″). These parameters will tell us whether the polymer performs like a gel, and therefore, whether it can act as a barrier to drug diffusion.

Fig. 3a shows the amplitude sweep of A-HEMA copolymer. The obtaining of this graph let us select the suitable deformation value in which frequency sweeps will be done. The selected value was $\gamma = 0.15$.

Next, to ascertain G′ and G″ at different strain frequencies, we performed a frequency sweep. Fig. 3b reveals that the storage modulus overcomes the loss modulus, $G' > G''$, and does not show any significant dependency on frequency, so the definition of gel is fulfilled.

This behavior allows us to confirm that A-HEMA graft copolymers fulfil the first necessary condition for good control of drug release.

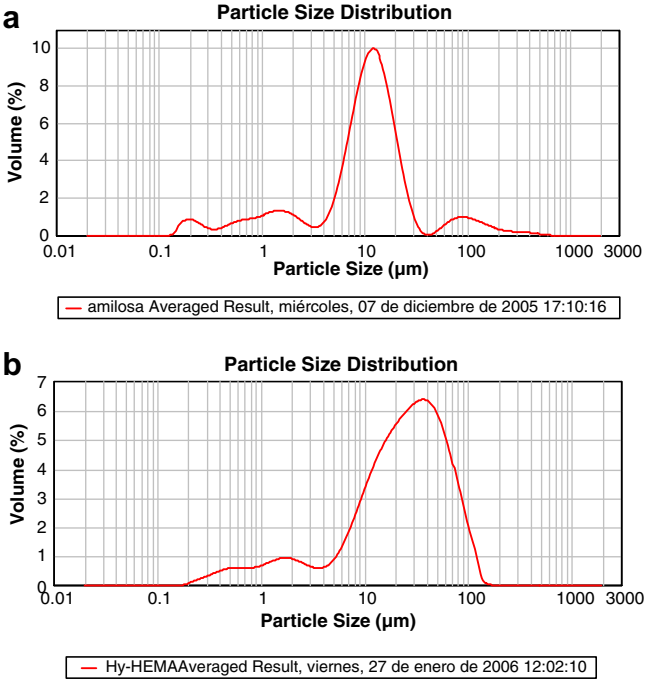

Fig. 1. Particle size distribution of (a) Amylose and (b) A-HEMA graft copolymer.

3.6. Enzymatic degradation

Fig. 4 shows the enzymatic hydrolysis kinetics of High-amylose starch and the hydrolysis of A-HEMA copolymer. As it is possible to see, the rate of hydrolysis of the graft copolymerized amylose increases considerably with respect to the carbohydrate.

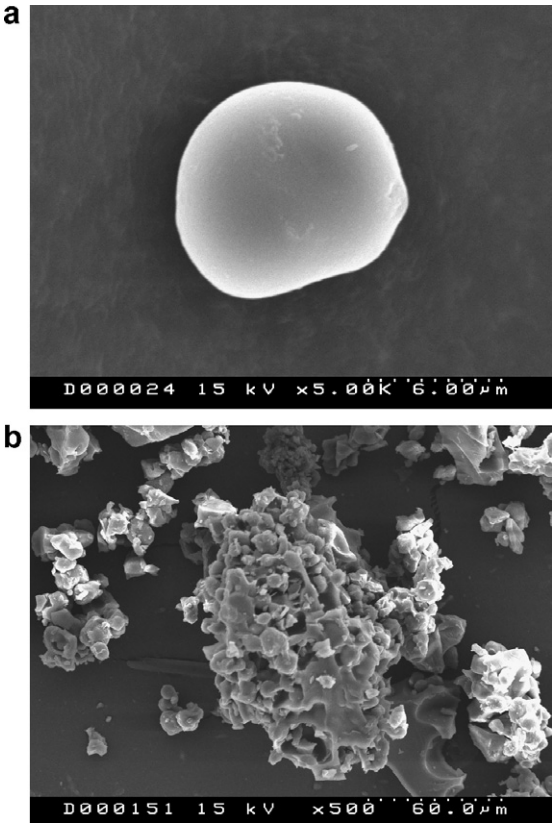

Fig. 2. SEM micrographs of particles: (a) Amylose and (b) A-HEMA graft copolymer.

As it can be observed in Calinescu, Mulhbacher, Nadeau, Fairbrother, and Mateescu (2005), high-amylose starch modifications alter amylolysis in α-amylase presence. In previous works (Alias et al., 2007) was verified that the graft increases the enzymatic resistance. But in this case, by grafting HEMA monomer to the carbohydrate, lower enzymatic resistance has been obtained. This behavior is justified due to hydrophilic character that the material obtains when carbohydrate is copolymerized. The copolymer has strong tendency to interact with the water in which enzymes are dispersed, forming and hydrogel where the enzymes are in direct contact with the carbohydrate chain which permits the hydrolysis.

In principle, this behavior is not desirable because the tablet could disaggregate at short times but copolymerizing with HEMA more plasticity is obtained, necessary quality to maintain tablet morphology. So, copolymerizing amylose with HEMA, two consequences are obtained, on one hand, lower enzymatic resistance and on the other hand, more plasticity that improve tablet morphology.

3.7. In vitro dissolution tests

Dissolution assays of Theophylline (Fig. 5) were performed at pH 1.5 (stomach), pH 5 (duodenum), pH 6.8 (small intestine). In general, it can be observed that this copolymer gives a good control to the controlled release, delivering 60–80% of the drug at 12 h. of testing.

Looking at the release profiles, substantial differences cannot be observed at different pH levels in either case.

In theory, PHEMA is not a polymer which we would expect to be influenced by pH, unlike Poly (Acrylic acid). But it is possible that other factors (swelling, disgregation, Theophylline dissolution...) could be affected when the pH is changed, and this could have a slight bearing on its ability to release the drug from the tablet.

Focusing now in procaine hydrochloride (Fig. 6), we van say that there is a light difference in the release at acidic pH, in which is faster than at basic pHs. This pH dependence can be due to the differences of procaine hydrochloride solubility when pH changes.

Fig. 7 shows the release kinetics of the BSA protein. In comparison with the kinetics of Theophylline and Procaine hydrochloride, it can be observed that the BSA release (Fig. 7) was a bit more slowly. Probably, this behavior is due to its high-molecular weight which hinders its diffusion through the matrix, because of that the release will be controlled by its low-diffusion through the gel layer that performs when water is absorbed (Korsmeyer, Gurny, Doelker, Buri, & Peppas, 1983). As it can be seen in Fig. 7, apart from releases at one pH (1.5, 6.8 and 8), release of BSA at a pH sequence (2 h at pH 1.5 and 10 h at pH 6.8) was carried out, in which the same tablet was placed in a pH 1.5 medium for 2 h. and later in a pH 6.8 medium till the end of the test, consecutively.

It can be observed that the release at acidic pH was more slowly and let us think that protein denaturalizes at this pH. This suggestion was ruled out because when pH sequences was performed, after 2 h. at acidic pH, the protein continues releasing at more basic pH, being perfectly detectable. So, we can conclude that using A-HEMA matrices, desired effect for protein release is obtained because quantities lower than 10% are released in the pH 1.5 (simulating stomach), keeping protein in the matrix to be released at pH 6.8 (simulating intestine). The fastest releases were at basic pHs. This big difference between acidic and basic medium could be related to the different protein solubility at different pHs. So, BSA has atypical pH dependence with respect to other proteins, because decreases when pH decreases (Khan, Roy, & Lalthantluanga, 1985).

Taking into account that the transit through the GI tract of the tablet is subject either to pH changes or enzymatic attack, dissolution tests at pH 6.8 were carried out also in the presence of pancreatin using BSA as a model protein. Pancreatin contains α-amylases that can hydrolyze the carbohydrate part of our copolymer. In view of the results observed for the enzymatic degradation of the copolymers, higher release than the obtained should be expected when pancreatin is added to the medium but Fig. 8 shows that the release presents no significant differences. It is possible to think that although

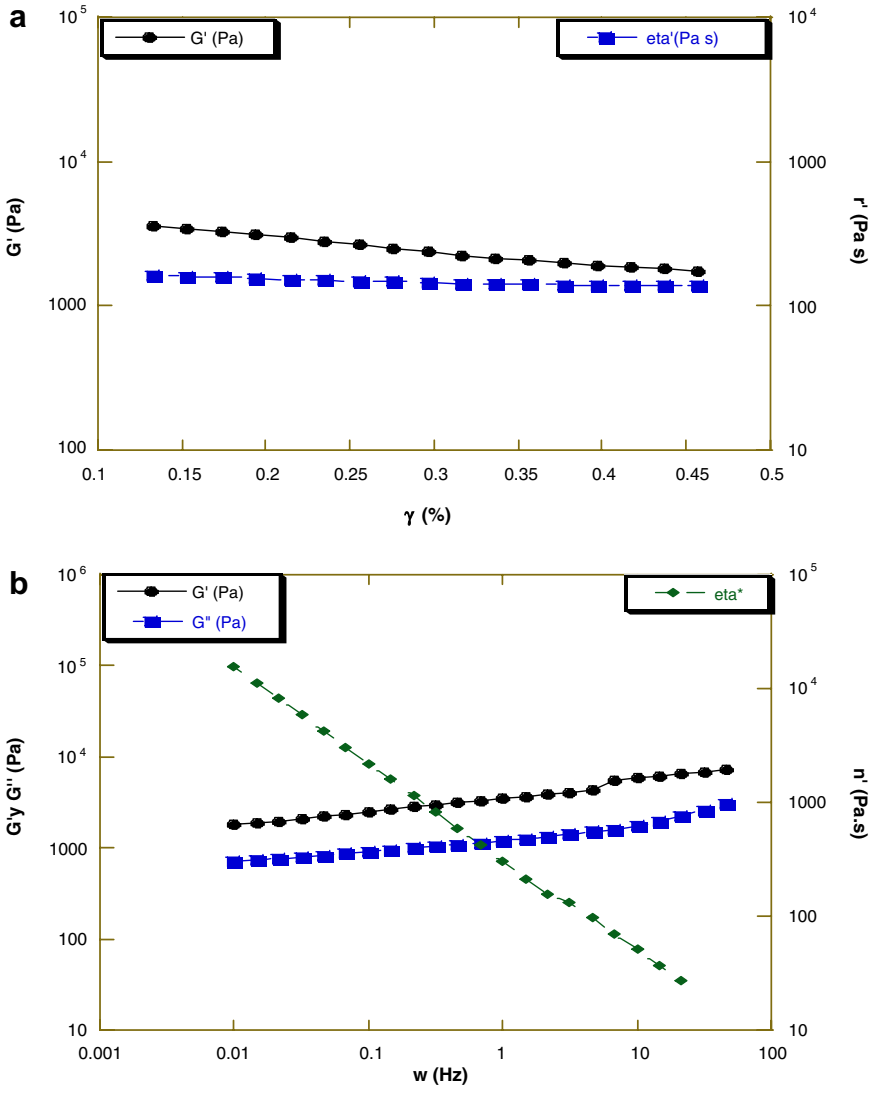

Fig. 3. (a) Amplitude sweep for A-HEMA copolymer at 37 °C, (b) frequency sweeps for A-HEMA copolymer at 37 °C under linear viscoelastic conditions. Storage modulus (G′), loss modulus (G″), dynamic viscosity (η') and complex viscosity (η^*).

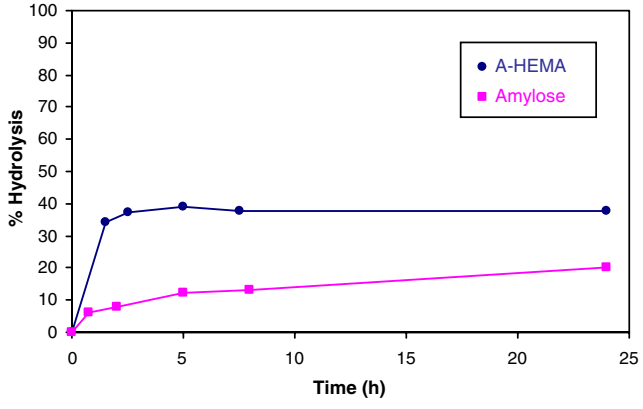

Fig. 4. α-Amylase enzymatic degradation of amylose and A-HEMA.

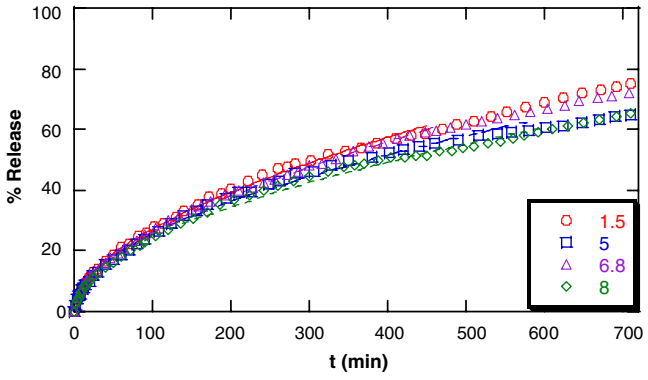

Fig. 5. Dissolution kinetics of theophylline at four different pHs from tablets of A-HEMA.

some copolymer degradation occurs, this is not enough to provoke higher protein release and diffusional effects are much more important than possible enzymatic degradation.

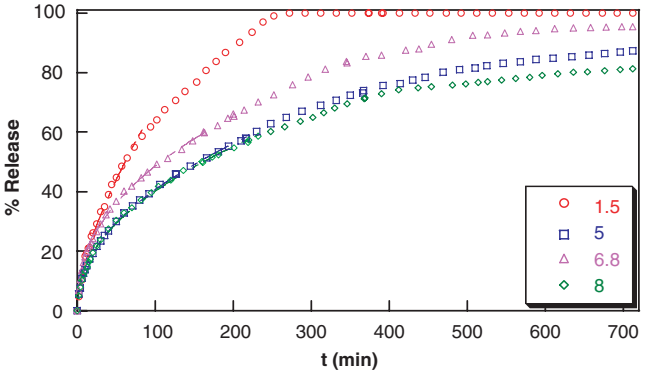

Fig. 6. Dissolution kinetics of procaine hydrochloride at four different pHs from tablets of A-HEMA.

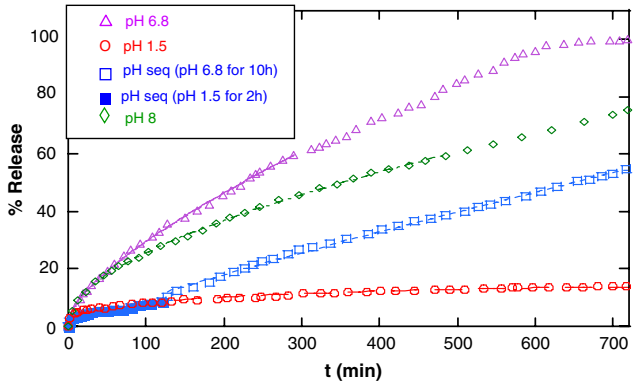

Fig. 7. Dissolution kinetics of BSA at different pHs from tablets of A-HEMA.

3.8. In vitro colonic fermentation by anaerobic bacteria

The test performed above pointed at the possibility of carrying proteins through the GI tract. Nevertheless, to obtain the necessary release in the colon tract, an evaluation of the digestibility of our copolymers under colonic conditions was required to verify if the carbohydrate part of the copolymers is degraded or not by colonic bacteria. Experiments using A-HEMA were carried out.

The degradation of the copolymer in 12% fecal homogenates with 40 mg added substrate/ml is illustrated in Fig. 9. The production of SCFA was subtracted from the production in control homogenates without added substrate, hence illustrating the formation of SCFAs from the added copolymer. The different SCFAs concentrations were calculated taking into account the amylose percentage is the system.

The in vitro production of SCFAs is observed which indicates that the carbohydrate fermentation is caused by the anaerobic bacteria (Nordgaard et al., 1995).

Looking at the graphs for the propionic and butyric acids, it can be observed that in all cases the amounts of acid produced are very small and detection is subject to experimental error. However, Acetic was the main acid produced in A-HEMA fermentation.

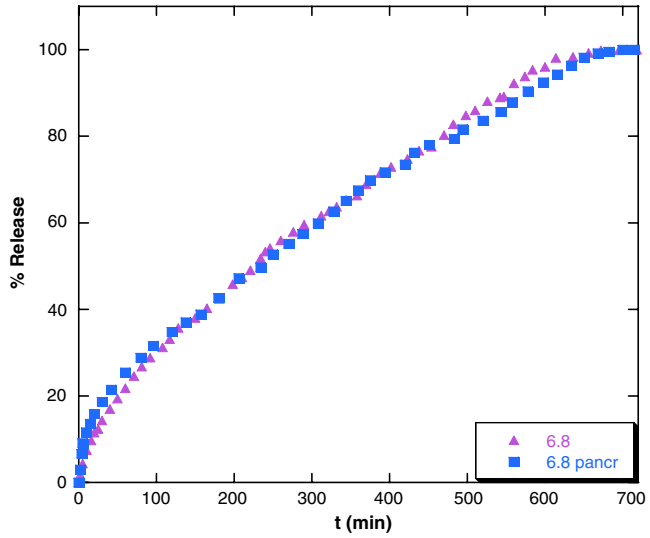

Fig. 8. Dissolution kinetics of BSA at pH 6.8 from tablets of A-HEMA adding pancreatin to dissolution medium.

4. Conclusions

Rheological tests reveals that the storage modulus overcomes the loss modulus, $G' > G''$, and does not show any significant dependency on frequency, so the definition of gel is fulfilled. This behavior allows us to confirm that A-HEMA graft copolymers fulfil the first necessary condition for good control of drug release.

Grafting HEMA monomer to the carbohydrate, lower enzymatic resistance has been obtained. This behavior is justified due to hydrophilic character that the material obtains when carbohydrate is copolymerized.

In general, it can be observed that this copolymer gives a good control to the controlled-release, delivering 60–80% of the Theophylline at 12 h. of testing. Substantial differences cannot be observed at different pHs with this drug. Using procaine hydrochloride, we van say that there is a light difference in the release at acidic pH, in which is faster than at basic pHs.

Using A-HEMA matrices, desired effect for protein release is obtained because quantities lower than 10% are released at pH 1.5 (simulating stomach), keeping protein in the matrix to be released at higher pHs (simulating intestine). The fastest releases were at basic pHs. This big difference between acidic and basic medium could be related to the different protein solubility at different pHs. In the test with added pancreatin, although some copolymer degradation occurs, this is not enough to provoke higher protein release and diffusional effects are much more important than possible enzymatic degradation.

The degradation of the material in the fecal fermentation system that simulated the luminal conditions in the human colon showed that A-HEMA generates amounts of SCFAs up to 48 h of fermentation. This indicates that the carbohydrate of A-EMA copolymers is susceptible to fermentation in spite of the HEMA coating around the

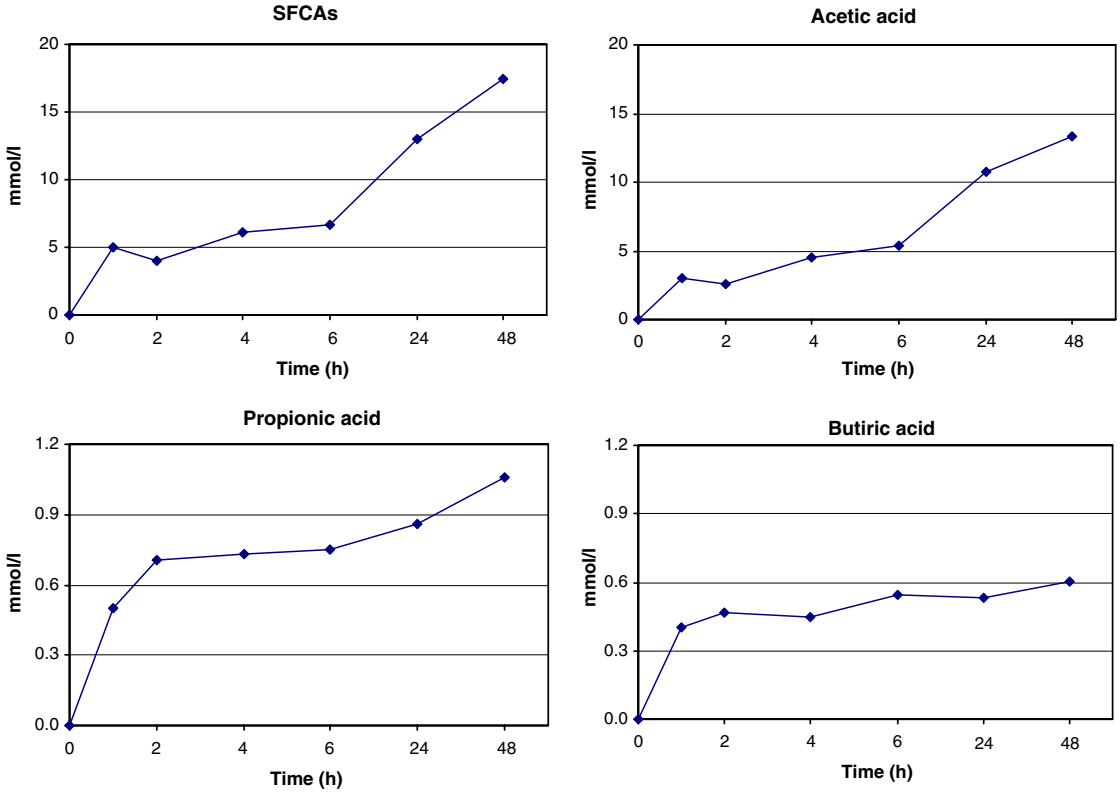

Fig. 9. Production of short-chain fatty acids (SFCAs), acetic acid, propionic acid and butyric acid in 12% fecal homogenate incubated with A-HEMA.

amylose backbone and that these materials could favor a colon targeted delivery.

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References

Alderman, D. A. (1984). A review of cellulose ethers in hydrophilic matrixes for oral controlled-release dosage forms. *International Journal of Pharmaceutical Technology & Product Manufacture, 5*(3), 1–9.

Alias, J., Goñi, I., & Gurruchaga, M. (2007). Enzymatic and anaerobic degradation of amylose based acrylic copolymers, for use as matrices for drug release. *Polymer Degradation and Stability, 92*(4), 658–666.

Benson, R. S. (2002). Use of radiation in biomaterials science. *Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms, 191*(1–4), 752–757.

Calinescu, C., Mulhbacher, J., Nadeau, E., Fairbrother, John Morris, & Mateescu, Mircea Alexandru (2005). Carboxymethyl high amylose starch (CM-HAS) as excipient for *Escherichia coli* oral formulations. *European Journal of Pharmaceutics and Biopharmaceutics, 60*(1), 53–60.

Casimiro, M. H., Leal, J. P., & Gil, M. H. (2005). Characterisation of gamma irradiated chitosan/pHEMA membranes for biomedical purposes. *Nuclear Instruments & Methods in Physics Research, Section B: Beam Interactions with Materials and Atoms, 236*, 482–487.

Hill, D. J. T., O'Donnell, J. H., Pomery, P. J., & Whittaker, M. R. (1995). A high resolution NMR investigation into the microstructure of HEMA and EEMA copolymers. *Polymer Gels and Networks, 3*(1), 85–97.

De Zárraga, M. A., (2001). Tesis Doctoral, UPV.

Echeverria, I., Silva, I., Goñi, I., & Gurruchaga, M. (2005). Ethyl methacrylate grafted on two starches as polymeric matrices for drug delivery. *Journal of Applied Polymer Science, 96*(2), 523–536.

Ferreira, L. M., Rocha, J. M. S., Andrade, M. E., & Gil, M. H. (1998). Preparation and characterization of polyethylene based graft copolymers. Applications in the immobilization of enzymes. *Radiation Physics and Chemistry, 52*(1–6), 207–212.

Fredriksson, H., Bjorck, I., Andersson, R., Liljeberg, H., Silverio, J., Eliasson, A.-C., et al. (2000). Studies on a-amylase degradation of retrograded starch gels from waxy maize and high-amylopectin potato. *Carbohydrate Polymers, 43*(1), 81–87.

Goni, I., Gurruchaga, M., Vazquez, B., Valero, M., & Guzman, G. M. (1992). Synthesis of graft copolymers of acrylic monomers on amylose: Effect of reaction time. *European Polymer Journal, 28*(8), 975–989.

Hebeish, A., El-Rafie, M. H., Higazy, A., & Ramadan, M. (1996). Synthesis, characterization and properties of polyacrylamide–starch composites. *Starch/Staerke, 48*(5), 175–179.

Khan, M. Y., Roy, Y. B., & Lalthantluanga, R. (1985). Anomalous salting-out behaviour of cyanogen bromide fragments of bovine serum albumin. *International Journal of Biological Macromolecules, 7*(4), 226–229.

Korsmeyer, R. W., Gurny, R., Doelker, E., Buri, P., & Peppas, Nikolaos A. (1983). Mechanisms of solute release from porous hydrophilic polymers. *International Journal of Pharmaceutics, 15*(1), 25–35.

Milojevic, S., Newton, John M. l., Cummings, John H., Gibson, Glenn R., Botham, R. Louise, Ring, Stephen G., et al. (1996). Amylose as a coating for drug delivery to the colon: Preparation and in vitro

evaluation using 5-aminosalicylic acid pellets. *Journal of Controlled Release, 38*(1), 75–84.

Nordgaard, I., Mortensen, P. B., & Langkilde, A. M. (1995). Small intestinal malabsorption and colonic fermentation of resistant starch and resistant peptides to short-chain fatty acids. *Nutrition (Burbank, Los Angeles County, California), 11*(2), 129–137.

Pourjavadi, A., & Zohuriaan-Mehr, Mohammad J. (2002). Modification of carbohydrate polymers via grafting in air. 2. Ceric-initiated graft copolymerization of acrylonitrile onto natural and modified polysaccharides. *Starch/Staerke, 54*(10), 482–488.

Trimnell, D., & Stout, E. I. (1980). Grafting acrylic acid onto starch and poly(vinyl alcohol) by photolysis. *Journal of Applied Polymer Science, 25*(10), 2431–2434.

Tuovinen, L., Peltonen, S., Liikola, M., Hotakainen, M., Lahtela-Kakkonen, M., Poso, A., et al. (2004). Drug release from starch-acetate microparticles and films with and without incorporated a-amylase. *Biomaterials, 25*(18), 4355–4362.