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Synthesis and Characterization of Crosslinked Maleyl Chitosan Microspheres Prepared by Coacervation Technique

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Crosslinked chitosan microspheres with different substitution degrees were prepared by the coacervation technique and chemically modified with maleic anhydride. The resultant systems were characterized by FT-IR, ^{13}C-NMR, elemental analysis, degree of swelling, thermogravimetry and scanning electron microscopy. The results obtained in the characterization suggest the formation of monoamide and monocarboxylic acid groups. The modified chitosan microspheres presented a high swelling degree at pH 1.2, one mass loss event at between 250 and 330°C, and a spherical shape. In summary, this system was shown to be a potential candidate for use in controlled drug delivery.

Keywords: Chitosan, microspheres, crosslinked, maleic anhydride

1 Introduction

Smart polymers have a variety of properties depending on their reactive functional groups and side chains. These groups can be responsive to pH, temperature, ionic strength, electric or magnetic fields and light. Some polymers are reversibly crosslinked by noncovalent bonds that can break and reform depending on the external conditions (1–5). Therefore, researchers are currently trying to find new and more efficient ways to administrate drugs through the development of polymeric controlled-drug-release systems (6, 7). Chitosan and its derivatives are receiving considerable attention in the development of formulations for several biomedical and pharmaceutical applications, due to their inherent biocompatibility, biodegradability, adsorption, and transmucosal drug transport properties, as well as their ability to interact with different substances, such as hydrophilic and hydrophobic drugs (8–15). The functional and biological properties of this polymer are related to their molecular weight, charge density and distribution, and degree of deacetylation, as well as to the pH value of the medium in which it will be placed (16). Thus, there is a practical necessity to synthesize chitosan derivatives with improved control over the solubility under different pH conditions, taking into consideration the particular envisaged biomedical application (17). The modification of the chitosan structure is usually achieved through the introduction of some specific chemical changes in the polymer chain molecule (18, 19). Therefore, the aim of this study was to synthesize crosslinked chitosan microspheres with maleic anhydride and investigate their physico-chemical properties.

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2 Experimental

2.1 Materials

Chitosan with a molecular weight of 122.740 Da and degree of deacetylation of 90% was purchased from Purifarma (São Paulo, Brazil). All other materials were at least of analytical grade.

2.2 Preparation of Chitosan Microspheres

Chitosan (3 g) was dissolved in 100 mL of 5% acetic acid (v/v) in order to produce a viscous 3% chitosan solution (m/v) and subsequently poured into a bath containing a 2.0 mol.L^{-1} NaOH solution through an Ismatec peristaltic

pump to obtain chitosan microspheres (MCT), which were washed until neutral pH.

2.3 Chemical Modification of Chitosan Microspheres with Maleic Anhydride

A sample of chitosan microspheres (10 g) was weighed and suspended in 20 mL of anhydrous ethanol, under constant stirring for 1 h. The ethanol was filtered off and then replaced with a fresh supply of ethanol (20 mL). The mixture was stirred for 30 min. This procedure was repeated three times. After treatment with alcohol, the microspheres were suspended in an ethyl alcohol solution and maleic anhydride and refluxed at 78°C. To produce microspheres with different degrees of N-substitution, the reflux times used were 2, 8 and 12 h, labeled MCT2, MCT8 and MCT12, respectively. For 1 g of wet microspheres, 0.6 g of maleic anhydride dissolved in 50 mL of ethyl alcohol was used. After reflux, the samples were filtered and washed 3 times with 96% ethyl alcohol for 15 min and dried at room temperature. The resultant microspheres were characterized by the following techniques.

2.4 Fourier Transform Infrared (FT-IR) Spectroscopy

Fourier transform infrared (FT-IR) spectra were recorded on a Perkin-Elmer Model 1600 spectrometer using KBr discs in the range of 4000–400 cm^{-1}.

2.5 Nuclear Magnetic Resonance (^{13}C NMR)

^{13}C-NMR spectra in solid phase were recorded on a Varian 400 Fourier transform spectrometer.

2.6 Determination of Substitution Degree (SD) by Elemental Analysis

The SD values of the modified chitosan samples were determined according to the method described by Inukai and co workers (20), and calculated as follows (Eq. 1):

$$DS = [(C/N)_r - (C/N)_o]/4 \quad (1)$$

where $(C/N)_r$ is the C/N of the modified microspheres and $(C/N)_\circ$ is the C/N of chitosan microspheres.

2.7 Degree of Swelling (S_w)

The degree of swelling was determined by maintaining 500 mg of the microspheres in 100 mL of 0.1 $mol.L^{-1}$ HCl (pH 1.2) and phosphate buffer (pH 6.8 and 9.0). The increase in the weight (W_t–W_0) of microspheres at different time intervals in comparison to the initial weight ($W_\circ$) was used to calculate the degree of swelling (Eq. 2).

$$S_w(\%) = (W_t - W_o)/W_o \times 100 \quad (2)$$

Where W_0 and W_t represent the weight of dry and wet modify chitosan microspheres, respectively.

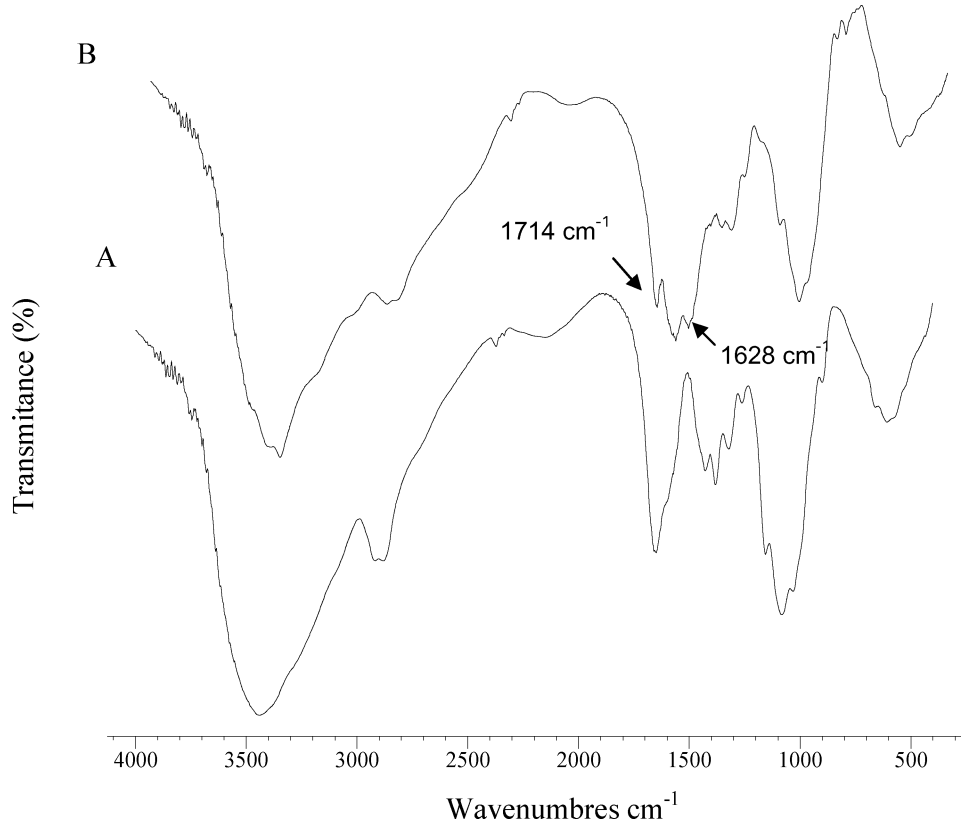

Fig. 1. Infrared spectra of MCT (A) and modified chitosan (B) microspheres.

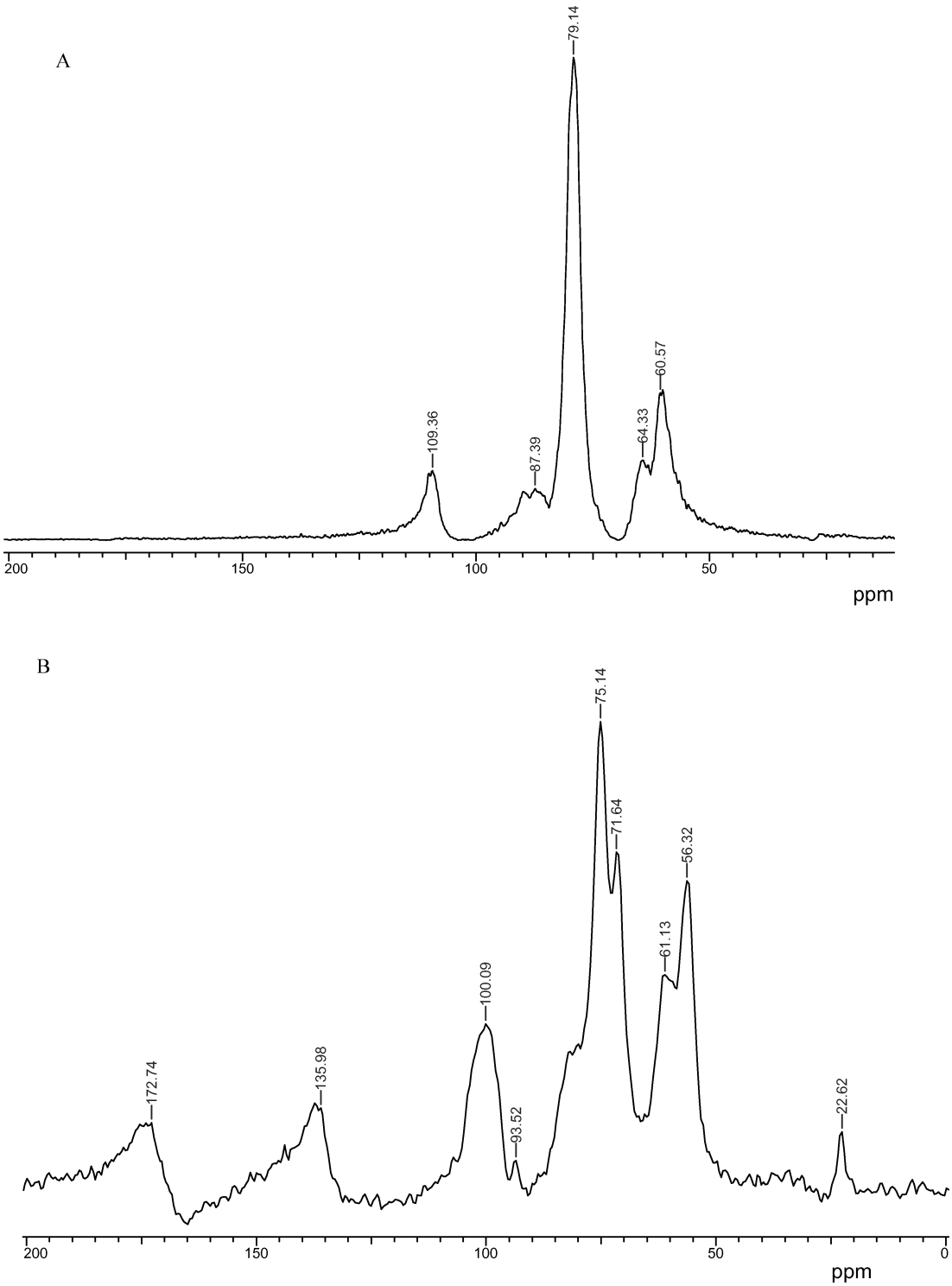

Fig. 2. ^{13}C-NMR spectra of MCT (A) and modified chitosan (B) microspheres.

2.8 Thermogravimetric Analysis (TG)

TG curves were obtained with a thermobalance, model TGA-50 (Shimadzu), in the temperature range of 25–600°C, using platinum crucibles with 4.0 ± 0.1 mg of sample, under dynamic N_2 atmosphere (50 mL.min^{-1}) at a heating rate of 10°C.

2.9 Scanning Electron Microscopy (SEM)

The microsphere morphology was observed by scanning electron microscopy (Phillips XL30). Samples were mounted onto metal stubs using double-sided adhesive tape, vacuum-coated with gold (350 Å) in a Polaron E-5000 and analyzed.

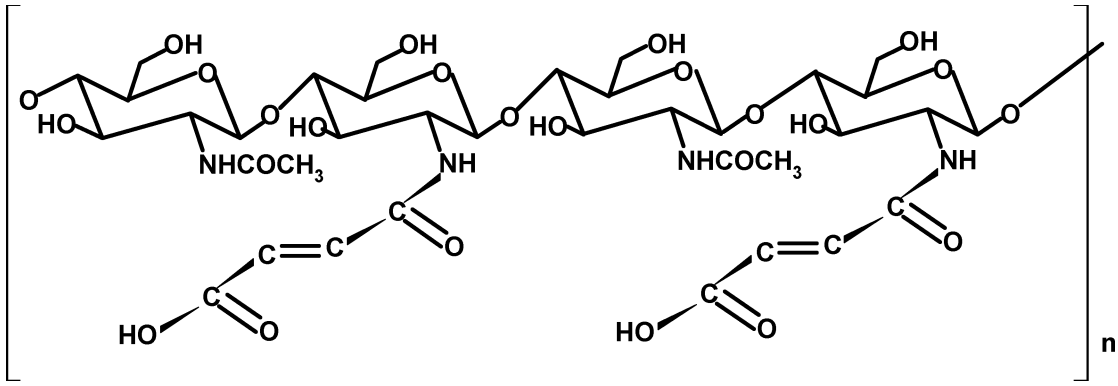

Fig. 3. Monomer structure of chemically modified chitosan

3 Results and Discussion

3.1 Fourier Transform Infrared (FT-IR) Spectroscopy and Nuclear Magnetic Resonance (^{13}C-NMR)

The infrared and ^{13}C-NMR spectra for all of the modified microspheres (MTC2, MCT8 and MCT12) are identical. The FT-IR spectra are shown in Figure 1. The absorption at 1070–1075 cm^{-1}, which appears for all samples, is attributed to the C-0 stretching vibration of the alcohol functional group present in samples of chitosan microspheres (MCT) and modified microspheres, since this group does not react with the maleic anhydride. In the MCT microspheres, a low intensity band at 2880 cm^{-1} was observed, produced by CH stretching of the remaining acetyl groups. This band is shifted to 2928–2932 cm^{-1} in the modified microsphere spectra. The main differences between the spectra of the MCT and modified microspheres were that the modified microspheres have absorption bands at 1628 cm^{1} attributed to vibrational deformation of NH (amide I) and at a frequency of 1714 cm^{-1}, the lower intensity band

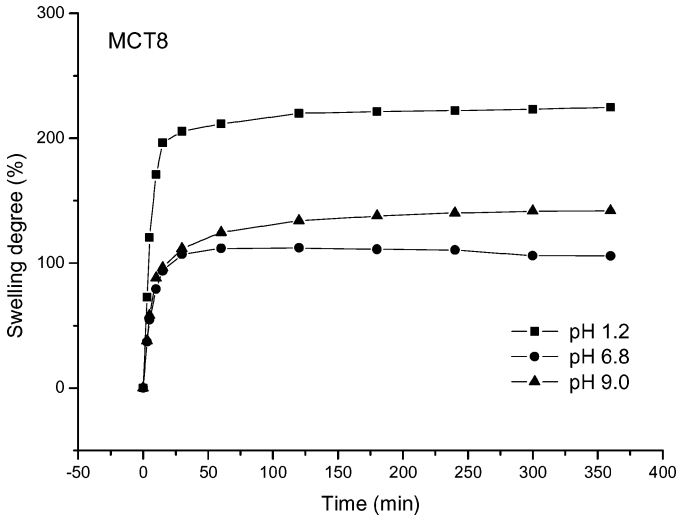

Fig. 5. The swelling degree of MCT8 at pH 1.2, pH 6.8 and pH 9.0

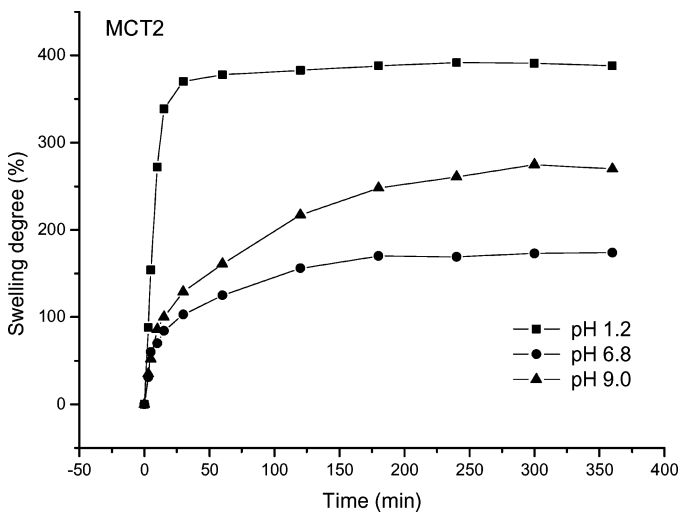

Fig. 4. The swelling degree of MCT2 at pH 1.2, pH 6.8 and pH 9.0

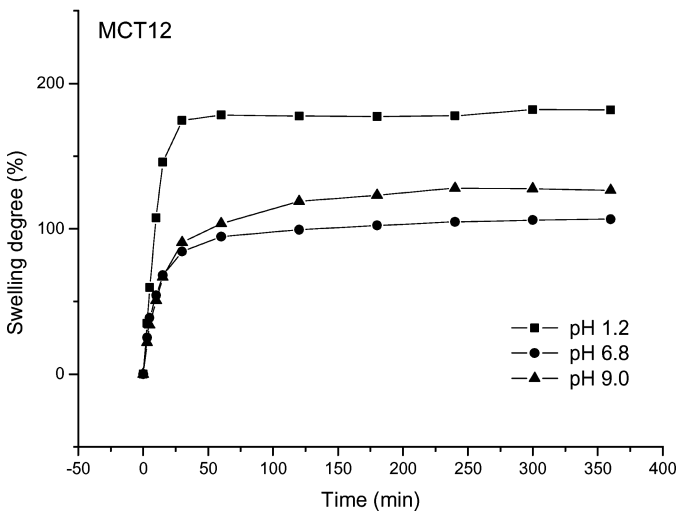

Fig. 6. The swelling degree of MCT12 at pH 1.2, pH 6.8 and pH 9.0

relating to carboxylic acids. These absorption bands suggest the formation of monoamide and monocarboxylic acid groups. These bands are not present in the spectra of MCT microspheres, indicating that the amidation reaction occurred successfully.

The ^{13}C-NMR analysis of the MCT (Fig. 2a) and modified (Fig. 2b) microspheres showed signals at δ 60.6–87.4 and δ 56.3–93.5, respectively, which indicate the presence of a sugar moiety in both samples. In addition, two signals for anomeric carbons at δ 109.4 and δ 100.09 were observed. Comparing the ^{13}C-NMR spectroscopic data of the MCT and modified microspheres, obvious differences were the additional signal of the carbonyl group in the modified chitosan microspheres at δ 172.74 (Fig. 2b). The amide, acid or ester functional groups have a signal relating to the carbonyl group in the range of 155–185 ppm. The appearance of a carbonyl carbon signal in the modified chitosan microspheres at δ 172.7 suggests the presence of a carbonyl function in the molecule, which can be attributed to an amide or acid carboxylic function.

3.2 Substitution Degree of the Microspheres

The substitution degree (SD) of the modified microspheres was calculated through the method described by Inukai (1998) (20). The results showed an increase in the carbon to nitrogen (C/N) atomic ratio for all synthesized microspheres (MCT2 (C/N = 6.94), MCT8 (C/N = 7.42) and MCT12 (C/N = 7.86)), in comparison with the original chitosan microspheres (C/N = 5.13) (Table 1). Also, they demonstrated that an increase in the reflux time produced microspheres with higher SD values, indicating that a greater number of NH_2 groups were substituted (crosslinked).

Table 1. Substitution degree for microsphere samples

Sample	*% C*	*% H*	*% N*	*SD %*
MCT	44.3	7.65	8.63	0
MCT2	47.32	7.52	6.81	45.46
MCT8	47.46	7.31	6.39	57.43
MCT12	47.60	7.06	6.05	68.25

Note: C = Carbon, H = Hydrogen, N = Nitrogen, SD = Substitution degree

Substitution by maleyl groups in the MCT microspheres can occur for both the more reactive hydroxy group at C6 and the amino group at C2 of chitosan. However, N-substitution is reported to occur preferentially to O-substitution (21). The characterization studies carried out through FT-IR, ^{13}C-NMR and elemental analysis indicated the amidation of chitosan, and the probable structure of the resultant product is given in Figure 3.

3.3 Swelling Properties of the Modify Microspheres

The principal characteristic of hydrophilic polymers is their ability to absorb and hold large amounts of liquid without dissolving. There is a relationship between the degree of swelling and the nature of both the polymer and the liquid (22). The release of a drug from polymeric microspheres is dependent on the amount of water associated with the system and the swelling properties. The release of drugs from a polymeric system may involve the penetration of water into the matrix and simultaneous release of drugs via diffusion, governed by Fick's Law (23–24). Generally, drugs are administered orally and are thus subject to the influence of different pH values in the gastrointestinal tract.

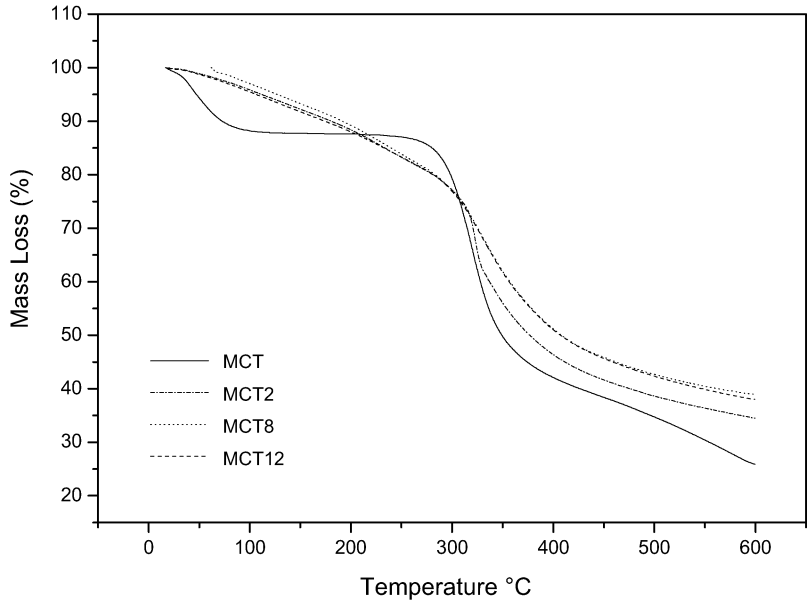

Fig. 7. TG Curves for MCT, MCTM2, MCTM8 and MCTM12

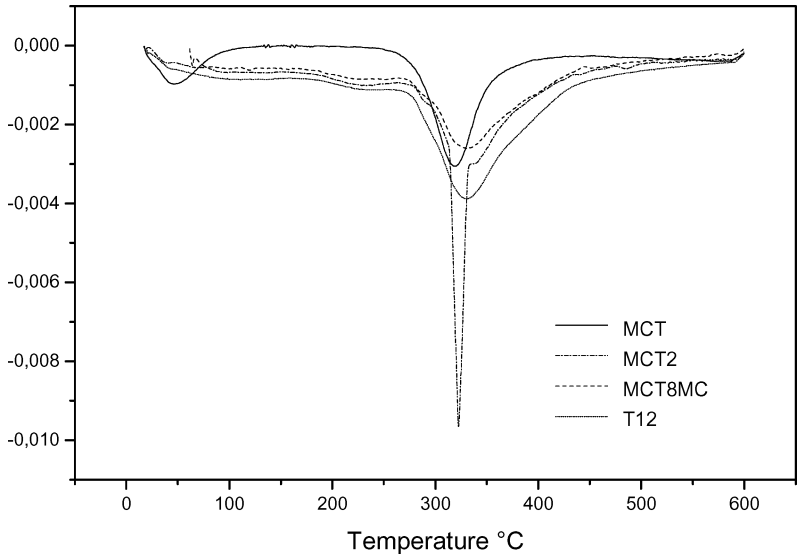

Fig. 8. DTG Curves for MCT, MCTM2, MCTM8 and MCTM12

The swelling degrees of MCT2, MCT8 and MCT12 were evaluated at pH 1.2, 6.8 and 9.0 (Figs. 4, 5 and 6). The results indicated that the S_w of modify microspheres was pH-dependent. All samples presented a greater swelling at pH 1.2, although MCT2 demonstrated a higher S_w value, probably due to it having less NH_2groups substituted in its polymeric chain. The NH_2groups are also responsible for the higher S_w values at acid pH, where it is in the protonated form NH_3^+.

3.4 Thermogravimetric Analysis

Figures 7 and 8 show the thermal profiles of the samples MCT, MCTM2, MCTM8 and MCTM12, with the following degradation peaks: 319.21°C, 322.34°C, 330.63°C and 330.38°C, respectively. Therefore, the thermal stability is dependent on the strength of the polymer crosslinking. A greater substitution degree of the groups promoted a stronger linking of the polymeric chains.

3.5 Scanning Electron Microscopy (SEM)

The pore structure at the surface of the microspheres was important in terms of their swelling degree. The microspheres generally had good sphericity (Figs. 9 and 10), although sample MQT12 had cracks on the surface which suggests that the sample with the highest degree of substitution had less mechanical strength, typical of reticulated

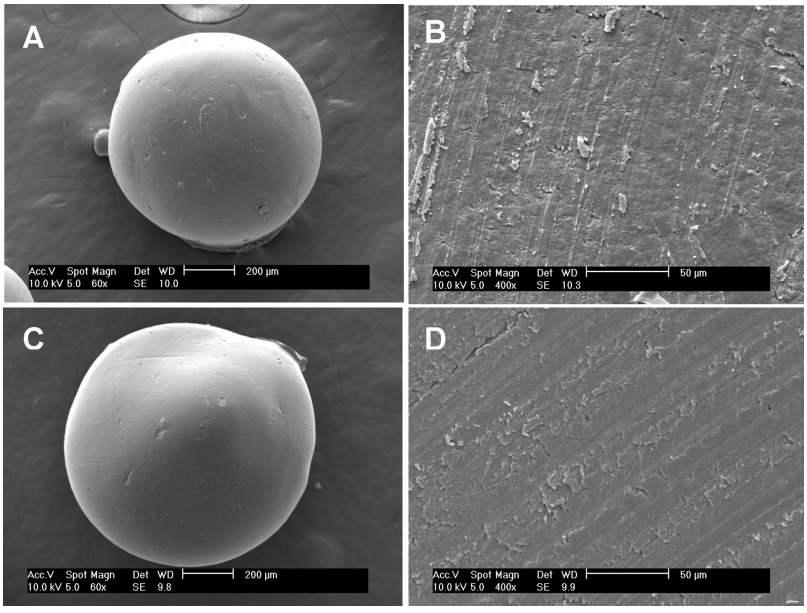

Fig. 9. Scanning electron microscopy of MCT (A) and MCT cross-section (B), MCT2 (C) and MCT2 cross-section (D), magnification of 60x and 100x, respectively.

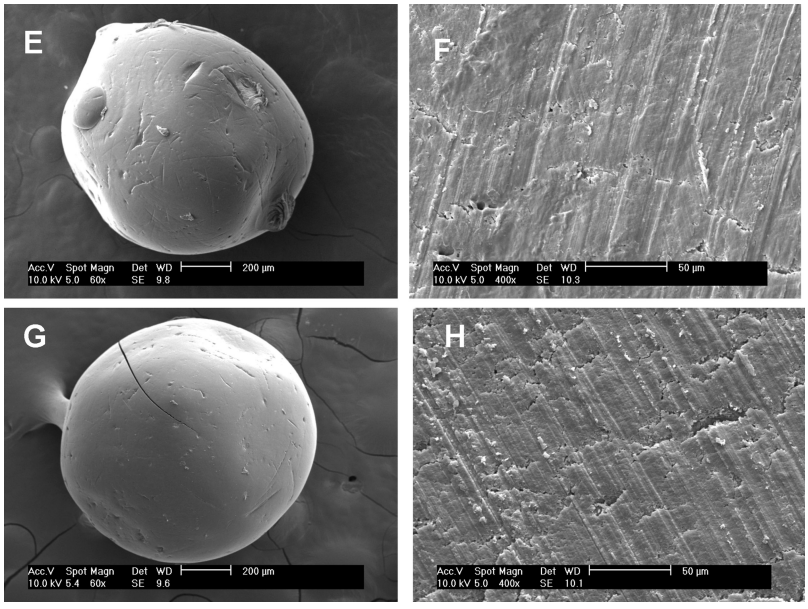

Fig. 10. Scanning electron microscopy of MCT8 (E) and MCT8 cross-section (F), MCT12 (G) and MCT12 cross-section (H), magnification of 60x and 100x, respectively.

films. The surface of the cross-section of the microspheres showed an increase in roughness proportional to the N-substitution level. The average size of the MCT, MCT2, MCT8 and MCT12 samples were 890, 999, 1020 and 1038 μm, respectively.

4 Conclusions

The synthesis and characterization of cross-linked chitosan microspheres with maleic anhydride were investigated. The systems developed suggest the formation of monoamide and monocarboxylic acid groups. The microspheres showed a hydrogel behavior with a high swelling degree, particularly for the MCT2 sample, which was shown to be a potential candidate system for use as a carrier in swelling-controlled drug delivery.

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