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Physical blends of starch graft copolymers as matrices for colon targeting drug delivery systems

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ABSTRACT

Colon targeting drug delivery systems have attracted many researchers due to the distinct advantages they present such as near neutral pH, longer transit time and reduced enzymatic activity. Moreover, in recent studies, colon specific drug delivery systems are gaining importance for use in the treatment of local pathologies of the colon and also for the systemic delivery of protein and peptide drugs.

In previous works, our group has developed different types of hydrophilic matrices with grafted copolymers of starch and acrylic monomers with a wide range of physicochemical properties which have demonstrated their ability in controlled drug release. Since the cost of synthesizing a new polymeric substance and testing for its safety is enormous, polymer physical blends are frequently used as excipients in controlled drug delivery systems due to their versatility. So, the aim of this work is to combine two polymers which offer different properties such as permeability for water and drugs, pH sensitivity and biodegradability in order to further enhance the release performance of various drugs. It was observed that these physical blend matrices offer good controlled release of drugs, as well as of proteins and present suitable properties for use as hydrophilic matrices for colon-specific drug delivery.

1. Introduction

Generally the small intestine is considered as the primary site for drug absorption and thus it is a preferred part of gastrointestinal tract (GI) for targeting the drugs with various controlled release technologies. Colon targeting drug delivery systems have attracted much research due to distinct advantages such as a near neutral pH, longer transit time and reduced enzymatic activity. Colon specific drug delivery not only increases the bioavailability of the drug target site, but also reduces the dose required as well as the site effects. Furthermore, colon targeted drug delivery systems are gaining importance in the treatment of local pathologies of the colon (Crohn's disease, inflammatory bowel disease, colonic cancer) and also for the systemic delivery of protein and peptide drugs (Ravi, Siddaramaiah, & Pramod Kumar, 2008).

For many reasons, oral drug delivery continues to be the preferred route of pharmaceutical administration of drug substances (Bae et al., 1991; Deshpande, Rhodes, & Shah, 1996). During the last two decades, polymers which swell in aqueous media have been used for preparation of oral sustained release dosage forms. Among solid dosage forms, tablets are very desirable owing to their easy preparation. Thus, a sustained release matrix tablet consists of a compressed compact form containing a mixture of one or more active ingredients, with one or more gel forming agents, which retard the release of the drug (Cardinal, 1984; Rao, Devi, & Buri, 1988).

In our group, we developed in previous works, different types of hydrophilic matrices with grafted copolymers of starch and acrylic monomers (Alias, Silva, Goñi, & Gurruchaga, 2008; Alias, Goñi, & Gurruchaga, 2007; Echeverria, Silva, Goñi, & Gurruchaga, 2005; Goni et al., 1992; Silva, Gurruchaga, & Goñi, in press), with a wide range of physicochemical properties which have demonstrated their versatility in controlling the release of drugs and good results including in in vivo testing (Ruiz Correa, 2005).

Since the cost of synthesizing a new polymeric substance and testing for its safety is enormous, a new focus has been to directly research the use of polymer blends of previously synthesized and tested polymeric materials as matrix excipients to retard drug release (Ebube & Jones, 2004). By simply varying the polymer blend ratio, broad ranges of system characteristics and thus, drug release patterns can be provided (Lecomte et al., 2005). Thus polymer blends are widely used in the pharmaceutical field to obtain controlled delivery systems (Lecomte et al., 2005; Karnichi et al., 1995; Nerurkar, Jun, Price, & Park, 2005; Patel & Patel, 2007; Raffin, Colome, Pohlmann, & Guterres, 2007; Samani, Montaseri, & Kazemi, 2003). This fact is demonstrated by the publication of numerous patents and research papers and their utilization in new products in the market place (Abbaspour, Sadeghi, & Afrasiabi Garekani, 2008; AlKhatib & Sakr, 2003; Cao, Langridg, & Van Gessel,

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2006; Dunbar & Sharma, 2000; Guo & Skinner, 2002; Hong & Oh, 2008; Piao & Chung, 2001).

In this work, the aim is to combine two polymers with different properties such as permeability for water and drugs, pH sensitivity and biodegradability to further enhance the release performance of various drugs.

Therefore, we prepared a new type of tablet with blends of two graft copolymers, which were synthesized previously in our laboratory, to obtain biodegradable copolymers suitable for the development of matrices which offer controlled release of proteins to be administered orally. We took high amylose starch (Am) based copolymers in which two kinds of acrylic monomers were grafted onto a carbohydrate backbone. The monomers chosen to be grafted were two different methacrylic monomers: on the one hand, methacrylic acid (MA), which is pH-sensitive because of its acid groups and on the other, 2-hydroxyethyl methacrylate (HEMA), with high hydrophilicity and of strong interest for biomedical applications. Using Am-HEMA copolymer as the hydrophilic matrix, we achieved very good results in controlling the release of drugs such as model proteins (Alias et al., 2008) as we have recently published. However, the graft copolymer Am-MA formed gels which do not offer adequate consistency and tend to disintegrate without being able to control the release of the drug. This finding led us to consider the possibility of using a physical blend of both copolymers in order to combine their characteristics separately in a single matrix and thus achieve a matrix with enough consistency to control the release of pH sensitive drugs and proteins and in this way enable us to modulate release depending on the place where the tablet was found. Thus, we formulated tablets with physical blends of both copolymers in different proportions.

2. Experimental

2.1. Materials

High amylose starch (70% of amylose content starch from maize, T_g = 110 °C, viscosity = 28–43 [specific viscosity], pH 4.0–7.0 [20% aqueous suspension]); HEMA and MA (Merck, Germany); Ceric ammonium nitrate (CAN) (Fluka, Germany); Potassium persulfate (K2S2O8) (Scharlau); Ethyl acetate (Oppac); Sodium phosphate dibasic heptahydrate (Sigma–Aldrich); Sodium phosphate monohydrate (Sigma–Aldrich); Sodium chloride (Panreac, Spain); Potassium chloride (Sigma–Aldrich); Hydrochloride acid (Panreac, Spain); Citric acid monohydrate (Sigma–Aldrich).

Anhydrous theophylline (Th) (Mw = 180.20) (Sigma–Aldrich); Procaine hydrochloride (Pr) (Mw = 272.80) (Sigma–Aldrich); Albumin from bovine serum (BSA) (Sigma); Pancreatin and α-amylase from porcine pancreas (Sigma); Maltose/Saccharose (Sucrose)/D-Glucose Boehringer enzymatic kit (Roche, Germany).

2.2. Methods

2.2.1. Synthesis of Am-HEMA and Am-MA copolymers

The synthesis of starch based graft copolymers by the Ce (IV) ion method has been extensively studied by our research group (Alias et al., 2007; Echeverria et al., 2005; Goni et al., 1992), obtaining very good results for many acrylic monomers, HEMA among these.

First, the polysaccharide Am (4 g) was dispersed in the reactor containing 290 ml of bidistilled water (Echeverria et al., 2005) and the assembly was placed in a thermostatic bath at 30 °C under a constant light source. Next, 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) was added.

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

Am-MA graft copolymer was synthesized using a potassium persulphate solution (5 g in 100 ml of bidistilled water) as the initiator (Bayazeed, Elzairy, & Hebeish, 1989). The reaction took place at 43 °C, under N_2 atmosphere and constant mechanical stirring.

In a typical experiment, 0.1765 mol of MA distilled monomer was dispersed in 100 ml of bidistilled water. The stirred mixture was deoxygenated by bubbling through a slow stream of N2 for 20–30 min. Next, 5 g of Am, 100 ml of bidistilled water and the initiator solution were added to the reaction medium. The mixture was allowed to react for a period of 4 h.

The graft copolymer was obtained by precipitation over a large volume of ethyl acetate. After filtration and washing, the reaction product was dried in an oven at 50 °C.

In both cases, as the dried product formed agglomerates, it was milled until a homogeneous powder was obtained.

2.2.2. Polymeric matrix blends

For this study, we made physical blends of both copolymers in different proportions, starting from a proportion of 50–50 and gradually increasing the acid copolymer: 60–40 and 70–30. Proportions of 80–20 and 90–10 were discarded from the outset because the amount of copolymer Am-HEMA was not enough to obtain tablets capable of withstanding the dissolution test without disintegrating.

2.2.3. FTIR

FTIR spectra measurements were recorded on a FTIR-Nicolet 50XC spectrophotometer. The graft copolymers and their physical blend spectra were obtained with potassium bromide (KBr) tablets.

2.2.4. Particle size distribution

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

2.2.5. Scanning electron microscopy (SEM)

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

2.2.6. Rheological study

To determine the viscosity of the different swollen graft copolymers, cone-plate viscometry was used (C60/2) with a diameter of 6.0 cm and a 2° angle. Every sample was tested at 37 °C. Deformation amplitude sweeps were carried out prior to the frequency sweeps (between 10^{-2} Hz y 50 Hz) to determine the zone of linear viscoelasticity. In this zone, storage modulus G′ and dynamic viscosity η′ remain constant and the shift equivalent to deformation, γ°, increases lineally when the deformation amplitude grows (De Zarraga et al., 2004).

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

2.2.7. Enzymatic degradation

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 this out (Fredriksson et al., 2000), 50 mg of carbohydrate or graft copolymer was 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 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.

2.2.8. In vitro release tests

Prior to the release tests, tablets of Th, Pr, BSA and mixture of graft copolymers (Table 1) were prepared by mixing and compressing the components in a hydraulic press until a crushing strength of 80 N was achieved. Three tablets of 500 mg of each composition were tested individually in 900 ml of buffer solution at 37 °C. The tablet composition was 25/75 (w/w): model drug/copolymers blends. Different dissolution media were used depending on the pH required: pH 1.5 (gastric fluid), pH 5, pH 6.8 and pH 8 (intestinal fluids).

In vitro release testing is an established method to test the release kinetics of drugs from solid matrices. The dissolution testing of Th, Pr and BSA, was performed in an USP apparatus 1 (basket method) at 60 rpm. In the paddle assembly the tablets were introduced in a basket to prevent floating (sink conditions). Th and Pr were used as low and high water soluble drugs, respectively. It is known that BSA is not a therapeutic protein but, as in much re-

Table 1
Polymeric matrix blends used in this study and release tests performed.

Polymeric matrix blend		Release tests
Am-MA	Am-HEMA	
50	50	Th; Pr; BSA
60	40	Th; Pr; BSA
70	30	Th; Pr; BSA
80	20	–
90	10	–

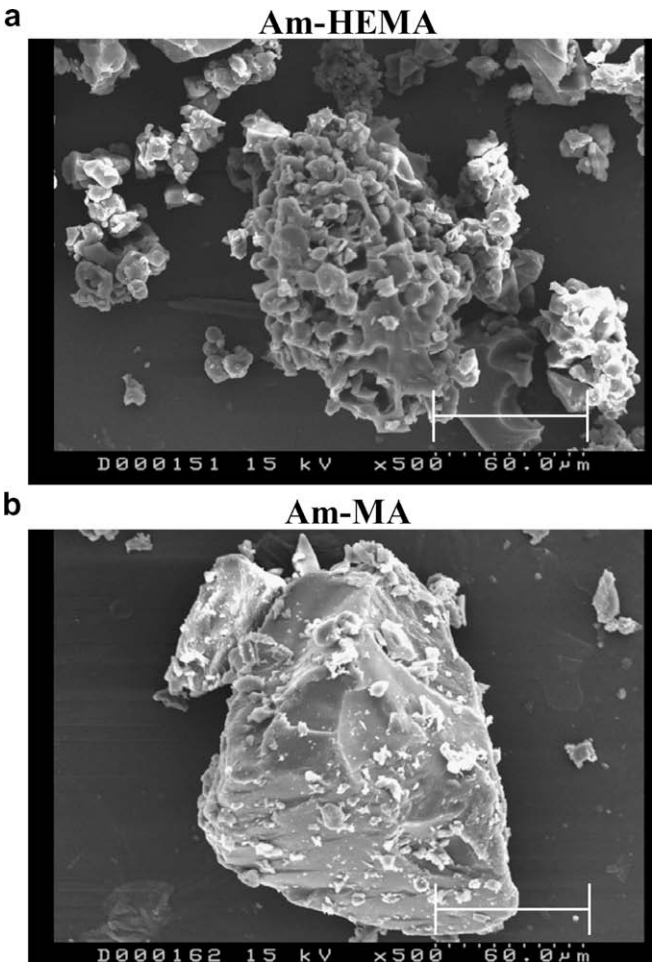

Fig. 2. SEM micrographs of (a) Am-HEMA and (b) Am-MA copolymers particles.

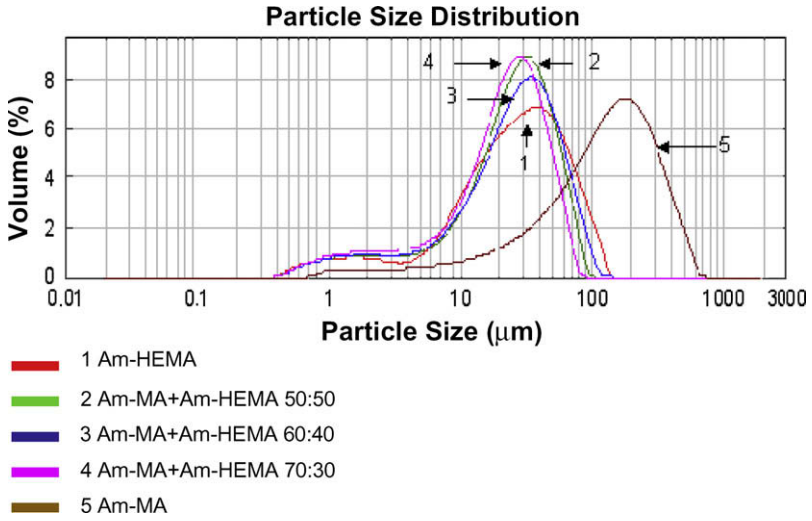

Fig. 1. Particle size distribution of Am-MA, Am-HEMA and their physical blends in different proportions such as 50:50, 60:40 and 70:30.

search on controlled release, BSA was used as the model protein due to its reasonable cost.

When the model drug was the protein, the release test was also carried out at a pH sequence, in which the tablet was placed at pH 1.5 for 2 h and subsequently at pH 6.8 medium till the end of the test.

A new test was performed to check possible enzyme influence in the release profiles. Thus, pancreatin (1750 U) was added to the dissolution medium at pH 6.8 as recommended by US Pharmacopoeia. Aliquots of 2 ml were manually taken from the dissolution vessels.

The concentration of the various delivered drugs was determined by UV–vis spectrophotometry at maximum absorbance: 271 nm for Th, 291 nm for Pr and 277 nm for BSA. Each data point is the average of five individual measurements.

3. Results and discussion

3.1. Synthesis of graft copolymers

The choice of the polymeric matrix is of obvious importance to achieve the desired release profile. To this aim, a good characterization of products is necessary, thus, some of the physicochemical properties of powders and other characteristics were studied in previous work (Alias et al., 2008; Silva et al., in press). These products offered a high percent grafting (%G = percentage weight of grafted acrylic polymer with respect to grafted carbohydrate): 249.09 ± 2.53% for Am-HEMA graft copolymers and 287.45 ± 2.07% for Am-MA. The high %G indicates the strong tendency of the polymer to graft onto the carbohydrate backbone.

3.2. Particle size distribution and SEM

Fig. 1 shows the particle size distribution of mixtures Am-MA + Am-HEMA 50:50, 60:40 and 70:30 along with that of Am-MA and Am-HEMA copolymers. We can see that by mixing both copolymers, particle size distributions more similar to the Am-HEMA copolymers, that is, presenting smaller particles, are achieved. This gives us an idea of good miscibility between both copolymers and even the possibility of interparticle interaction.

As we mentioned in previous works (Alias et al., 2008; Silva et al., in press), the particle size measurements were taken after an analogous milling process of the dry reaction product. Therefore, the small differences found in the size distribution of both

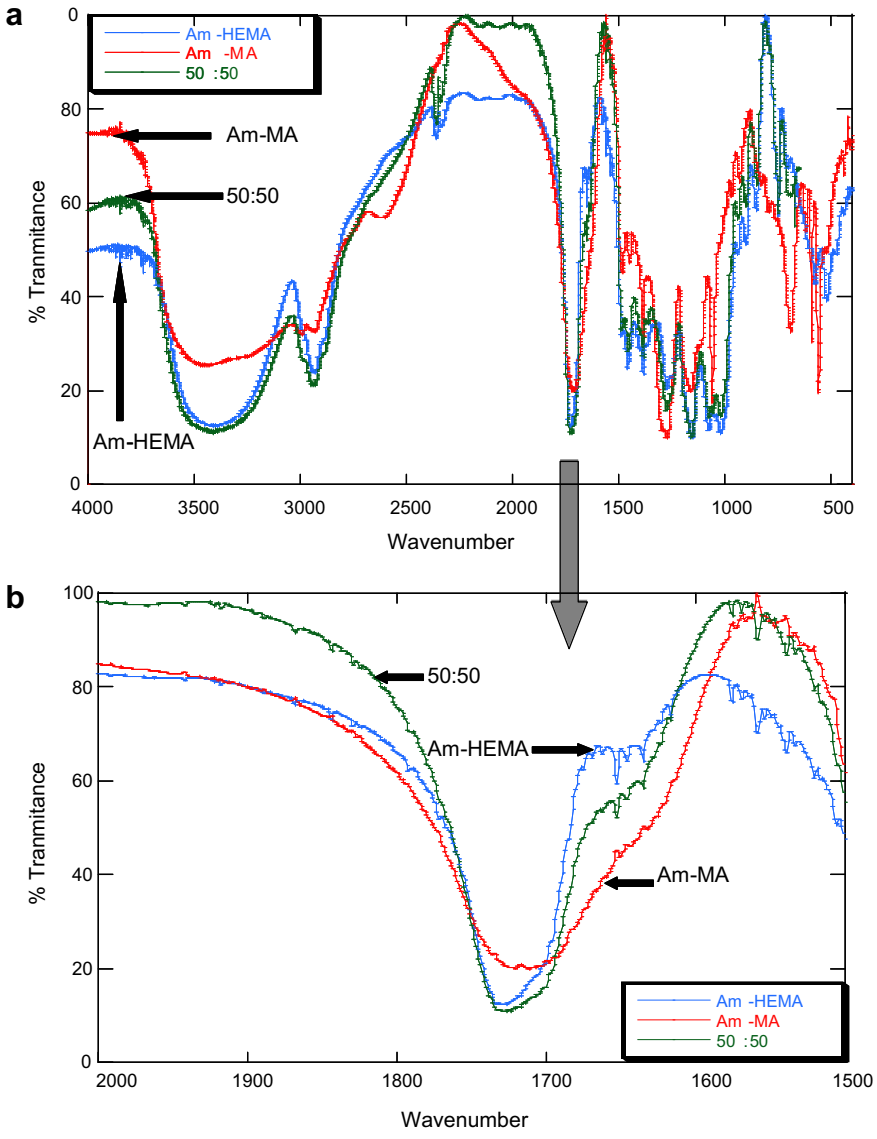

Fig. 3. (a) IR spectra of the Am-HEMA, Am-MA and the mixture 50:50. (b) The carbonyl zone extended.

graft copolymers were attributed to the higher porosity of the Am-HEMA particles. Fig. 2 shows the surface and the inside of the graft copolymers particles by SEM.

3.3. FTIR

Interacting forces are involved when different particles approach each other. In this case, the contribution of hydrogen bonds could appear enhanced due to the mixing of particles with OH groups of the Am-HEMA on the one hand and with carboxyl groups of the Am-MA on the other. Thus, IR spectra of each product and of the mixture are registered to see if this kind of interaction is noteworthy.

Fig. 3a represents the IR spectra of both copolymers and a 50/50 mixture of them. At 2500 cm^{-1}, a broad band can be observed in the Am-MA spectrum attributed to OH groups and to the intramolecular H bonds. Fig. 3b allows us to see that this signal disappears in the mixture as the number of methacrylic acid units decreases. Thus, we can say that the mixtures Am-MA + Am-HEMA give rise to H bonds and ionic interaction.

3.4. 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. Rheological measurements allow us to evaluate the gel characteristics of the swollen polymers.

In previous publications (Alias et al., 2008; Silva et al., in press) we proved that Am-MA and Am-HEMA copolymers performed as gels. In this study, we measured storage and loss modulus, G′ and G″ of the different mixtures in order to check their gel formation capacity. First, we selected a suitable deformation value to perform the frequency sweeps. Fig. 4a shows the amplitude sweep of Am-MA + Am-HEMA mixture 50:50; we take only this mixture as a representative example. Thus, the selected value was $\gamma = 0.10$.

Fig. 4b reveals that the storage modulus overcame the loss modulus, G′ > G″, and did not show any significant dependency on frequency, so the definition of gel was fulfilled. This behaviour

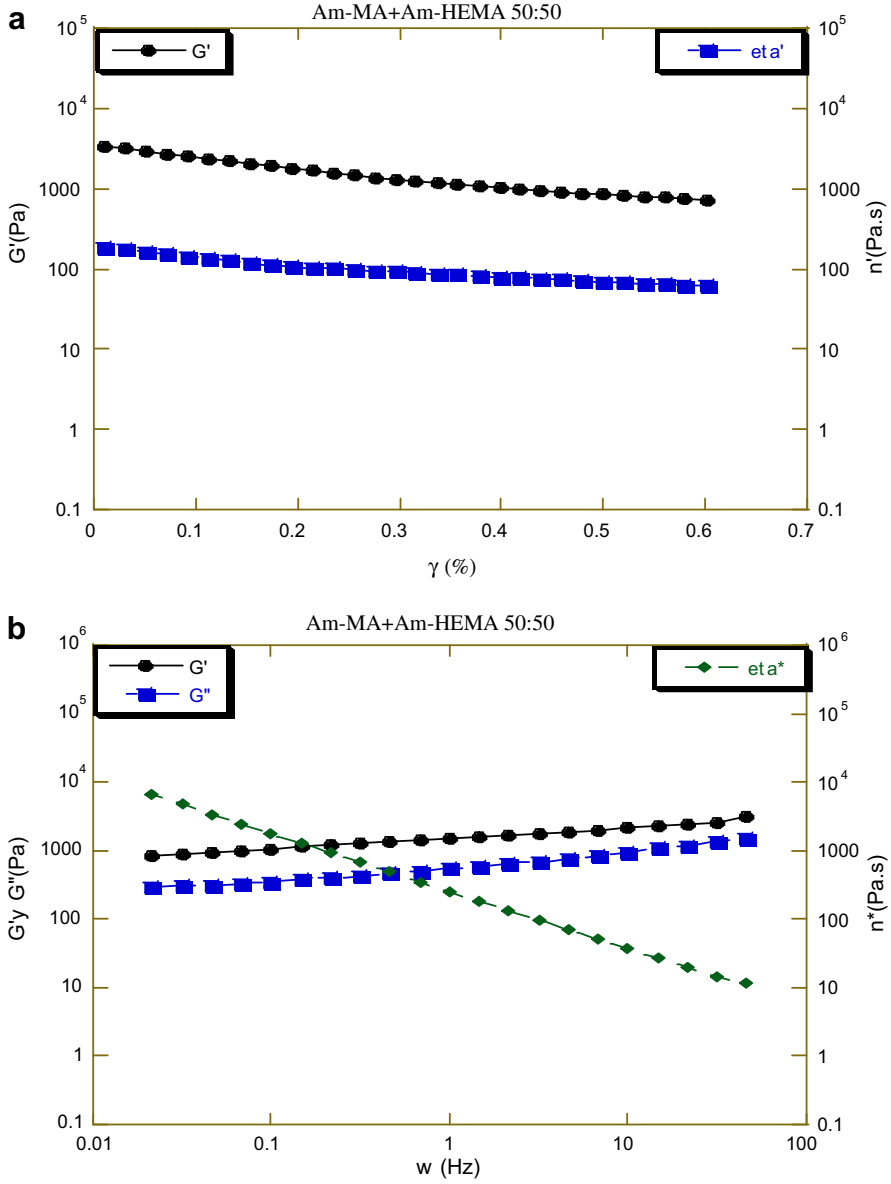

Fig. 4:. (a) Amplitude sweep for Am-MA + Am-HEMA 50:50 mixture at 37 °C, G′ (●) and η′ (■). (b) Frequency sweeps for mixture of copolymers at 37 °C under linear viscoelastic conditions, G′ (●), G″ (■) and η* (◆).

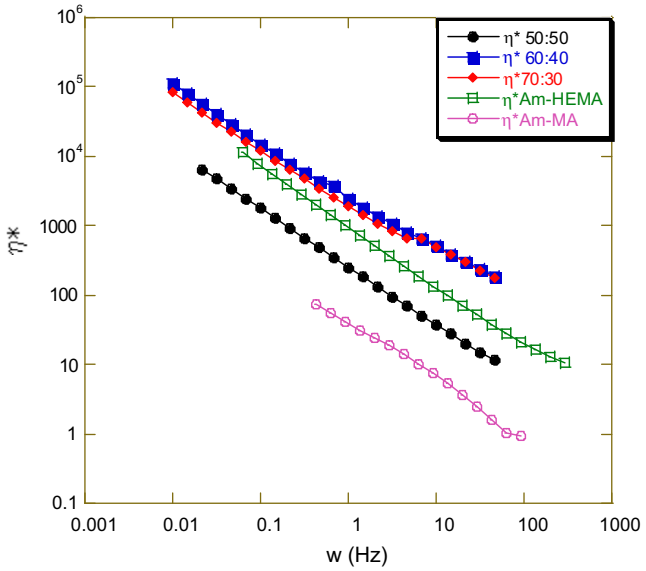

Fig. 5. Complex viscosity vs frequency sweeps for Am-MA and Am-HEMA copolymers and their mixtures. Am-MA (O), Am-HEMA (□), 50:50 (●), 60:40 (■) and 70:30 (◆).

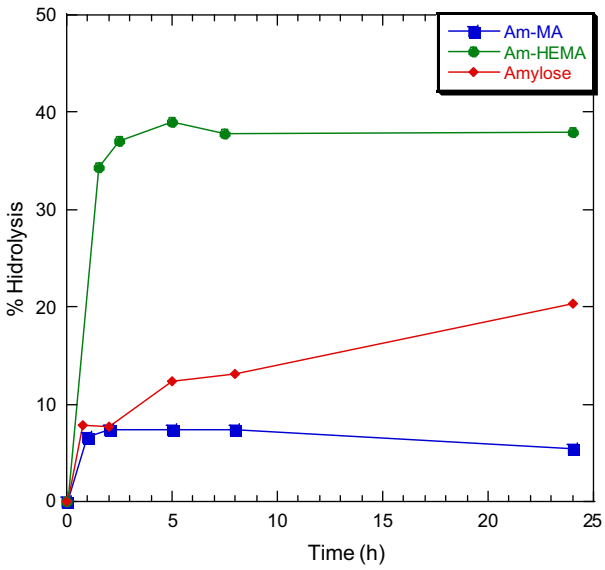

Fig. 6. α-Amylase enzymatic degradation of high amylose starch, Am-MA and Am-HEMA copolymers. Am-HEMA (●), Am-MA (■) and high amylose starch (◆).

allows us to confirm that the mixtures of Am-MA and Am-HEMA graft copolymers fulfilled the first necessary condition for good drug release control.

In order to compare how the mixture affects gel viscosity, Fig. 5 shows the plot of η^* vs ω(Hz) for both copolymers and their blends. We can see that the viscosity of the 50:50 mixture lies halfway between the viscosity values of the pure copolymers. But, as the mixture is enriched with the Am-MA copolymer, the viscosity of the mixture increases, surpassing even that of the Am-HEMA. This may be due to increased interaction between H bonds resulting from increased numbers of carboxyl groups and HEMA hydroxyl groups, leading to greater entanglement and thus generating an increase in viscosity.

3.5. Enzymatic degradation

The characteristic enzymatic degradation of starch can be modified by graft copolymerization. In a previous work (Alias et al., 2007), we demonstrated that the formation of grafted acrylic branches gave rise to coating which wrapped the carbohydrate backbone protecting it against the enzymatic attack. As we can see in Fig. 6, in the case of Am-HEMA we cannot apply the same theory. However, in the case of Am-MA copolymer, the rate of hydrolysis of the copolymer decreased considerably with respect to that of the ungrafted carbohydrate. The two graft copolymers studied are highly hydrophilic materials. Thus, both copolymers have a strong tendency to interact with water in which enzymes are dispersed thus forming a hydrogel where the enzymes are in direct contact with the carbohydrate. This could increase the starch degradation that offers a disrupted structure owing to the grafting reaction. However, looking at the Am-MA hydrolysis, its values do not reach 10%. This seems contradictory but we must take into account that in the case of MA grafting, other factors come into play. Given that the mechanisms of enzymatic attack on carbohydrates are multiple and complex, they are going to influence countless factors (Calinescu et al., 2005; Mazur & Nakatani, 1993). One particularly important factor is the particle size which may limit the accessibility of enzymes (Colonna, Leloup, & Buleon, 1992; Eerlingen, Jacobs, & Delcour, 1994; Fredriksson et al., 2000). In our case, the larger particle size of the Am-MA copolymer together, mainly, with the morphology of the particle, limit the accessibility of the enzymes. As we have seen, the particles of Am-MA copolymers had a totally non-porous solid inside, while those of the Am-HEMA had a porous structure that understandably promote the accessibility to α-amylase. Therefore, this solid and non-porous interior of acid copolymer will be an important factor that will hinder enzymatic attack.

Moreover, another very important factor that can influence the lower biodegradability of this copolymer are the carboxylic groups of acid monomer. As it is well known, enzymes are a special class of proteins and are thus composed of amino acids, which present amine and carboxylic groups capable of reacting with the acid group of MA creating strong interaction between them, thus resulting in lower enzyme activity (Aksoy, Tumturk, & Hasirci, 1998). In other words, the α-amylase may attack the glycosidic linkages of amylose or interact with copolymer carboxylic groups resulting in a decrease of enzyme activity on the carbohydrate. To this end, together with the competition between the carbohydrate and the monomer for the enzyme, we must also take into account the decrease in pH that also occurs in the environment when MA acid groups are present. Recent studies concerning the stability of α-amylase have shown that activity of the enzyme drops by 78% and 90% at pH 4 and 3.8, respectively, and remains idle at pH 3.6 (Babacan & Rand, 2007). This leads us to believe that the decrease in pH of the MA copolymer environment is also influenced by the slightest action of the enzyme in this copolymer.

3.6. In vitro release tests

As we have explained before, these tests were carried out using tablets formulated with the various copolymers and three different model drugs. To perform the test, the tablet must not disintegrate. When we used only Am-MA copolymer, the tablet does not withstand the release test when the drugs are Th and Pr. For this reason, a mixture of two graft copolymers, Am-MA and Am-HEMA, were used, with the aim of combining the pH sensitivity of the Am-MA copolymer with the consistency that Am-HEMA brings to the matrix and thus form tablets that do not disintegrate during the release test.

Fig. 7 shows the release assays for the tablets formulated with the mixture of Am-MA + Am-HEMA in proportions of 50:50,

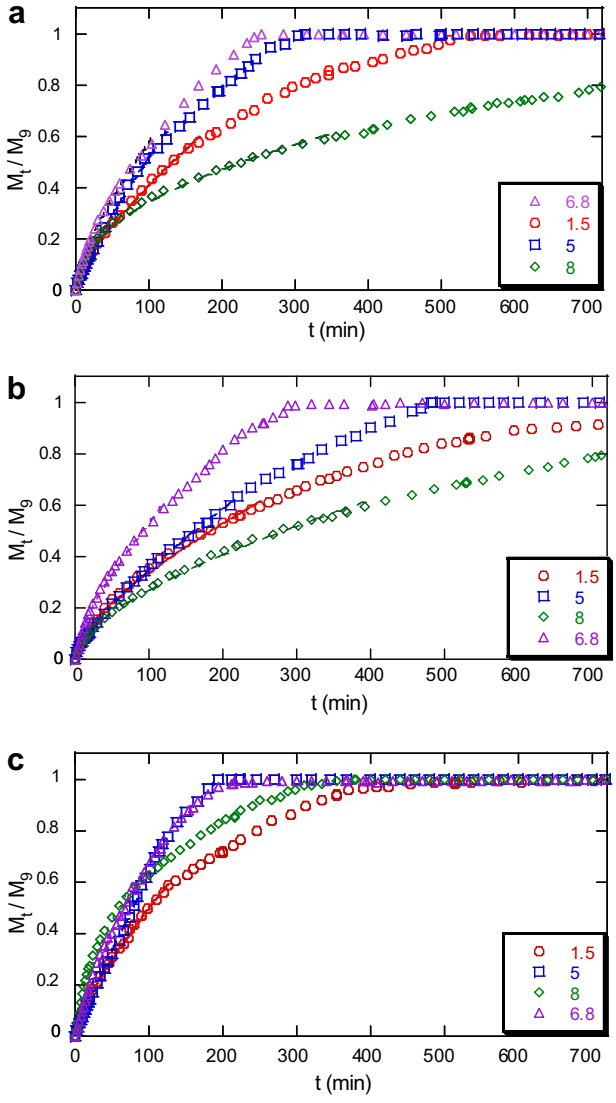

Fig. 7. Dissolution kinetics of theophylline at four different pHs (1.5 ○; 5 □; 6.8 △ and 8 ◊) from tablets of Am-MA + Am-HEMA mixtures: 50:50 (a), 60:40 (b) and 70:30 (c).

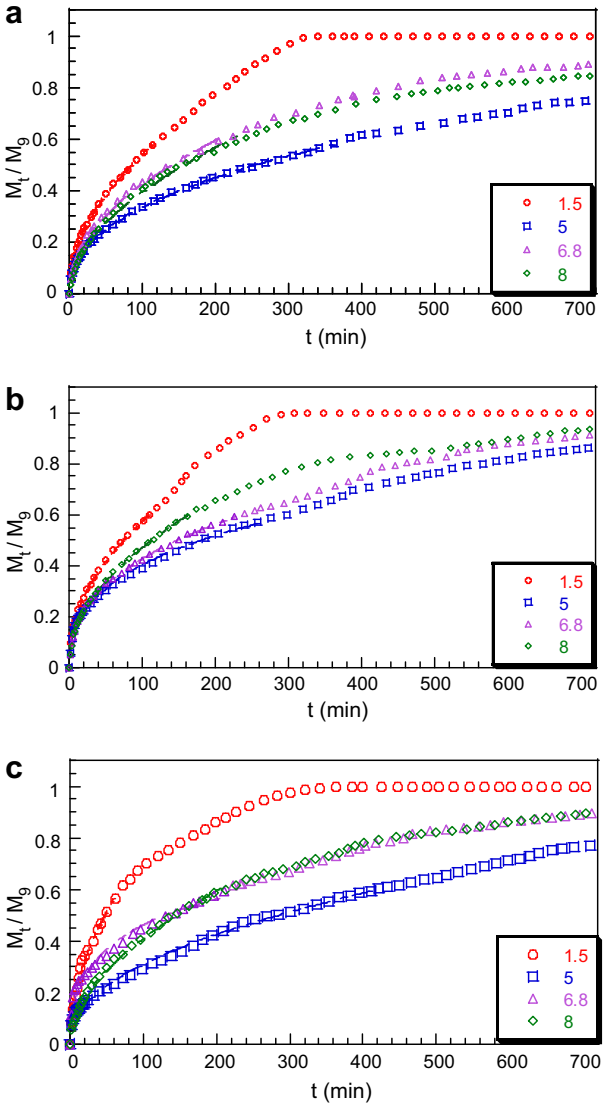

Fig. 8. Dissolution kinetics of procaine hydrochloride at four different pHs (1.5 ○; 5 □; 6.8 △ and 8 ◊) from tablets of Am-MA + Am-HEMA mixtures: 50:50 (a), 60:40 (b) and 70:30 (c).

60:40 and 70:30, respectively, for the different pHs using Th as the model drug. If we look at the release kinetics of these mixtures, we can say that in all cases, by adding the Am-MA copolymer we are bringing a degree of pH sensitivity to the formulation that was not present with the Am-HEMA copolymer alone (Alias et al., 2008). At it is well known, polymethacrylic acid swells at basic pHs and collapses at acid pHs. However, it was noted, when the proportion of the Am-MA in the mixture is greater than 60%, the pH sensitivity is reduced. It might be logical to think the greater the amount of the pH sensitive component added, the more pH sensitive the release of the model drug. However, when more Am-MA is added, less Am-HEMA copolymer is added to the matrix thus creating a less resistant tablet which disintegrates quickly and accelerates kinetic release of the model drug thus negating any anticipated differences due to pH. Despite this, controlled dosage of Th was achieved in all the different proportions over the 12 h that the test lasted. Release was consistently faster at neutral–basic pHs, whereas more basic and acid pHs slowed it down. This may also be due to the effect of the concentration of the Am-MA copolymer acid groups in the matrix.

With reference to Pr release (Fig. 8), the first important noteworthy conclusion is that in all cases, this matrix is capable of controlling the dosage of this highly water-soluble drug during the 12 h of the test. This finding is very sought after by the pharmaceutical industry.

Another clear conclusion that can be observed is that with the three different ratio mixtures, almost identical release profiles are achieved so that we can say that enriching the mixture with Am-MA does not affect its drug release capacity. However, the kinetics for these mixtures were somewhat slower than the profiles for the Am-HEMA matrix. This may be due to the influence of the particle size of the drug component which makes diffusion through the matrix more difficult, counteracting thus the accelerating effect of the acid copolymer as noted in the case of Th. In all cases, the release kinetics are faster in acid pH media, whereas at more basic pHs, the three kinetics are very similar. This sensitivity to pH is caused by two contributions mainly, on the one hand, the presence of the Am-MA copolymer in the matrix, and the other hand, the increased solubility of the Pr with respect to acid pHs.

As it can be seen in Fig. 9, apart from the release profiles at various pHs, the release of BSA at a pH sequence was carried out too. By comparing these kinetics with those of Th and Pr, it can be observed that the protein release was a bit slower. Probably, this per-

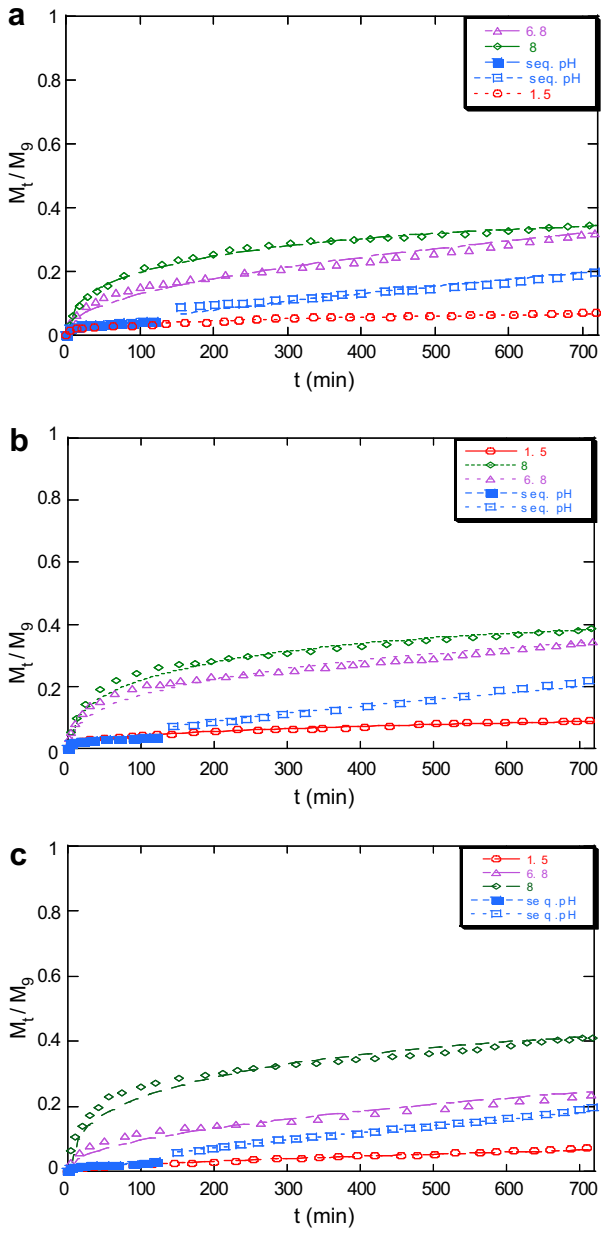

Fig. 9. Dissolution kinetics of BSA at different pHs from tablets of mixtures Am-MA + Am-HEMA: (a) 50:50, (b) 60:40 and (c) 70:30. pH 1.5 (○), pH 6.8 (△), pH 8 (◊) and sequence of pH (□).

formance is due to its high molecular weight which hinders its diffusion through the gel layer formed when water is absorbed (Korsmeyer, Gurny, Doelker, Buri, & Peppas, 1983). Moreover, as happened with Th and Pr, the addition of large quantities of Am-MA to the mixture does not cause great differences in the release kinetics of the protein.

Additionally, it can be observed that the release at acid pH was slower than at the more basic pHs and could suggest that protein denaturalizes at acid pHs. This suggestion was ruled out because when pH sequence testing was performed, after 2 h, at high acid pH, the protein was still being released at a more basic pH and was clearly detectable. In a previous paper, the released protein was checked by circular dichroism (CD) and size exclusion chromatography (SEC) and both analysis confirmed that most of the protein remains intact (Silva et al., in press).

The release differences of the matrix formulations in acid and basic media could also be attributable to the different protein solubility capacity at different pHs. BSA has an atypical pH dependence with respect to other proteins, because the solubility of BSA increases as the pH increases (Khan, Roy, & Lalthantluanga, 1985). So, at basic pHs, the solubility of the protein in the media is high and release is favoured. On the other hand, at acid pH, the lower solubility of the BSA coupled with the reduced swelling of the matrix makes release much slower.

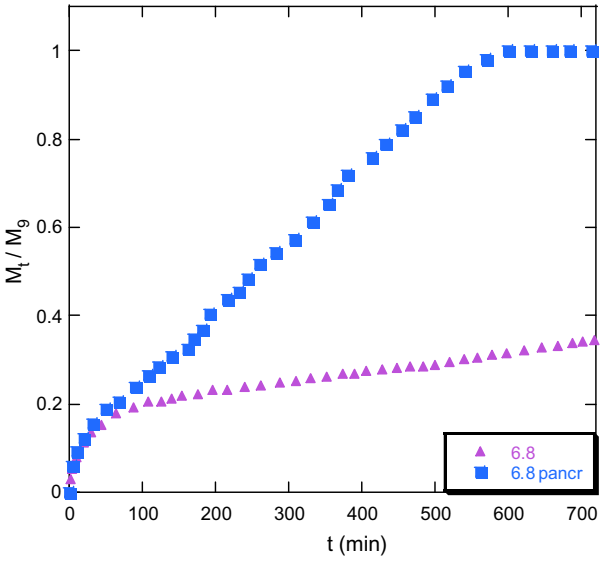

Fig. 10. Dissolution kinetics of BSA at pH 6.8 from tablet of Am-MA + Am-HEMA 60:40 adding pancreatin to dissolution medium (■) and without pancreatin in dissolution medium (□).

When Am-HEMA copolymers kinetics (Alias et al., 2008) were compared with the other mixtures at more basic pHs, release was seen to be somewhat slower than when the matrix only contained Am-HEMA. This may be due to the fact that the Am-MA copolymer lends greater swelling to the formulation at these pHs, which may slow down in part diffusion of the drug.

Taking into account that the transit through the GI tract of the tablet is subject either to pH changes or enzymatic attack, release tests at pH 6.8 were carried out also in the presence of pancreatin. Pancreatin contains α-amylases that can hydrolyze the carbohydrate part of the copolymer. As we can see in Fig. 10, the presence of pancreatin in the release test is very noticeable. From 2 h after the digestion process begins, a high acceleration of the release of the protein is observed. This effect is very different to the observed for both graft copolymers alone (Alias et al., 2008; Silva et al., in press). In the case of Am-HEMA the effect of the pancreatin was negligible, while in the case of Am-MA the execution of the test was not possible. Thus, we can say that the pancreatin causes the disintegration of the Am-MA copolymer in the blend, so the release of the drug is controlled primarily by the Am-HEMA.

4. Conclusions

Through this work, we have seen that the physical blends of the two graft copolymers of starch synthesized in our group offered interesting results in the release of the various model drugs. Using a matrix mixture of Am-MA and Am-HEMA copolymers, a combination of the properties of both copolymers such as permeability for water and drugs, pH sensitivity and biodegradability was obtained.

Furthermore, a modification of some of the properties of both copolymers was observed after the mixing and compaction process attributed to the interaction between the different particles. This presented very good results in the controlled release of different

drugs as well as proteins. These results were not obtained when the copolymers were used separately.

So, we can conclude that the physical blends of these two graft copolymers present good properties for use as hydrophilic matrices for colon-specific drug delivery.

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