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The Influence of Crosslinking Amylose-Methacrylic Acid Graft Copolymers on the Release of BSA

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Summary: One of the biggest challenges in the drug delivery field is to obtain oral systems for the release of peptides and proteins, enabling their use as therapeutic agents for clinical applications. The aim of this work is to obtain biodegradable copolymers suitable for the development of matrices which offer controlled release of proteins to be administered orally.Graft copolymers of amylose-methacrylic acid were synthesized using different amounts of the crosslinker N, N′-methylenediacrylamide. The influence on the release profiles of bovine serum albumin (BSA) was researched.

Keywords: crosslinking; drug delivery systems; graft copolymers; proteins; starch

Introduction

The availability of large molecular weight protein and peptide based drugs, due to recent advances in the field of molecular biology, represents an important breakthrough in the treatment of a number of illnesses such as Crohn's disease, diabetes etc.[1]

Up to now, the only way of administrating proteins has been parenteral, but this system presents several clear disadvantages. Oral delivery is always the preferred route of administration over injection because it increases patient compliance and comfort, is easy to administer, reduces costs and potentially improves efficacy.[2–8] In this case it has the added advantage of closely simulating the physiological delivery of proteins.

The proper carrier for the oral delivery of a protein drug depends on where the drug is intended to act, how the gastrointestinal (GI) tract acts on the drug and how the drug acts on the GI tract before reaching this site of action. The carrier should be used to ensure that the drug does not cause toxicity to the lining of the digestive tract prior to reaching the colon, does not become sequestered along the way in an organ like the stomach and, therefore, not reach its target, and does not become inactivated or altered in any way by the environment of the GI tract.[9–10]

Bearing in mind that the use of crosslinked polymers increases the resistance to digestion, the aim of this work is to obtain biodegradable crosslinked copolymers to form an appropriate matrix for the controlled release of proteins for oral administration. The influence of the amount of crosslinking agent in the release kinetics was also researched.

Materials

Starch with high amylose content (Am), was used as received. Methacrylic acid (MA) (Merck) was distilled under suitable conditions to remove the inhibitor. N, N′-methylenediacrylamide (MB) (Merck) as crosslinking agent[11] and potassium persulphate (Scharlau) as initiator were used as supplied.

The initiator solution was prepared by dissolving 5 g of potassium persulphate in 100 ml of bidistilled water.

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Bovine serum albumin (BSA) (Sigma) was used as the model drug.

Experimental Methods

The graft copolymer (Am-MA) was synthesized using potassium persulphate as the initiator.[12] The crosslinking agent was added in 2%, 1%, 0.5% and 0.3% over the total number of moles of monomer. The reaction takes place at 43 °C, under N_2 atmosphere and constant mechanical stirring.

In a typical experiment, 0.1765 mol of MA and the desired amount of crosslinking agent were dispersed in 100 ml of bidistilled water. The stirred mixture was deoxygenated by bubbling through a slow stream of N_2 for 20–30 minutes. 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 hours.

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

As a model drug for our study we used BSA, which is a protein of high molecular weight and high water solubility. A mixture of 75% of copolymer and 25% of model drug were compressed in a hydraulic press (Graseby Specac) to obtain tablets with an average mass of 500 ± 5 mg and a crushing strength of 8 kp.

The rate of absorption of an orally administered solid drug is often controlled by the rate of dissolution of the drug in the G.I. So we assessed the release kinetics of the drug in an ''in vitro'' dissolution test at 37 °C. The dissolution testing was performed with a mechanical stirrer with a paddle that operated at 60 rpm. In the paddle assembly, the tablets were introduced in a basket to prevent their floating.[13] To study the effect of the pH, different dissolution tests were carried out. Thus, we studied the behaviour of tablets in three different buffered solutions: pH = 1.5 (gastric fluid), 6.8 and 8 (intestinal fluids). In addition, a dissolution test was carried out performing a pH sequence: pH = 1.5 for two hours and 6.8 until the end of the experiment. The volume of dissolution media was 300 ml.

The concentration of the protein delivered was determined by UV-VIS spectrophotometry at their maximum absorbance 279 nm. The dissolution kinetics was adjusted to the model of Peppas for hydrophilic matrices.[14]

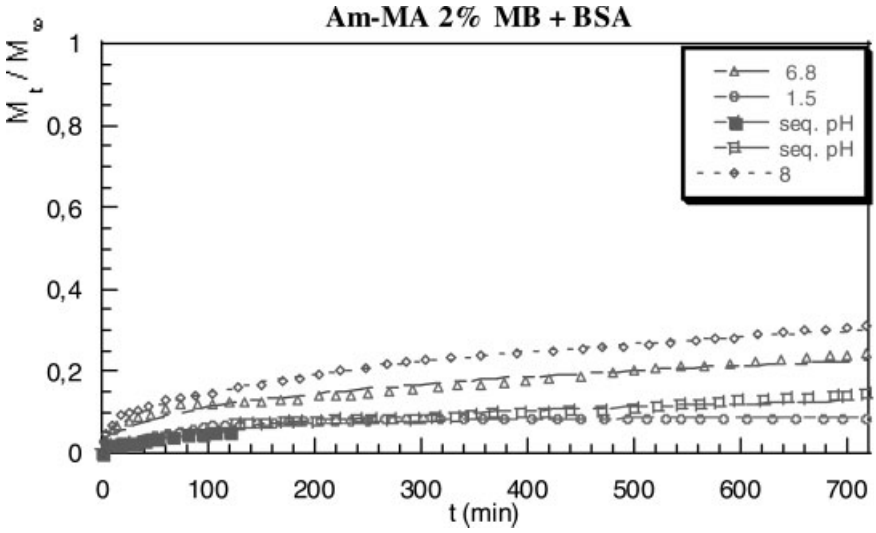

Figure 1.
Release kinetics of BSA from copolymer Am-MA with 2% of MB.

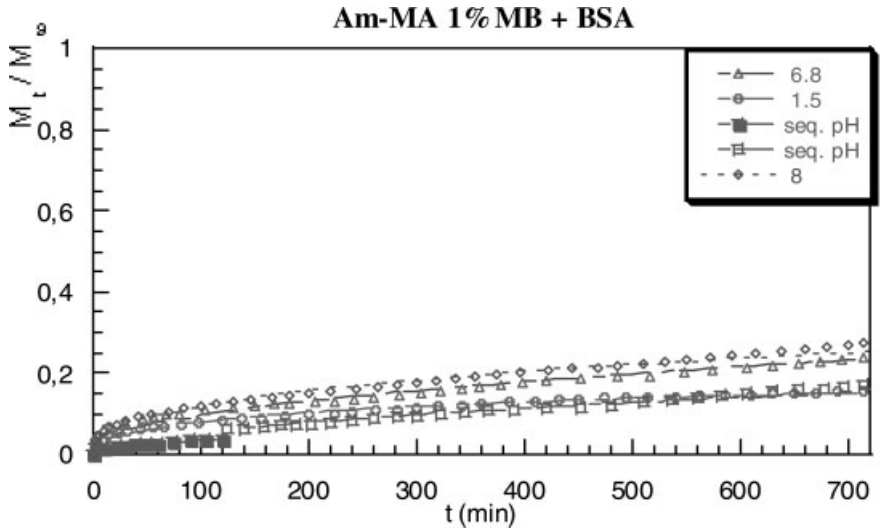

Figure 2.
Release kinetics of BSA from copolymer Am-MA with 1% of MB.

Results and Discussion

Figures 1–5 show the release kinetics of BSA from matrices obtained using the copolymer Am-MA without crosslinking agent and copolymers with different amounts of MB.

In all cases, the release kinetics under the most acid pH is the slowest, and as the pH becomes more basic, release occurs at a faster dissolution speed. Since the swelling of copolymers containing MA is pH-dependent, this behaviour is a consequence of the high swelling produced at high pH.

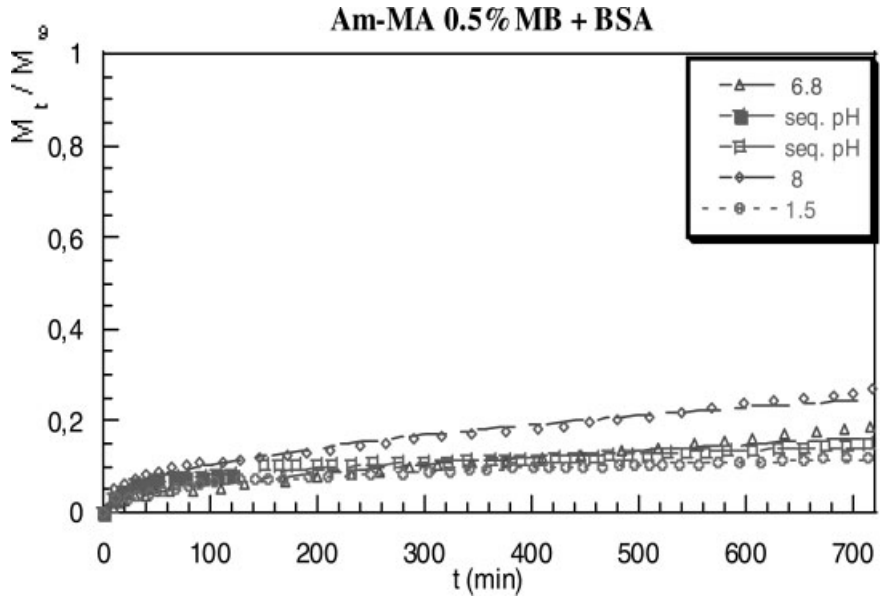

Figure 3.
Release kinetics of BSA from copolymer Am-MA with 0.5% of MB.

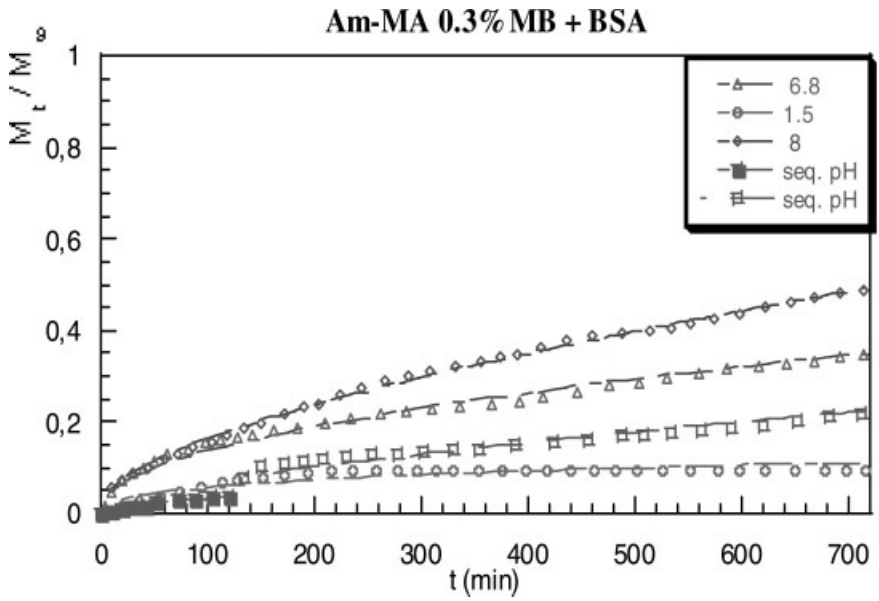

Figure 4.
Release kinetics of BSA from copolymer Am-MA with 0.3% of MB.

Thus, we can assume that the high compaction obtained with these copolymers and the small swelling performed at acid pH give rise to a very tight tablet structure that encloses the protein. This swelling increases considerably at high pH forming a gel layer that makes it easier for the BSA to diffuse through it. This behaviour can be observed in Figure 6, when swollen tablets are shown at different pHs: 1.5, 5 and 8 respectively.

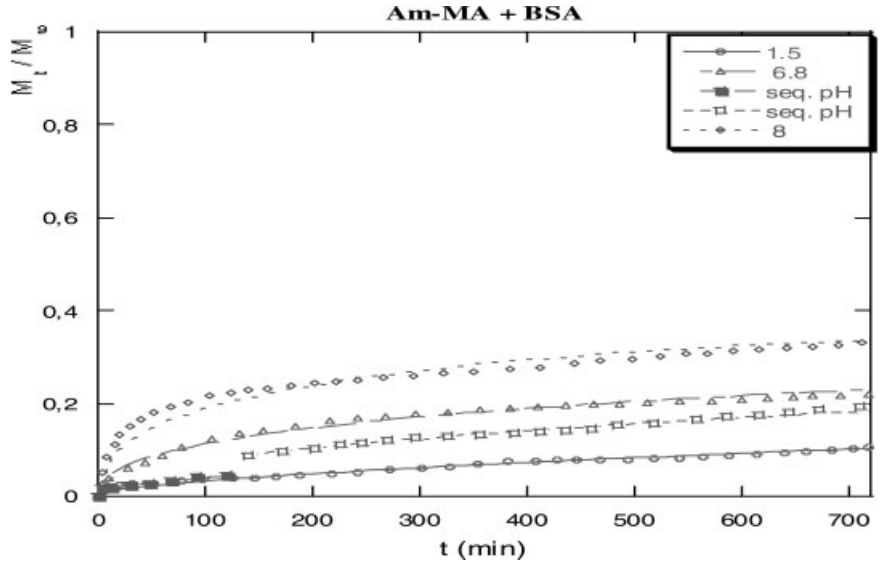

Figure 5.
Release kinetics of BSA from copolymer Am-MA without crosslinking agent.

Figure 6.
Swollen tablets in three different buffered solutions, pH 1.5, 5 and 8 in the top, middle and bottom rows respectively.

When observing the above figures, we can affirm that when more than 0.3% of the crosslinking agent was added to the copolymer Am-MA, the slowest release of drug was achieved. These differences could be attributed to differences in the swelling capacity owing to the amount of crosslinking agent.

Furthermore, when the amount of crosslinking agent is reduced to 0.3%, we achieve the fastest release of BSA and the influence of the pH is more noticeable. Comparing these results with those obtained with the uncrosslinked copolymer, we observe that small crosslinking contributes to slower dissolution and to obtaining a more compact tablet throughout the whole test.

Conclusions

The tablets prepared using Am-MA copolymers with different amounts of crosslinking agent present attractive properties for use as hydrophilic matrices showing optimum pH sensitivity for the controlled release of proteins.

In all cases, the release of BSA at acid pH is low enough to protect the protein for the 2 hours that simulate the stay of the tablets in the stomach, and when the tablet reaches the more basic colon conditions, release increases to reach the desired rate enabling the slow continuous dissolution of the protein.

By adding the appropriate amount of crosslinking agent, it has been shown that it is possible to achieve improved dosage control of proteins.

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