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Poly(3-hydroxybutyrate)/chitosan/ketoprofen or piroxicam composite microparticles: Preparation and controlled drug release evaluation

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ABSTRACT

Poly(3-hydroxybutyrate)/chitosan/piroxicam or ketoprofen composite microparticles were prepared by the solid-in-water-in-oil emulsion-solvent evaporation technique with the aim of reducing the burst effect and controlling the drug release. Reservoir-type microparticles, composed of poly(3-hydroxybutyrate) microspheres embedded in a chitosan matrix were prepared. The size and morphological characteristics of the composite microparticles were evaluated in relation to the chitosan concentration and cross-linking with glutaraldehyde. Reservoir-type composite microparticles were obtained using 2.0% and 3.0% w/v chitosan solutions. A significant reduction in the burst effect and prolonged drug release were observed, particularly when higher chitosan and glutaraldehyde concentrations were used.

1. Introduction

Polymer-based drug-delivery systems, such as microspheres, are relatively simple to produce and can be administered by various routes, including oral, pulmonary and parenteral injection. Microspheres using a single polymer generally lead to high initial drug release (burst effect) and are unable to sustain the release for long periods. Burst release, which may have serious side effects (Morita, Horikiri, Suzuki, & Yoshino, 2001), is generally difficult to control and may occur due to a number of phenomena, including the presence of the drug on the microsphere surface, porosity of the microparticles and physical–chemical nature of the polymeric matrix (Hasan et al., 2007). However, it may be prevented by developing more complex drug-loaded delivery systems, such as composite microspheres, having multiple cores of one polymer dispersed in a second continuous polymeric matrix (reservoir-type), or core-shell microparticles consisting of a single core surrounded by a polymeric layer. These systems present additional barriers to drug diffusion, reducing the initial burst and prolonging drug release (Pollauf, Kim, & Pack, 2005; Wang, Ye, Zheng, Liu, & Tong, 2007).

Naturally occurring polymers, such as poly(3-hydroxybutyrate) [PHB] and chitosan, show increasing importance in the development of controlled drug-delivery systems and implantable biomaterials. Chitosan is a polysaccharide that occurs principally in the exoskeleton of insects and the shell of crustaceans. Various drug-delivery systems have been developed using this polymer, including micro- and nanoparticles, due to its excellent biocompatibility, biodegradability, bioactivity, mucoadhesivity and nontoxicity (Anal, Stevens, & Remuñán-López, 2006; Gupta & Jabrail, 2007; Zhou et al., 2006). Drug release from chitosan microspheres can be controlled by cross-linking the polymeric matrix using physical or chemical agents, such as glutaraldehyde (Ge, Chen, Xie, & Zhang, 2007; Zhou et al., 2006).

PHB is a biodegradable and biocompatible polyester synthesized by numerous bacteria as intracellular carbon and energy storage compounds (Zinn, Witholt, & Egli, 2001) and offers a high potential for application in drug-delivery systems such as microparticles. However, its high degree of crystallinity leads to the formation of porous microspheres, which release the drug rapidly (Bazzo, Lemos-Senna, Gonçalves, & Pires, 2008; Bidone et al., 2009; Conway, Eyles, & Alpar, 1997; Martin, Miguens, Rieumont, & Sanchez, 2000).

To overcome this disadvantage of PHB microspheres, this paper proposes the preparation of composite microparticles consisting of PHB microspheres incorporated into a chitosan matrix, using the anti-inflammatory drugs ketoprofen (KET) and piroxicam (PXC) as models. A modified emulsion-solvent evaporation technique is proposed to obtain the composite microparticles. The influence of chitosan concentration and cross-linking with glutaraldehyde on the reduction of the initial burst effect and on the drug release was evaluated by statistical analysis.

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2. Materials and methods

2.1. Materials

Poly(3-hydroxybutyrate) (M_n = 312,800 g mol^{-1} and polydispersity degree of 1.23, determined by gel permeation chromatography) was kindly supplied by PHB Industrial S.A. (Serrana, São Paulo, Brazil). Ketoprofen was purchased from All Chemistry (São Paulo, Brazil), piroxicam from Henrifarma (São Paulo, Brazil), chitosan (medium molecular weight and deacetylation degree of 75%) from Sigma–Aldrich (USA) and poly(vinyl alcohol) (M_n = 92,000 g mol^{-1}, according to the manufacturer) from Vetec (Rio de Janeiro, Brazil). Glutaraldehyde was acquired from Nuclear (São Paulo, Brazil) and Span 80 from Beraca (São Paulo, Brazil). All chemicals were used without further purification.

2.2. Preparation of drug-loaded PHB microspheres

PHB microspheres containing either piroxicam (PHB/PXC) or ketoprofen (PHB/KET) were prepared by the oil-in-water emulsion-solvent evaporation technique, as previously described (Bazzo et al., 2008). The polymer (500 mg) and the drug (200 mg) were dissolved in dichloromethane (internal phase) and then emulsified in 200 mL of an aqueous phase containing 0.15% (w/v) of poly(vinyl alcohol) as the stabilizer and 6% (v/v) of isopropanol (external phase). The emulsion was stirred at 700 rpm, at room temperature, until the complete evaporation of the organic solvent. The microspheres were washed with distilled water, dried and stored under vacuum at room temperature.

2.3. Preparation of PHB/chitosan composite microparticles

A solid-in-water-in-oil (SWO) emulsion-solvent evaporation technique was proposed to obtain PHB/chitosan composite microparticles. The PHB/drug microspheres were dispersed in 25 mL of chitosan solution (acetic acid 2.0% v/v). The dispersed microspheres were then added to 200 mL of liquid paraffin containing 2.0% of Span 80 as the emulsifier, under magnetic stirring. After the evaporation of the aqueous phase, the microparticles were washed with n-hexane and dried at 25 °C. The composite microparticles were denoted by CM1, CM2 and CM3, corresponding to chitosan solution concentrations of 1.0%, 2.0% and 3.0% w/v, respectively.

2.4. Preparation of PHB/cross-linked chitosan composite microparticles

A quantity of composite microparticles containing 100 mg of chitosan were transferred to glutaraldehyde aqueous solution (0.1% or 1.0% w/v), maintained under stirring for 1 h and then washed with distilled water, dried and stored under vacuum at room temperature.

2.5. Determination of the drug content and encapsulation efficiency

The PXC and KET content in the microparticles was determined by maintaining 10 mg of microparticles in a mixture of 10 mL of acid acetic solution (2% v/v) and 10 mL of dichloromethane for 1 h, under stirring. The drug concentration in each solvent was determined by UV–vis spectrophotometry (Perkin Elmer lambda 11/Bio spectrophotometer). The experiments were previously validated in terms of linearity, accuracy, inter-day precision and specificity. The drug content was calculated from the calibration curves and expressed as encapsulation efficiency (EE%), according to Eq. (1).

$$EE\% = \frac{\text{drug found in microparticle (mg)}}{\text{drug initially added to the formulation(mg)}} \times 100 \qquad (1)$$

Table 1
Independent variables and their levels investigated in the preparation of the microparticles.

Independent variables	Low level (−)	Central level (0)	High level (+)
Chitosan concentration (%)	1.0	2.0	3.0
Glutaraldehyde concentration (%)	0	0.1	1.0

2.6. Scanning electron microscopy (SEM)

The morphology of the microspheres was examined under a Philips XL30 scanning electron microscope. To observe the internal structure, frozen particles were cut with a blade. The samples were vacuum-coated with gold in a Polaron E-5000 instrument and then analyzed. The arithmetic mean diameter was determined from at least 100 particles measured on micrographs obtained by SEM.

2.7. Thermal analysis

DSC curves were obtained using a Shimadzu DSC-50 differential scanning calorimeter by heating from 25 to 200 °C at 10 °C min^{-1} in a nitrogen atmosphere (50 mL min^{-1}).

2.8. In vitro drug release

The *in vitro* drug release was carried out in phosphate buffer solution pH 7.4 at 37 ± 1 °C. A total amount of microspheres containing 45 mg of the drug was suspended in 30 mL of phosphate buffer and maintained in a thermostated oscillating bath for one week. After pre-determined time intervals an aliquot of the release medium (4 mL) was withdrawn and the samples were immediately returned to the dissolution vessels after analysis. The samples were then analyzed by UV–vis spectrophotometry (λ_{max} = 360 nm for PXC and λ_{max} = 260 nm for KET) and the amount of drug released was calculated. In order to determine the effect of chitosan and glutaraldehyde concentration on drug release, the experiments were performed using a 3^2 factorial design. Table 1 gives the independent variables and their levels which were investigated in the preparation of the microparticles. The percentage of drug release in 45 min (Q_{45}) and the dissolution efficiency at 168 h (DE) were used to compare the drug release profiles. The dissolution efficiency is given by Eq. (2). The data were evaluated by analysis of variance (ANOVA) followed by the application of the Tukey test, using the software GraphPad Prism®.

$$DE = \frac{\int y dt}{t} \qquad (2)$$

where y denotes the percentage of dissolved drug and t the time.

3. Results and discussion

3.1. PHB/drug microspheres

The PHB/KET and PHB/PXC microspheres showed a spherical shape, mean diameter of 32.5 ± 11.2 μm and 31.1 ± 9.2 μm, respectively, and a rough polymeric matrix, as can be seen in Fig. 1. As expected, the *in vitro* release profiles (Fig. 2) showed an initial burst and a rapid drug release from the PHB microspheres. The rapid release of both drugs from the microspheres may be due to the rapid diffusion of the drug through the porous polymeric matrix and the drug distribution preferentially at the surface of the microparticles.

A previous paper (Bazzo et al., 2008) indicated through DSC experiments the predominant crystalline form of piroxicam in the PHB microspheres. However, the ketoprofen is predominantly

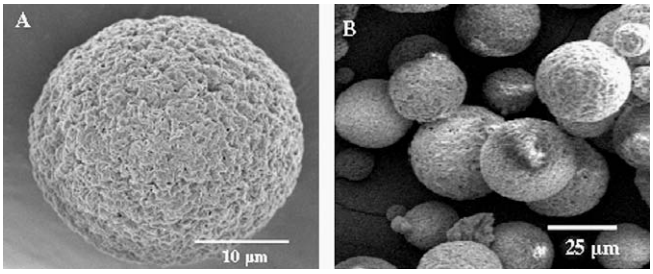

Fig. 1. Scanning electron micrographs of PHB microspheres containing piroxicam (A) and ketoprofen (B).

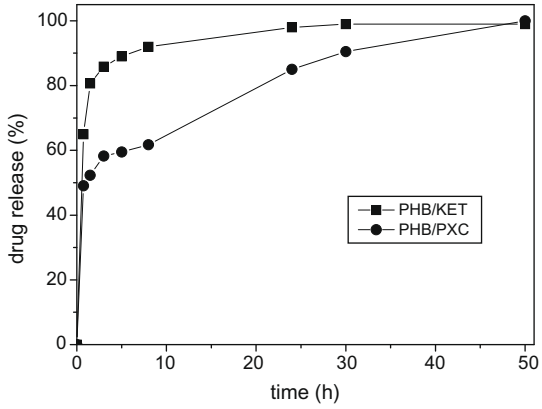

Fig. 2. Release profiles of PXC and KET from the PHB microspheres, in phosphate buffer pH 7.4.

present in the amorphous form, as shown in Fig. 3. The PHB shows two different melting temperatures at 166 °C and 176 °C ($\Delta H_f = 88\ J\ g^{-1}$), the KET had a melting temperature at 96 °C ($\Delta H_f = 108\ J\ g^{-1}$), and for the PHB/KET microspheres the melting temperature peak had the same value as the pure component. On the other hand, the fusion enthalpy value for the drug in the system was lower ($\Delta H_f = 15\ J\ g^{-1}$) than that for the pure KET. It should be considered that KET was predominantly dispersed in the PHB matrix in the amorphous form, which has a greater solubility than the crystalline form. This is one of the factors that could explain the faster release of KET from the microspheres compared to PXC. Also, the higher solubility of KET in the release medium may have contributed to its faster release.

3.2. PHB/chitosan composite microspheres

The methodology used in this study to incorporate the PHB/drug microspheres into the chitosan matrix includes three steps:

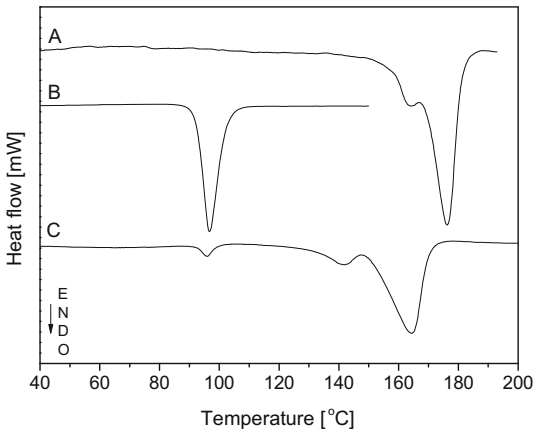

Fig. 3. DSC curves of: (A) PHB; (B) ketoprofen and (C) PHB/KET microspheres.

(i) immersion of the microspheres in aqueous chitosan solution; (ii) dispersion into liquid paraffin; and (iii) evaporation of the aqueous phase.

Fig. 4 shows the optical transmission micrographs of the emulsion when PHB microspheres dispersed in the chitosan solution were poured into the oil phase at CM1, CM2 and CM3, corresponding to chitosan solution concentrations of 1.0%, 2.0% and 3.0% w/v, respectively. For the chitosan solution of 1.0% w/v there is only a small domain of chitosan, which is not sufficient to cover the PHB microspheres (Fig. 4A). However, at a higher concentration of chitosan solution we can observe the PHB microspheres surrounded by chitosan matrix (Fig. 4B and C), and originating reservoir-type microparticles.

The scanning electron micrographs of the composite microparticles are shown in Fig. 5. There were no differences with regard to the morphological characteristics of microparticles according to the drug used as the model. However, the aqueous chitosan solution concentration influenced the size and morphology of the composite microparticles. It was not possible to obtain composite microparticles when a chitosan concentration of 1.0% w/v was used. As can be seen in the micrographs (Fig. 5A and B), uncoated PHB and chitosan microspheres were obtained. On the other hand, on increasing the concentration of the chitosan solution, composite microparticles were formed with the inclusion of numerous PHB/drug microspheres in a single composite microparticle (Fig. 5C and F). The mean size of composite microparticles increased from 89 μm to 410 μm when the chitosan concentration was increased from 2.0% to 3.0% w/v. The large particle size is associated with the viscosity of the chitosan solution. When using a high chitosan concentration, the polymer solution becomes more viscous and, consequently, more resistant to fragmentation into small droplets which, in turn, leads to the generation of a coarser emulsion and, subsequently, to the formation of larger microspheres (Thompson

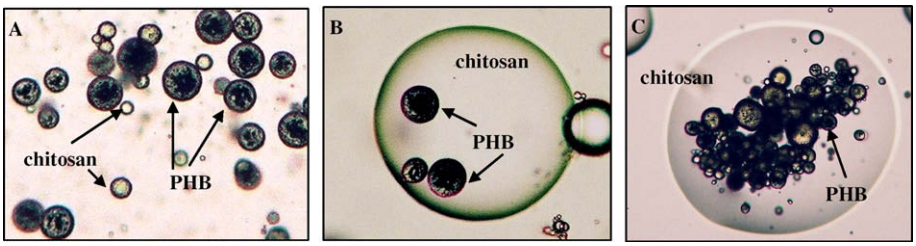

Fig. 4. Optical micrographs of the PHB microspheres after their dispersion into the oil phase during the preparation of the composite microparticles using different chitosan solution concentrations: (A) 1.0%; (B) 2.0% and (C) 3.0%.

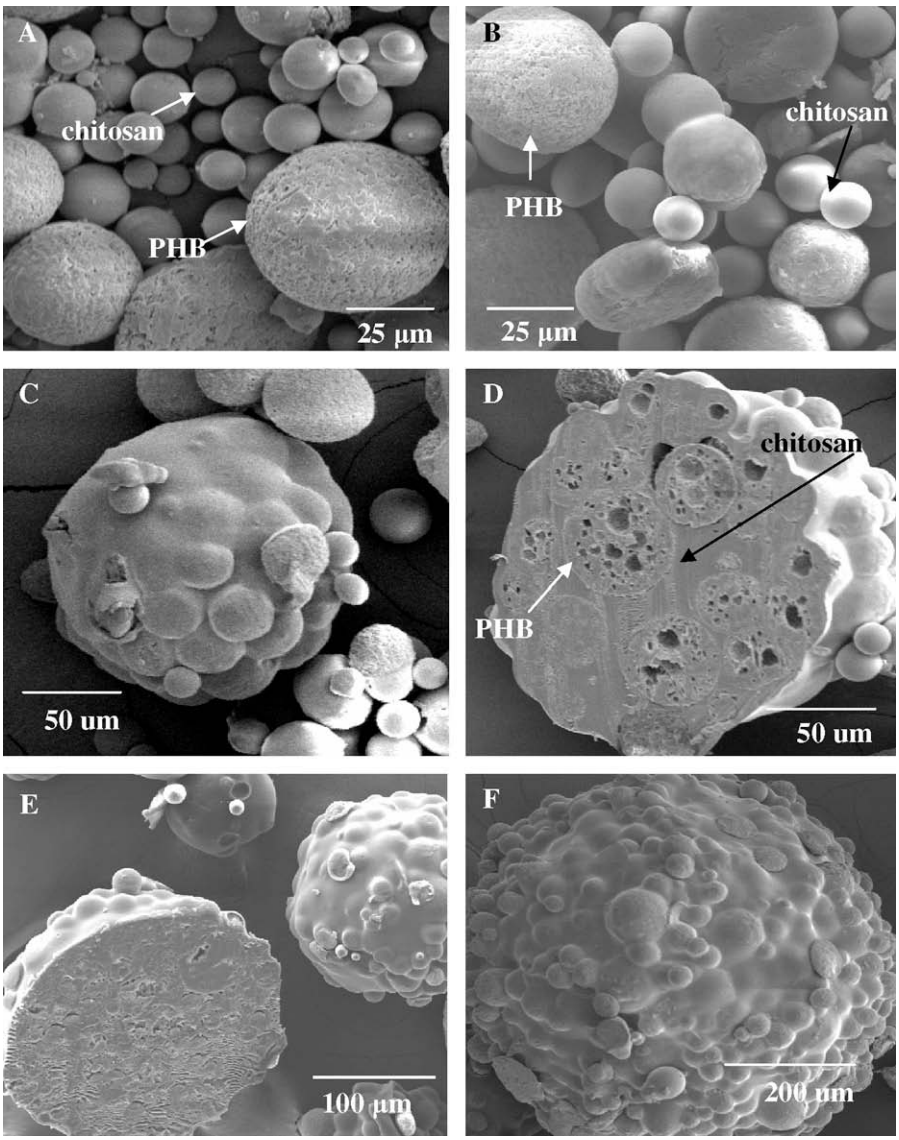

Fig. 5. Scanning electron micrographs of PHB/chitosan composite microparticles prepared using PXC and chitosan solutions at concentrations of (A) 1.0%, (C), 2.0%, (E) 3%, and KET and chitosan solutions at concentrations of (B) 1.0%, (D) 2.0%, (F) 3%.

et al., 2007). No differences were observed in the morphology and size of the composite microparticles after the cross-linking of chitosan with glutaraldehyde.

Fig. 6 shows that the KET and PXC encapsulation efficiency decreased from 74.9% to 34.6% and from 33.5% to 23.3%, respectively, when the chitosan concentration decreased from 3.0% to 1.0% w/v. The encapsulation efficiency of both drugs was less than 100%, indicating that some of the drug dissolved in the acid aqueous phase and then diffused to the external oil phase during the preparation of the composite microparticles. The increase in the solution viscosity when higher chitosan concentration was used could be a major contributor to the EE% increase. The viscous chitosan solution can act as a barrier to drug diffusion into the external oil phase. It has been reported that the enhancement of the polymeric solution viscosity enhances the EE% because it is more difficult for the drug to diffuse into the outer phase (Hasan et al., 2007). In fact, it has previously been demonstrated that a high chitosan concentration hinders the encapsulation of model drugs (Gan & Wang, 2007). The higher encapsulation efficiencies for KET may be due to it having a different solubility in the acidic and oil phase compared with PXC.

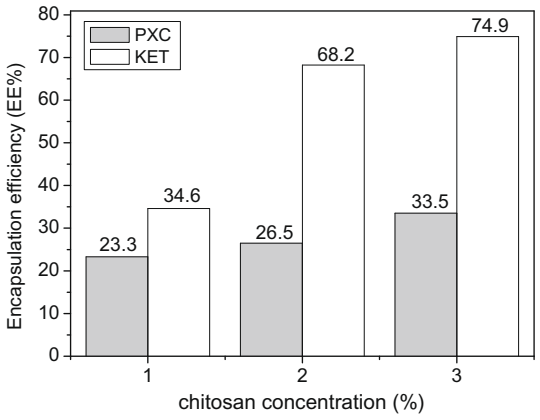

Fig. 6. Efficiencies of piroxicam and ketoprofen encapsulation into the PHB/chitosan composite microspheres.

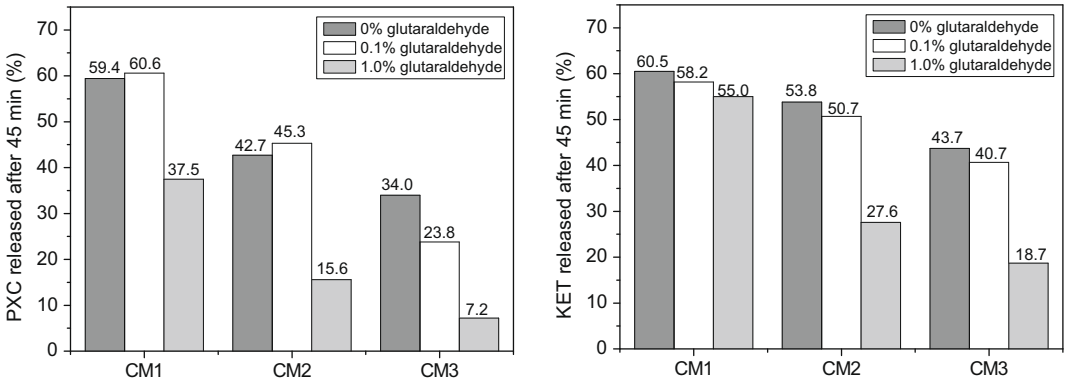

Fig. 7. Percentages of piroxicam and ketoprofen released after 45 min from the composite microparticles prepared with 1.0%, 2.0% and 3.0% w/v of chitosan solution (CM1, CM2 and CM3, respectively).

3.3. In vitro drug release

The percentages of drug release after 45 min (Q_{45}) presented in Fig. 7 were used to evaluate the effect of chitosan and glutaraldehyde concentration on the PXC and KET initial burst release. Analysis of variance shows that both variables influenced the initial drug release ($F_{calc} > F_{tab}$; $\alpha = 0.05$).

As previously described, the PHB microspheres showed an immediate initial drug release in the first 45 min (51.4% for PXC and 64.9% for KET), as shown in Fig. 2. The release of model drugs from the composite microparticles CM1 proved to be very similar to that from the PHB microspheres, with a pronounced burst effect, due to the microparticles being no coated and embedded in the chitosan matrix, as shown in the micrographs of Fig. 5. On the other hand, a decrease in the amount of drug released after 45 min was obtained for microparticles CM2 and CM3. The incorporation of the PHB microspheres into the chitosan matrix significantly reduced the PXC and KET burst release, particularly in the case of the reservoir-type microparticles CM3, related with the size of the composite microparticles. It has been reported that an increase in the size of chitosan microspheres, which is related to higher viscosity of the chitosan solution, leads to a reduction in the burst effect (Gan & Wang, 2007).

The release of drugs from microparticles prepared with chitosan can be modified through the cross-linking of the polymer, which makes the matrix more rigid, compact and hydrophobic (Yuan et al., 2007). In this study, we used glutaraldehyde, the most commonly used cross-linker agent, in order to evaluate the influence of chitosan cross-linking on the release profile of the PXC and KET. As can be seen in Fig. 7, a substantial reduction in the initial burst was obtained by cross-linking the chitosan, thus creating an additional barrier to drug diffusion. The application of the Tukey test showed that there was no statistically significant difference between the Q_{45} values of the microparticles in the absence and presence of cross-linking with 0.1% of glutaraldehyde ($P > .05$), regardless of the concentration of chitosan used. However, when the concentration of cross-linker was increased to 1.0% a significant reduction in Q_{45} ($P < .05$) was observed, indicating a greater reduction of the burst effect when using a higher concentration of glutaraldehyde.

Aiming to evaluate the effect of the concentration of chitosan and cross-linker on the ability of microparticles to prolong the drug release, the values of dissolution efficiency (Fig. 8) were evaluated by analysis of variance, indicating that both variables affect the DE at 168 h ($F_{calc} > F_{tab}$; $\alpha = 0.05$).

Through the application of the Tukey test it was shown that the increase in the chitosan concentration from 1.0% to 2.0% or 3.0% w/v decreased significantly the ED, regardless of the concentration of glutaraldehyde used. As discussed above, the incorporation of PHB microspheres into the chitosan matrix, particularly when the microparticles had a larger size (CM3), caused an additional barrier to the diffusion of the drug, prolonging its release. However, no differences were observed in the DE values of both drugs when the concentration of chitosan was increased from 2.0% to 3.0% ($P > .05$). The application of the Tukey test also showed that the addition of cross-linker agent decreased significantly the DE, regardless of the concentration of chitosan, leading to prolonged release of both drugs, as a result of the greater rigidity and hydrophobicity of the matrix. The increase in glutaraldehyde concentration from 0.1% to 1.0% caused a significant reduction in the DE ($P < .05$) and therefore the prolonging of the release of PXC and KET was dependent on the concentration of the cross-linker agent.

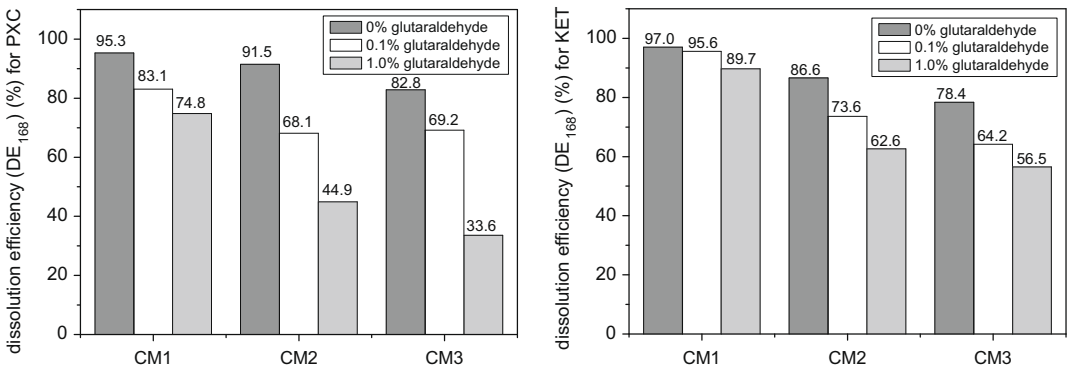

Fig. 8. Dissolution efficiencies of composite microparticles, prepared with 1.0%, 2.0% and 3.0% w/v of chitosan.

4. Conclusions

The solid-in-water-in-oil emulsion-solvent evaporation method proposed in this study was suitable for the production of PHB/chitosan/drug composite microparticles and can be considered as an efficiency procedure to obtain reservoir-type microparticles. The chitosan concentration in the internal aqueous phase is an important processing parameter that affects the size and morphological characteristics of the microparticles. It was possible to control the initial burst release and to prolong the piroxicam and ketoprofen release by varying the chitosan and glutaraldehyde concentrations.

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