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Carbohydrate Polymers 73 (2008) 490–497
Carbohydrate Polymers
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Synthesis and characterization of cross-linked malonylchitosan microspheres for controlled release of acyclovir

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Received 20 July 2007; received in revised form 11 December 2007; accepted 17 December 2007
Available online 30 January 2008

Abstract

The purpose of the present study was to obtain a polymeric system for delayed release of the drug acyclovir (ACV), which can be used for treatment of *Herpes simplex* and *Varicella Zoster*. The gelled chitosan (GCT) microspheres were obtained by coacervation-phase separation. They were treated with malonic acid to obtain malonylchitosan (MLCT) microspheres, which were characterized by, Fourier transform infrared spectroscopy (FT-IR), nuclear magnetic resonance spectroscopy (^{13}C NMR), elemental analysis (CHN), thermogravimetric analysis (TG/DTG) and scanning electron microscopy (SEM). The drug was encapsulated in MLCT microspheres by a contact adsorption technique, and the final formulation (MLCT-ACV), was analyzed for loading efficiency, degree of swelling and in vitro release profiles. The results obtained support the N-substitution of malonyl groups in the MLCT microspheres. The loading efficiency increased with impregnation time and a major amount of drug was encapsulated after 24 h. The swelling rate was higher in acid pH. The median release time was 5.5 h in pH 1.2 and 6.8. The mechanism involved in release was non-Fickian ($0.43 < n < 0.85$, $n = 0.8474$) and Super Case II kinetics ($n > 1$, $n = 1.0491$) at pH 1.2 and 6.8, respectively.

Keywords: Acyclovir; Chitosan microspheres; Controlled release

1. Introduction

There has been considerable interest in recent years in developing controlled or sustained drug delivery systems by using biopolymers. Controlled or sustained release drugs provide many advantages in comparison with conventional forms including reduced side effects, drug concentration kept at effective levels in plasma, improved utilization of drug and decrease the dosing times (Desai & Park, 2005).

Chitosan is currently gaining a great deal of attention for medical applications as well as for the controlled release of drugs (Paul & Sharma, 2000; Sinha et al., 2004). In drug delivery applications, chitosan has been used as a vehicle for drug, protein and gene delivery. This compound is a partially deacetylated polysaccharide obtained by alkaline treatment of chitin, one of the most abundant biopolymer in nature (Chen et al., 2004; Sinha et al., 2004). The use of microspheres based therapy allows drug release to be carefully tailored to the specific treatment site through the choice of appropriate formulation variables. Using innovate microencapsulation technologies and by modifying the polymeric matrix, microspheres can be developed into an optimal drug delivery system which will provide the desired profile (Desai & Park, 2005).

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doi:10.1016/j.carbpol.2007.12.012

The success of chitosan microspheres as carriers is due to the following features: (i) they can dissolve poorly soluble drugs and thus increase their bioavailability, (ii) they can stay in the body (in the blood) long enough to provide gradual accumulation in the required area, (iii) their size permits them to accumulate in body regions with leaky vasculature, (iv) they can be tailored to achieve targeting or other desired properties by attachment of a specific ligand to the outer surface, (v) they have low toxicity and a high loading capacity, as well as minimize drug degradation and loss and (vi) they can be easily produced in large quantities (Dodane & Vilivalam, 1998; Guliyeva, Oner, Ozsoy, & Haziroglu, 2006).

Acyclovir (2-amino-1,9-dihydro-9-[(2-hydroxyethoxy) methyl]-6*H*-purin-6-onez) (ACV) (Fig. 1) is an acyclic nucleoside analogue of guanosine which exhibits an antiviral effect on *Herpes simplex* virus HSV-1, HSV-2 and *Varicella Zoster* (VZV) virus through interfering with DNA synthesis and inhibition viral replication. Various reports have indicated that acyclovir is as effective as or even superior to other antiviral agents with lower host toxicity and milder side effects (Giammona, PuglishI, Cavallaro, Spadaro, & Pitarresi, 1995; Rossel, Carrenõ, Baeza, & Alderete, 2000).

According to the type of infection, it can be administered by intravenous infusion, in a final concentration not greater than 5 mg/mL, by oral administration in a dose of 200 mg five times daily, or by topical administration as ointment or cream containing 5% of drug, to be applied 5–6 times daily. Thus, it has a low oral bioavailability (20%) and a short circulation half-life ($t_{1/2}$, 2, 5 h) (Woo & Challacombe, 2007).

Due to its relative short half-life and low solubility, researchers have been developed different systems containing ACV. These studies include the poly (D, L-lactide-co-glycolide) microspheres for long-term intravitreal delivery, ciclodextrines, liposomes for ocular delivery, sustained release ethylcellulose microspheres, microspheres of acrylamide grafted dextran/chitosan and hydrophilic matrix tablets. (Cheu, Chen, Chen, & Lin, 2001; Conti et al., 1997; Jalón, Blanco-Príeto, Ygartua, & Santoyo, 2001; Law, Huang, & Chiang, 2000; Pavelic, Skalko-Basnet, Filipovic-Grcic, Martinac, & Jalsenjak, 2005; Rossel et al.,2000; Sancho, Vanrell, & Negro, 2003).

The aim of this study was to produce malonylchitosan microspheres by amidification of gelled chitosan microspheres with malonic acid. The resulting system trigger could be used for the controlled release of ACV in a pH sensitive way.

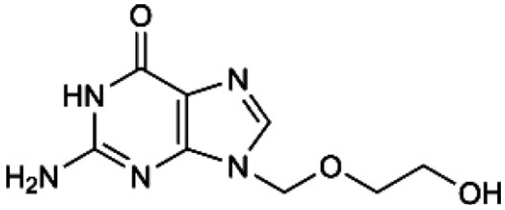

Fig. 1. Chemical structure of ACV.

2. Experimental

2.1. Materials and methods

The ACV reference substance was received from Shenyang Fine Chemical Co., China. Chitosan molecular weight of 122.740 Dalton 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 cross-linked malonylchitosan microspheres (MLCT)

Chitosan (5 g) was dissolved in 100 mL of acetic acid 5% (m/v) in order to produce a viscous 5% (m/v) chitosan solution and subsequently poured into a bath containing NaOH 2.0 mol L^{-1} solution through an Ismatec peristaltic pump to obtain gelled chitosan microspheres (GCT). A sample corresponding to 5 g of GCT microspheres were suspended in 20 mL of ethanol and stirred for 1 h. The ethanol was filtered off and then replaced with a fresh supply of ethanol (30 mL). The mixture was stirred for 30 min. This procedure was repeated three times. The GCT microspheres were suspended in 20 mL of ethanol and 450 mg of malonic acid was added into this suspension. The reaction mixture was refluxed for 36 h at 78 °C and then cooled to room temperature. The modified chitosan microspheres (MLCT) obtained were washed twice with 30 mL portions of ethanol and then, they were placed in contact with glutaraldehyde 2.5% (m/v) solution to allow cross-linking to take place for a period of 12 h at room temperature. The resulting material was washed with distilled water to remove the excess of cross-linking agent. The cross-linked MLCT microspheres were dried at room temperature (Glasser & Jain, 1999; Valgas, Gonçalves, Pedroza, Fávere, & Laranjeira, 2005).

2.3. Acyclovir loading on cross-linked MLCT microspheres

The cross-linked MLCT microspheres (1 g) were impregnated with ACV using a contact adsorption technique. ACV (0.2 g) was dissolved in a HCl 0.3 mol L^{-1} solution and the resulting solution was put in contact with cross-linked MLCT microspheres for 6, 12 and 24 h, under stirring of 100 rpm at room temperature. The excess of ACV was removing with HCl 0.3 mol L^{-1}, the cross-linked MLCT microspheres containing ACV (MLCT-ACV) were dried at room temperature.

2.4. Characterization

2.4.1. Fourier transform infrared (FT-IR)

FT-IR spectra was recorded on a Perkin-Elmer Model 1600 apparatus using KBr discs in the range of 4000–400 cm^{-1}.

2.4.2. Nuclear magnetic resonance (^{13}C NMR)

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

2.4.3. Determination of substitution degree by elemental analysis (SD)

The substitution degree was determined in accordance with the method describes by Inukai and co workers, it was calculated as follows (Eq. (1)):

$$DS = [(C/N)_r - (C/N)_o]/3 \quad (1)$$

where $(C/N)_r$ is the C/N of the cross-linked MLCT microspheres and $(C/N)_o$ is the C/N of GCT microspheres.

2.4.4. Thermogravimetric analysis (TG/DTG)

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

2.4.5. Scanning electron microscopy (SEM)

Microspheres morphology and size were 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.

2.5. Determination of loading efficiency

The drug content in MLCT-ACV was determined spectrophotometrically ($\lambda_{max} = 254$ nm; Shimadzu Model 1601, Japan). A sample of MLCT-ACV microspheres (100 mg) was triturated and dissolved in 10 mL of HCl 0.3 mol L^{-1}, under sonication for 20 min. The solutions were filtered through 0.22 μm Millipore filters and the amount of ACV was measured. Experiments were performed in triplicate ($n = 3$) and loading efficiencies were calculated using Eq. (2).

$$\%\ \text{Drug entrapment} = \frac{\text{Mass of drug present in microspheres}}{\text{Theoretical mass of acyclovir}} \times 100 \quad (2)$$

2.6. Degree of swelling (S_w)

The degree of swelling was determined by keeping 500 mg of the microspheres in 100 mL of HCl 0.1 mol L^{-1} pH 1.2, phosphate buffer pH 6.8 and 9.0. The increase in weight ($W_t - W_0$) of microspheres at different time intervals in comparison to initial weight (W_o) of microspheres was used to calculate the degree of swelling (Eq. (3)).

$$S_W(\%) = (W_t - W_o)/W_o \times 100 \quad (3)$$

where W_0 and W_t represent the weight of dry and wet MLCT-ACV microspheres, respectively.

2.7. In vitro release

The in vitro release of ACV was evaluated using dissolution methodology (Apparatus I, 100 rpm, 37 °C), in 500 mL of HCl 0.1 mol L^{-1} pH 1.2 and in phosphate buffer pH 7.4 to simulate gastrointestinal fluid. An aliquot of the release medium (5 mL) was withdrawn through a sampling syringe attached with a 0.22 μm membrane filter (Milipore, USA) at pre-determined time intervals (5, 10, 15, 30, 60, 90, 120, 150, 180, 210, 240, 270, 300, 330 and 360 min) and an equivalent amount of fresh dissolution media which was prewarmed at 37 °C was replaced. The samples were centrifuged and analyzed using an UV spectrophotometer at 254 nm.

3. Results and discussion

3.1. Characterization

3.1.1. Fourier transform infrared spectroscopy (FT-IR)

FT-IR spectra of cross-linked MLCT and GCT microspheres were showed in Fig. 2. The absorption bands at 3424–3446 cm^{-1} of cross-linked MLCT microspheres (A) are rather intense, as a consequence of –OH and water

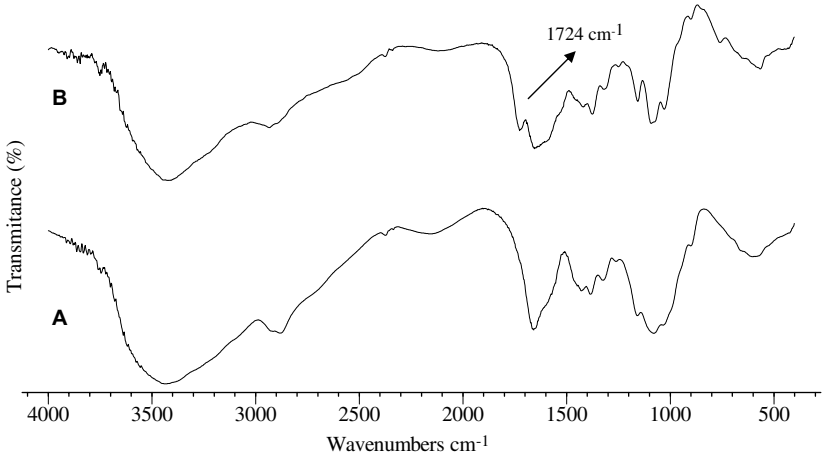

Fig. 2. Infrared spectrum of GCT (A) and cross-linked MLCT (B) microspheres.

stretching vibrations. The bands assigned to primary amine and amide groups appear in the FT-IR spectrum in the region between 1560 and 1650 cm^{-1} (Silverstein, Webster, & Kiemle., 1994). In both spectral a band appears in this region, although in GCT microspheres it was more intense than MLCT. In this way, the amide signal in the spectrum was not clear, because the signal was coupled with the amine signal. The absorption band at 1721 cm^{-1} is assigned to aliphatic carboxylic acid, which is not observed in the GCT microspheres. This suggests the formation of a monoamide group and a monocarboxylic acid group after 36 h for the resulting malonyl derivative.

3.1.2. Nuclear magnetic resonance ^{13}C NMR

^{13}C NMR analyses of samples GCT (Fig. 3a) and cross-linked MLCT (Fig. 3b) showed signals at δ 60.6–87.4 and δ 59.1–79.0, 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 105.0 were observed. Comparing the ^{13}C NMR spectroscopic data of GCT and cross-linked MLCT, the obvious difference was the additional signal at the carbonyl group in the cross-linked MLCT at δ 178.3 (Fig. 3b).

The functional groups amide, acid or ester presents a signal referent a carbonyl group in the range 155–

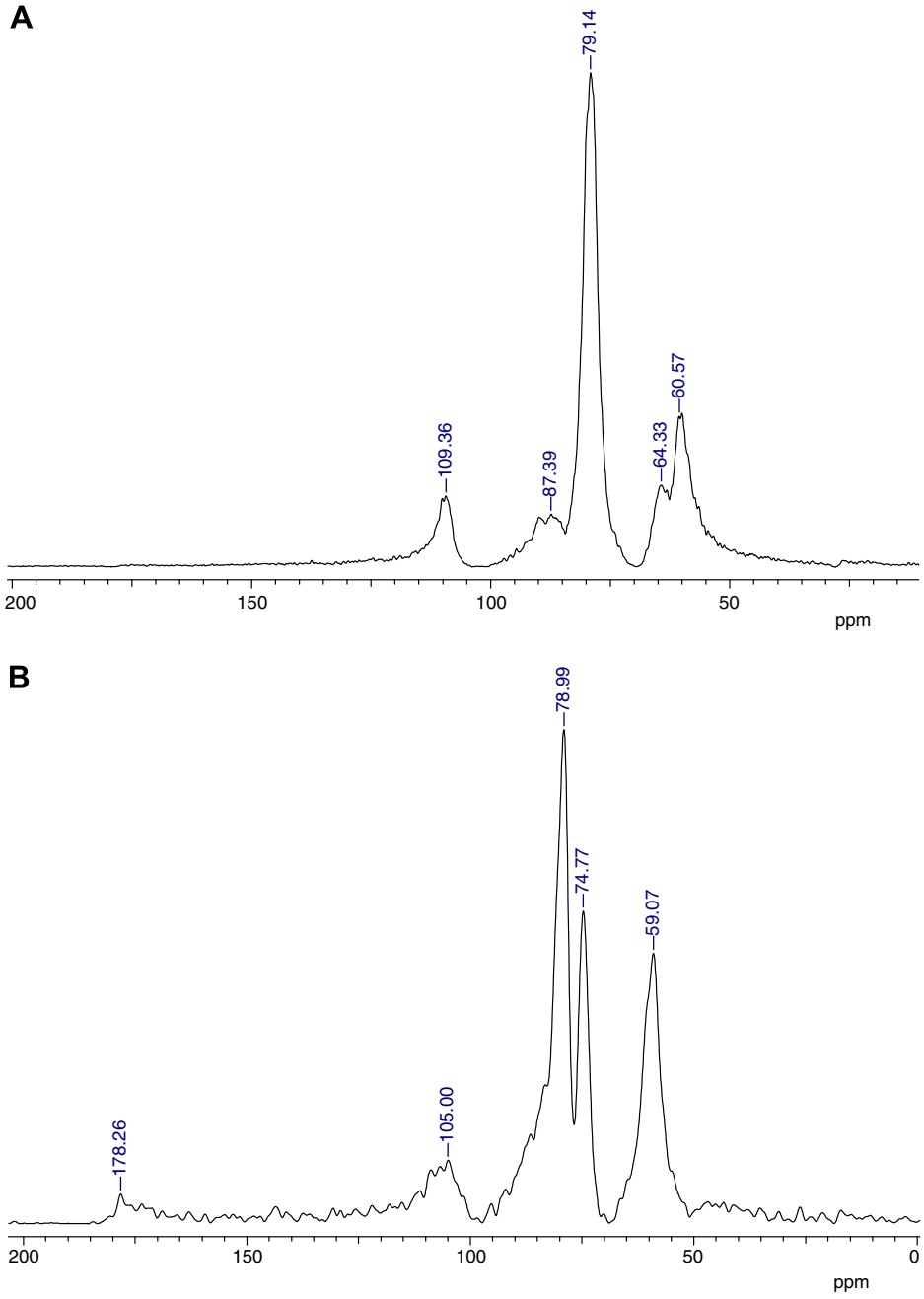

Fig. 3. ^{13}C NMR spectrum of GCT (A) and MLCT (B) microspheres.

185 ppm. The appearance of carbon carbonylic signal in the cross-linked MLCT at δ 178.3, suggested the presence of carbonyl function in the molecule, that can be attributed to amide or acid carboxylic function.

3.1.3. Substitution degree (SD)

The results present an increase of atomic ratio of carbon to nitrogen (C/N) for cross-linked MLCT microspheres (C/N = 7.46) in comparison with GCT microspheres (C/N = 5.47). The results of elemental analysis were: C 38.2%, H 7.05% and N 5.12% for cross-linked MLCT microspheres (36 h) and C 38.5%, H 7.91% and N 7.0% for original GCT microspheres. The degree of substitution calculated was 66.3%. The characterization studies carried out through FT-IR, RMN ^{13}C and elemental analysis indicated the amidification of chitosan and the probable structure of amidification product is illustrated in Fig. 4.

The substitution by malonyl groups in the original GCT 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 be preferred over O-substitution (Inukai, Chinen, Matsuda, Kaida, & Yasuda, 1998).

3.1.4. Thermogravimetric analysis (TG/DTG)

TG/DTG curves illustrated in Fig. 5, demonstrate that GCT microspheres has one mass loss event between 235 and 335 °C ($\Delta m = 30.32\%$). For MLCT microspheres the curves showed two overlapping steps of degradation in the temperature range from 165 to 221 °C ($\Delta m = 22.54\%$) and 265 to 333 °C ($\Delta m = 21.67\%$).The first mass loss was related to volatile products formed in cross-linked process (Rocha, Schefft, Greil, Bressiani, & Bressiani, 2005). In this way the modified polymers presented less thermal stability than natural chitosan, which can be attributed to ramify lateral chain in polymeric structure. The decomposition product around 400 °C was characterized as a carbonaceous material in both cases.

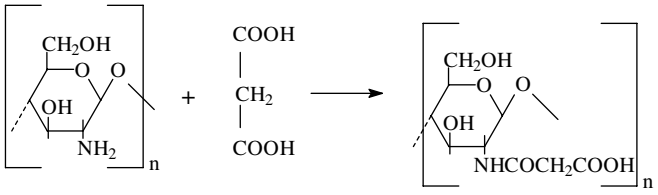

Fig. 4. Structure of chemically modified chitosan with malonic acid.

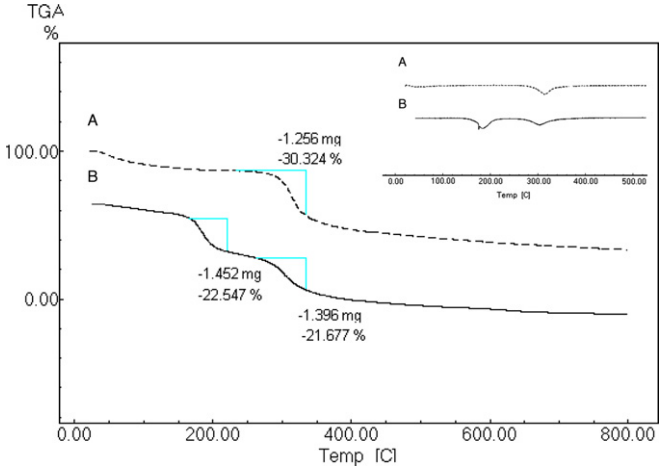

Fig. 5. TG/DTG curves of GCT (A) and MLCT microspheres (B).

3.1.5. Scanning electron microscopy (SEM)

The photomicrographs of MLCT microspheres demonstrated that these were spherical, smooth, with medium size of 882 μm. In the surface of MLCT-ACV microspheres was observed the residual drug adhered (Fig. 6A, B and C), that was rapidly dissolved in contact with the dissolution medium. This initial amount of the drug dissolved results of an immediate release (“burst effect”) motivated for the release of the existing drug in the microspheres surface. This effect was observed for others delayed release biopolymers systems (Zolnik & Burgess, 2007).

The photomicrographs of transversal section of MLCT-ACV microspheres indicated that it has a compact structure with only a few pores (Fig. 6D and E).

3.2. Loading efficiency

The loading efficiency was 23.9%, 45.8% and 69.7% for impregnation times of 6, 12 and 24 h. The most efficient time of loading efficiency was 24 h, where a major amount of drug was encapsulated.

3.3. Degree of swelling (S_w)

The swelling properties of ionic polymers are due the ionization of their pendent functional groups. Physiological parameters, such as pH, can change drastically the equilibrium degree of swelling by several orders of magnitude (Pasparakis & Bouropoulos, 2006). S_w is an important parameter associated with the mechanism of release and kinetics. The sample of MLCT-ACV microspheres that was submitted in a cumulative pH solution medium (1.2, 6.8 and 9.0), present a final *SD* of 2.300% (± 0.6) after removing the surface solution medium using an absorbent paper (Table 1). In assays of individual pH values, the MLCT-ACV microspheres present different swelling rates. The percentage of swelling was 2018% (± 0.89), 1060% (± 0.58) and 1178% (± 0.91) for pH values of 1.2, 6.8 and 9.0, respectively, indicating that the better swelling rate was in acid pH.

3.4. In vitro drug release

The percentages of drug released from MLCT-ACV microspheres in simulated gastric fluid and intestinal fluid are shown in Fig. 7. The controlled release characteristic was prominent in both fluids, the media release time was

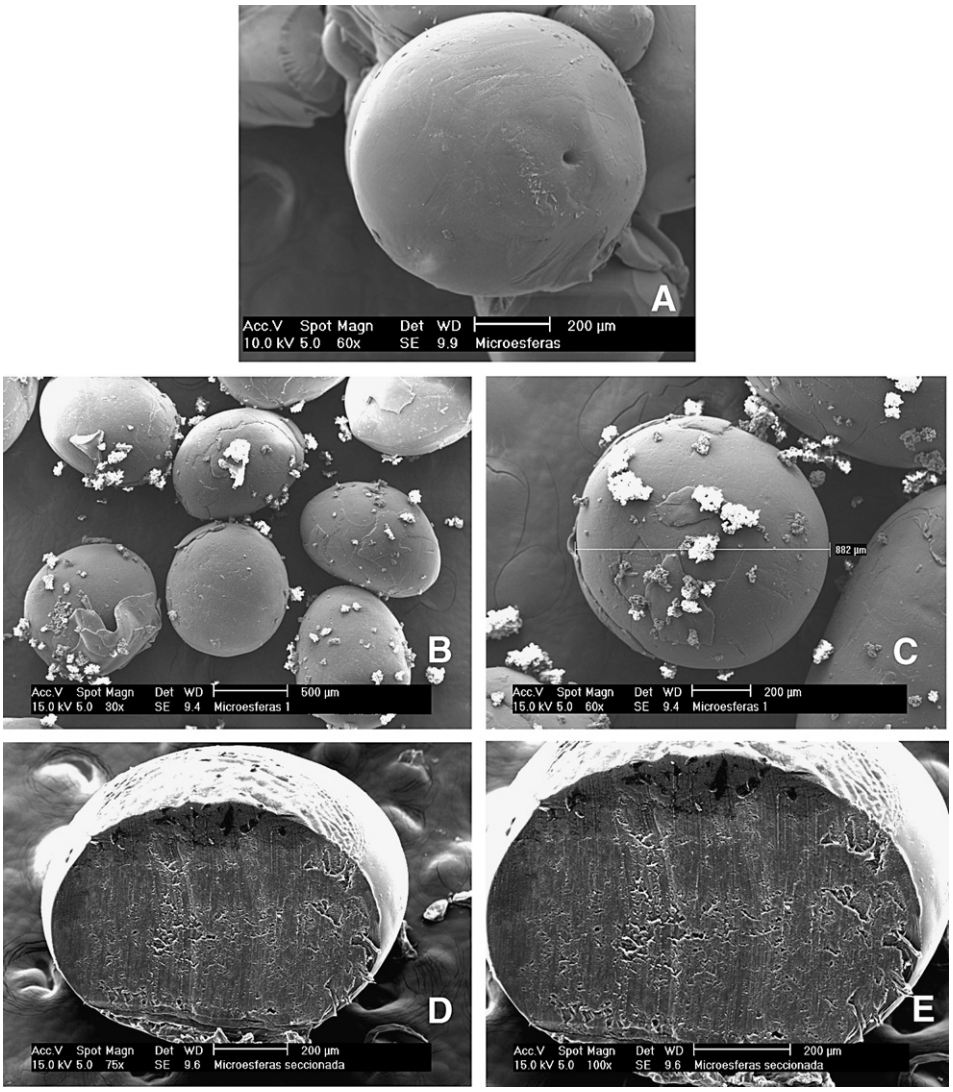

Fig. 6. Scanning electron microscopy of cross-linked MLCT microspheres (A) magnification of 60×, MLCT-ACV microspheres (B and C) magnification of 30 e 60×, respectively, transversal section of MLCT-ACV microspheres (D and E) magnification of 75 and 100×, respectively.

Table 1
Results of % swelling degree for cumulative and individual pH values

Cumulative swelling				Individual swelling					
pH	Time (min)	Mass (g)	Swelling degree (%)	Mass (g) pH 1.2	Swelling degree (%)	Mass (g) pH 6.8	Swelling degree (%)	Mass (g) pH 9.0	Swelling degree (%)
1.2	0	0.5	0	0.5	0	0.5	0	0.5	0
	30	7.85	1470	7.52	1440	1.26	152	1.36	172
	60	9.73	1846	9.65	1830	1.38	176	1.41	182
	90	10.51	2002	10.48	1996	1.82	264	1.96	292
	120	10.58	2016	10.48	1996	2.39	378	2.65	430
6.8	150	11.39	2178	10.52	2004	3.65	630	4.23	746
	180	11.41	2182	10.53	2006	4.12	724	4.89	878
	210	11.54	2208	10.55	2010	4.23	746	5.11	922
	240	11.62	2224	10.56	2012	4.89	878	5.13	926
9.0	270	11.73	2246	10.57	2014	5.01	902	5.69	1038
	300	11.79	2258	11.58	2016	5.13	926	6.12	1124
	330	11.80	2260	10.59	2018	5.32	964	6.53	1206
	360	12.00	2300	10.59	2018	5.80	1060	6.39	1178

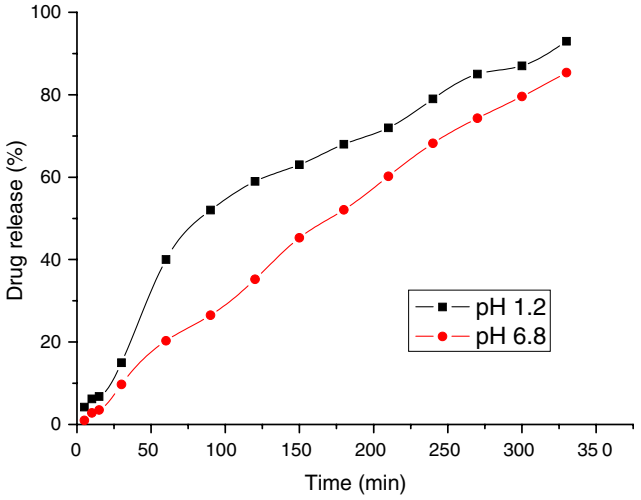

Fig. 7. Release of acyclovir from MLCT-ACV microspheres in pH 1.2 and pH 6.8.

5.5 h. In the first 15 min was observed a immediately release of ACV which was probably the drug present in the surface of MLCT-ACV, that was previously identify by SEM assays.

3.5. Mechanism and mathematical modeling for drug release from MLCT-ACV microspheres

The mechanism of drug release from microspheres is determined by different physical–chemical phenomena. According to Nixon (1983), three steps lead to drug release from microparticles in aqueous medium: (i) imbibition of the release medium into microparticles, (ii) dissolution of the drug inside microparticles and (iii) drug release by a diffusion process into the aqueous medium. The Korsmeyer–Peppas model (Eq. (4)), a semi-empirical model, correlating drug release to time by a simple exponential equation for the fraction of release drug <0.6, has been used to evaluate drug release from controlled release polymeric devices, especially when the drug release mechanism is unknown or when there are more than one release mechanism (Costa & Lobo, 2001; Korsmeyer, Gurny, & Doelker, 1983).

$$\frac{M_t}{M_\infty} = k \cdot t^n \tag{4}$$

M_t/M_∞ is the proportion of drug released at time t, k is the kinetic constant and the exponent n has been proposed as indicative of the release mechanism. In this context, $n \leqslant 0.43$ indicates Fickian release and $n = 0.85$ indicates a purely relaxation controlled delivery which is referred as Case II transport. Intermediate values $0.43 < n < 0.85$ indicate an anomalous behavior (non-Fickian kinetics corresponding to coupled diffusion/polymer relaxation, (Ritger & Peppas, 1987a, 1987b). Occasionally, values of $n > 1$ has been observed, which has been regarded as Super Case II kinetics (Munday & Cox, 2000; Ranga, Devi, & Buri, 1988). With the linear form of Eq. (4), plotting of ln M_t/M_∞ against ln t, yielded the diffusion exponential (n), the Pearson coefficient (r^2) and the diffusion constant (k), the results are summarized in Table 2.

Table 2
Analysis of release data from MLCT-ACV microspheres

MLCT microspheres	n	k (min^{-n})	r^2
pH 1.2	0.8474	1.5756	0.9863
pH 6.8	1.0491	1.4404	0.9975

Inspection of the results shown in Table 2 indicates that the release of MLCT-ACV microspheres in pH 1.2 presented an n value of 0.8474 indicative of an anomalous behavior. The non-Fickian kinetics corresponds to the coupling of diffusion and polymer relaxation. On the other hand, MLC-ACV microspheres in pH 6.8 presented an n value of 1.0491, indicating that the mechanism for release was included diffusion, swelling, relaxation and erosion process.

The k values were related with the kinetics of acyclovir release, in both medium the values were similar.

4. Conclusions

The results obtained in characterization suggested the N-substitution of malonyl groups in the MLCT microspheres. The system developed showed hydrogel behavior with a high degree of swelling, which may be used as carrier for swelling-controlled drug delivery.

The median release time was 5.5 h, and the mechanism implicated in this process was non-Fickian and Super Case II kinetics. Hence, these microspheres may be considered as suitable candidate for the oral delivery of ACV.

Acknowledgements

The authors acknowledge to Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for fellowships and Conselho Nacional de Desenvolvimento Científico and Tecnológico (CNPq) for the financial support.

References

Chen, S. C., Wu, Y. C., Mi, F. L., Lin, Y. H., Yu, L. C., & Sung, H. W. (2004). A novel pH-sensitive hydrogel composed of N,O-carboxymethyl chitosan and alginate cross-linked by genipin for protein drug delivery. *Journal of Controlled Release, 96*, 285–300.

Cheu, S. J., Chen, R. R. L., Chen, P. F., & Lin, W. J. (2001). *In vitro* modified release of acyclovir from ethyl cellulose microspheres. *Journal of Microencapsulation, 18*, 559–565.

Conti, B., Bucolo, C., Giannavola, C., Puglishe, G., GiunchedI, P., & Conte, U. (1997). *European Journal of Pharmaceutical Sciences, 5*, 287.

Costa, P., & Lobo, J. M. (2001). Modeling and comparison of dissolution profiles. *European Journal of Pharmaceutical Sciences, 13*, 123–133.

Desai, K. G. H., & Park, H. J. (2005). Preparation of cross-linked chitosan microspheres by spray drying: Effect of cross-linked agent on the properties of spray dried microspheres. *Journal of Microencapsulation, 22*(4), 377–395.

Dodane, V., & Vilivalam, V. D. (1998). Pharmaceutical applications of chitosan. *Pharmaceutical Science and Technology Today, 1*, 246–253.

Giammona, G., PuglishI, G., Cavallaro, G., Spadaro, A., & Pitarresi, G. (1995). Chemical stability and bioavailability of acyclovir coupled to α, β-poly (N-2-hidroxyethyl)–dl-aspartamide. *Journal of Controlled Release, 33*, 261.

Glasser, W. G., Jain, R. K. (1999). Method of making ester-crosslinked chitosan support materials and products thereof. United States Patent 5874551.

Guliyeva, U., Oner, F., Ozsoy, S., & Haziroglu, R. (2006). Chitosan microparticles containing plasmid DNA as potential oral gene delivery system. *European Journal of Pharmaceutics and Biopharmaceutics, 62*, 17–25.

Inukai, Y., Chinen, T., Matsuda, T., Kaida, I., & Yasuda, S. (1998). Selective separation of germanium (IV) by 2, 3-dihydroxypropyl chitosan resin. *Analytica Chimica acta, 371*, 187–193.

Jalón, E. G., Blanco-Príeto, M. J., Ygartua, P., & Santoyo, S. (2001). Topical application of acyclovir-loaded microparticles: Quantification of the drug in porcine skin layers. *Journal of Controlled Release, 75*, 191–197.

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

Law, S. L., Huang, K. J., & Chiang, C. H. (2000). Acyclovir-containing liposomes for potential ocular delivery corneal penetration and absorption. *Journal of Controlled Release, 63*, 135–140.

Munday, D. L., & Cox, P. L. (2000). Compressed xanthan and karaya gum matrices hydration, erosion and drug release mechanisms. *International Journal of Pharmaceutics*, 179–192.

Nixon, J. R. (1983). Release characterization of microcapsules. In F. Lim (Ed.), *Biomedical Applications of Microcapsulation*. Boca Raton, FL: CRC Press.

Pasparakis, G., & Bouropoulos, N. (2006). Swelling studies and in vitro release of verapamil from calcium alginate and calcium alginate–chitosan beads. *International Journal of Pharmaceutics, 323*, 34–42.

Paul, W., & Sharma, C. P. (2000). Chitosan, a drug carrier for the 21st century: A review. *Società Française des Sciences et Techniques Pharmaceutics. Pharmaceutical Sciences, 10*, 5–22.

Pavelic, Z. P., Skalko-Basnet, N., Filipovic-Grcic, J., Martinac, A., & Jalsenjak, I. (2005). Development and in vitro evaluation of a liposomal vaginal delivery system for acyclovir. *Journal of Controlled Release, 106*, 34–43.

Rocha, R. M., Schefft, M., Greil, P., Bressiani, J. C., & Bressiani, A. H. A. (2005). Ceramic tapes of Si–Al–O–N–C compounds using mixtures of polysiloxane and Si-Al2O3 fillers. *Cerâmica, 51*, 42–51.

Rossel, C. V., Carrenõ, J. S., Baeza, M. R., & Alderete, J. B. (2000). Inclusion complex of the antiviral drug acyclovir with cyclodextrin in aqueous solution and in solid phase. *Química Nova, 23*, 749–752.

Ranga, R., Devi, K. D., & Buri, P. (1988). Cellulose matrices for zero-order release of soluble drugs. *Drug Development and Industrial Pharmacy, 14*, 2299–2320.

Ritger, P. L., & Peppas, N. A. (1987a). A simple equation for description of solute release. I. Fickian and non-Fickian release from non-swellable devices in the form of slabs, spheres, cylinders or discs. *Journal of Controlled Release, 5*, 23–36.

Ritger, P. L., & Peppas, N. A. (1987b). A simple equation for description of solute release. II. Fickian and anomalous release from swellable devices. *Journal of Controlled Release, 5*, 37–42.

Sancho, C. M., Vanrell, R. H., & Negro, S. (2003). Poly (D, L-lactide-co-glycolide) microspheres for long-term intravitreal delivery of acyclovir: Influence of fatty and non-fatty. *Journal of Microencapsulation, 20*, 799–810.

Sinha, V. R., Singla, A. K., Wadhawan, S., Kaushik, R., Kumria, R., Bansal, K., et al. (2004). Chitosan microspheres as a potential carries for drugs. *International Journal of Pharmaceutics, 274*, 1–33.

Silverstein, R. M., Webster, F. X., & Kiemle, J. (1994). *Identificação Espectrométrica de Compostos Orgânicos* (5th ed.). RJ: Guanabara Dois.

Valgas, S., Gonçalves, V., Pedroza, R. C., Fávere, V. T., & Laranjeira, M. C. (2005). Malonylchitosan microspheres as a matrix for oral enrofloxacin delivery. *Macromolecular Symposia, 229*, 246–252.

Woo, S. B., & Challacombe, S. J. (2007). Management of recurrent oral herpes simplex infections. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontics, 103*, 1–18.

Zolnik, S. B., & Burgess, J. D. (2007). Effect of acidic pH on PLGA microsphere degradation and release. *Journal of Controlled Release, 122*, 338–344.