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RESEARCH ARTICLE

Design and characterization of sustained release ketoprofen entrapped carnauba wax microparticles

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Abstract

Context: Ketoprofen is a non-steroid anti-inflammatory drug (NSAID) used in the treatment of rheumatic diseases and in mild to moderate pain. Ketoprofen has a short biological half-life and the commercially available conventional release formulations require dosages to be administered at least 2–3 times a day. Due to these characteristics, ketoprofen is a good candidate for the preparation of controlled release formulations.

Objectives: In this work, a multiparticulate-sustained release dosage form containing ketoprofen in a carnauba wax matrix was developed.

Methods: Particles were prepared by an emulsion congealing technique. System variables were optimized using fractional factorial and response surface experimental design. Characterization of the particles included size and morphology, flow rate, drug loading and *in vitro* drug release.

Results: Spherical particles were obtained with high drug load and sustained drug release profile. The optimized particles had an average diameter of approximately 200 µm, 50% (w/w) drug load, good flow properties and prolonged ketoprofen release for more than 24 h.

Conclusions: Carnauba wax microspheres prepared in this work represent a new multiparticulate-sustained release system for the NSAID ketoprofen, exhibiting good potential for application in further pharmaceutical processes.

Keywords: Carnauba wax, microparticles, ketoprofen, factorial design, sustained release, dissolution

Introduction

The NSAID ketoprofen was first described by the French company Rhone Poulenc[1]. Indications include rheumatoid arthritis, osteoarthritis, gout and psoriasis related arthritis, dysmenorrhea, pain associated with non-rheumatic inflammatory conditions, vascular headache and fever[2–7]. Posology for adults is from 100 to 200 mg daily, in 2 or 3 doses[8].

When administered in conventional immediate release formulations, ketoprofen is rapidly absorbed in the gastrointestinal (GI) tract, reaching plasma concentration peaks in 0.5–2 h. Ketoprofen half-life in plasma is approximately 2–3 h[9–11] causing blood concentrations to drop abruptly to sub-therapeutic levels[12]. Adversely, one single dose of 150 mg in an immediate release formulation leads to plasma concentrations of 15–25 µg/mL, above the therapeutic levels[12]. In order to maintain plasma levels within the therapeutic range, frequent administrations are necessary[10–12], leading to adverse effects that may limit clinical usage of ketoprofen[9,13]. These characteristics make ketoprofen a good candidate for the development of sustained release drug delivery systems[14–19].

The commercially available modified release oral formulations for ketoprofen utilize soluble or non-soluble polymeric coatings[20–22]. Disadvantages of polymer coatings include the use of organic solvents and potential compatibility issues[21]. In addition, in the event of a defective film coating, high doses of the drug can be released in a short time in a limited area, leading to severe adverse effects[23,24]. Sustained release formulations are designed

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to maintain the blood concentration of the drug within the therapeutic range for a prolonged time, increasing therapeutic efficacy, reducing adverse effects and contributing to improve patient compliance[25–28].

Multiparticulate oral dosage forms have been investigated as potential sustained release formulations[26,29–36]. Following their administration, the particles spread over a large surface area of the GI tract. This increases the safety of the therapy, especially when the drug induces local irritation[27,37–39]. Microparticle formulations also exhibit a prolonged time of residence in the small intestine, which contributes for their properties of sustaining drug release[40,41].

Lipophilic products such as waxes and lipids can be very attractive as formulation ingredients due to their low cytotoxicity and their ability to provide both sustained release and protection of the drug against chemical degradation. Examples of this class of materials include glyceryl monostearate (GMS), cetyl ester wax, cetyl alcohol, yellow beeswax, glycerol, glyceryl palmitostearate and stearic acid[36,42–44]. However, these products often present low flowability, limiting their application in industrial pharmaceutical processes[45].

Multiparticulate systems can be prepared by the emulsion congealing technique. Despite this being a relatively simple and inexpensive technique[46–48], little is known about its application in the preparation of spherical particles (pellets or microspheres) using waxes or lipids[7,36,49–52].

In this work, a novel sustained release multiparticulate system, consisting of carnauba wax microspheres containing the NSAID ketoprofen was prepared using the emulsion congealing technique. Carnauba wax was chosen due to its biocompatibility, wide availability and low cost from a pharmaceutical formulation point of view. Formulation and process variables affecting pellet diameter, drug loading and morphology were investigated. Drug release profile and physical-chemical properties of the particles, such as repose angle and apparent density were evaluated.

Materials and methods

Materials

Ketoprofen was purchased from Hubei Wuxue Xunda Pharmaceutical, Wuxue, China. Potassium phosphate monobasic and polysorbate 80 (Tween 80) were purchased from Vetec Química Fina, Duque de Caxias, Brazil. Carnauba wax (tipe I, micronized) was supplied from Tropical Ceras, Parnaíba, Brazil.

Experimental design

Formulation and process variables were defined as speed of agitation (rpm), drug/wax ratio (w/w), cooling time onset (s), wax/ aqueous phase ratio (g/mL), and surfactant concentration (% v/v). Levels of variations were defined by a fractional factorial design 2 III$^{5-2}$[53] and are presented in Table 1. After the definition of the most influential variables, two experimental designs were conducted according to the response surface method (2^2 with central point[53]).

Preparation of microspheres

Particles were prepared by the emulsion congealing technique. Drug loaded wax matrix microspheres were prepared by adding different amounts of ketoprofen to the formulation. Briefly, carnauba wax was melted at 90 ± 2°C in a water bath, and appropriate amount of ketoprofen was added. The mixture was then poured into 200 mL of an aqueous solution of polysorbate 80 (0.2 or 0.5%) heated at 90 ± 2°C and under continuous stirring (200, 300, 400 or 600 rpm) (Fisatom, model 710). After

Table 1. Fractional factorial experimental design 2_{III}^{5-2} to study diameter, drug load and morphology of ketoprofen entrapped carnauba wax microparticles.

		Level	
	Variable	–	+
1	Speed of agitation(rpm)	300	600
2	Ketoprofen/carnauba wax ratio(w/w)	1/1	3/1
3	Cooling onset(seconds)	15	120
4	Wax/aqueous phase(g/mL)	3/200	6/200
5	Surfactant concentration (%.v/v)	0.2	0.5

Particles	1	2	3	4	5	Ketoprofen load in particles (%.w/w)			Average	Diameter (μm)			Average	Morphology			Average
A_1	–	–	–	–	+	48.47	38.87	43.11	43.48	252.73	236.11	194.46	227.77	+++	+++	+++	+++
B_1	+	–	–	+	–	34.68	58.45	43.87	45.67	132.53	125.54	177.8	145.29	+++	+++	+++	+++
C_1	–	+	–	+	–	12.13	11	16.07	13.07	306.53	327.33	267.47	300.44	+++	++++	+++	+++
D_1	+	+	–	–	+	7.47	8.87	9.46	8.6	91.01	70.86	80.89	80.92	++++	++++	++++	++++
E_1	–	–	+	+	+	25.54	30.19	30.82	28.85	278.3	209.54	276.08	254.64	+++	+++	++	+++
F_1	+	–	+	–	–	17.47	23.76	14.19	18.47	118.76	91.09	127.89	112.58	++	++	++	++
G_1	–	+	+	–	–	8.66	8.2	7.47	8.11	128.22	176.42	211.15	171.93	++	+++	++++	+++
H_1	+	+	+	+	+	3.95	3.77	3.74	3.82	112.33	164.63	231.93	169.63	++	++	++	++

Morphology (spherical) = +: bad, ++: regular, +++: good, ++++: very good.

mixing both phases, a rapid cooling of the system was induced after 5, 15, 25 or 120 seconds by adding 50 mL of water at 5°C following the addition of ice cubes in a volume corresponding to 50% of the original aqueous phase. Agitation was maintained until the emulsion had reached room temperature. Particles were then washed three times with water, filtered and allowed to dry.

Evaluation of pellet size and morphology

Particle size and size distribution were measured using an optical microscope (Leica DME, Leica Microsystems, Germany) and the morphometry software LAZ EZ (Leica Microsystems). From each batch of microspheres, a randomly selected sample was collected and placed on a glass slide. Diameter of at least 200 particles from each batch was measured. Morphology of the particles was also evaluated with the aid of a stereomicroscope (Leica MZ6, Leica Microsystems, Germany) and a scanning electron microscope (SEM) (JEOL, JSM-840, Japan).

Flow rate, repose angle, and density of microspheres

Flow rate and angle of repose were determined according to the fixed height and variable cone aperture method, using a Granulate and Powder Flow Tester (GTB Flow meter, Erweka GmbH, Germany). A fixed amount of particles was allowed to flow through a funnel (8.3 cm upper diameter and 11.3 mm inferior diameter) without agitation. The average of three determinations was calculated.

Density was determined using an automated compactor (SVM 203, Erweka GmbH, Germany) equipped with a 15 mL glass column. The volume of a fixed amount of particles was measured before and after compaction (300 strokes per minute, during 10 min). The average of three determinations was calculated.

Flow properties of the particles were represented by calculating Carr (%) index and Hausner ratio[28,54–56]:

$Carr = 100\dfrac{V_B - V_T}{V_B}$	where V_B is the freely settled volume of the batch of particles, and V_T is the tapped volume of the same batch.
$Hausner = \dfrac{\rho_T}{\rho_B}$	where ρ_B is the freely settled bulk density of the batch of particles, and ρ_T is the tapped bulk density of the same batch.

Efficiency of drug loading

To determine the amount of ketoprofen loaded in the particles, wax matrix microspheres were submitted in triplicates to a quantitative drug assay. Extraction of ketoprofen from the pellets was conducted as follows: samples of each batch of particles were crushed in a mortar and pestle. Following this, 10 mg of the powdered samples were accurately weighed, transferred to a beaker containing 10 mL of water:acetonitrile (40:60, v/v) and placed in an ultrasonic bath for 30 s. A volume of 4 mL of the solvent was removed and centrifuged at 5000 rpm for 5 min. Then, 100 µL of the supernatant was diluted with 0.9 mL of the mobile phase, filtered and placed in an HPLC vial. The extraction procedure was validated by combining a given amount of micronized carnauba wax with a known quantity of ketoprofen, followed by the same quantitative assay.

HPLC was performed in a Varian, Prostar 240 Liquid Chromatographer (Palo Alto, CA), equipped with a Microsorb MV100-5 C18, 250 × 4.6 mm column. Mobile phase was 37% water, 60% acetonitrile and 3% potassium phosphate buffer 0.5 Mol/L (pH 3.5), elution was isocratic. Injection volume was 10 µL, mobile phase flow was 1.7 mL/min and UV detection was at 260 nm wavelength. A calibration curve was obtained using ketoprofen reference standard solutions in the mobile phase at a concentration range of 6 to 40 mg/L.

In vitro release of ketoprofen from carnauba wax matrix microspheres

In order to study the dissolution of ketoprofen from the carnauba wax microspheres, hard gelatin capsules were filled with a mass or pellets corresponding to 200 mg of ketoprofen. Dissolution test was also conducted with similar capsules filled with 200 mg of pure ketoprofen.

Dissolution test was conducted in sextuplicates. Dissolution medium was 1000 mL of potassium phosphate buffer 0.05 Mol/L, pH 7.4 at 37 ± 0.5°C, in an USP apparatus I (basket[57]), 100 rpm, using a Varian VK7000 Dissolution Tester attached to a Cary 50 WinUV Total Solution System (Varian, Cary, NC). Sink conditions were maintained throughout the experiment, since ketoprofen solubility in pH 7.4 was approximately 12.6 mg/mL.

The amount (%) of drug dissolved was determined according to the analytical method described in section 2.6. A calibration curve ($r^2 = 0.9998$) was previously obtained with ketoprofen reference standard solutions in concentrations ranging from 1 to 250 mg/L, in triplicate.

Statistical analyses of results

Response surface models (software SYSTAT® 12) were adjusted by ANOVA. Statistical significance of each variable studied on the properties of the system, as well as comparisons of dissolution profiles were determined by applying *t* test for independent samples, with a confidence interval of 95% (software OriginPro® 8).

Results and discussion

The characteristics of the particles prepared in this study by the emulsion congealing technique are presented in Table 1, according to the 2_{III}^{5-2} fractional factorial design. It can be observed that particle diameter was mainly influenced by the speed of agitation of the emulsion, and to a lesser extent, by the ratio between the wax phase (containing carnauba wax and ketoprofen) and the aqueous phase in the system. Similar findings were presented by

Adeyeye and Price[49] and Al-Kassas et al.[50] when studying ibuprofen microspheres. Al-Kassas et al.[50] also observed that increasing the rate of cooling of the system contributed to the obtention of smaller particles, which diverged from Draper and Becker[46] results from sulfa ethyl thiadiazole wax formulations. In the present work, similar test were performed, gradually reducing the temperature of the emulsion; however, this procedure lead to the formation of numerous agglomerates and wax accumulation on the beaker walls. When a rapid cooling method was used, these problems were not observed. The initiation of the cooling after 5, 15, 25 or 120 seconds of mixing wax and aqueous phase did not seem to influence the final diameter of the particles.

Results demonstrated that ketoprofen load in the particles depended mostly on the amount of ketoprofen added to the wax phase. This variable was also considered to have significant influence on particles morphology.

Varying the surfactant concentration from 0.2 to 0.5% in the aqueous phase did not cause significant modifications in the characteristics of the microspheres. However, when no surfactant was used, pellets could not be obtained in a reproducible manner. Similar observations were made by Hassan et al.[52], when working with different wax materials used surfactant concentrations from 0.2 to 0.8%. In this study, polysorbate 80 concentration was fixed at 0.5% (v/v) in the aqueous phase, since higher concentrations can interfere with the dissolution tests due to the presence of surfactant residues in the particles[46].

The formation of irregular and large flakes or masses which do not allow the identification of individual particles is related to the type of wax or lipid used in the preparation process[49,51]. Usually, the higher the melting point of the wax, the lower the possibility of the appearance of agglomerates[48]. This is an advantage of carnauba wax, which has a melting point around 85°C, when compared to other wax materials, such as beeswax used by Hassan et al.[52]. After the preparation of pellets, the melting point of beeswax is markedly reduced (63.2 to 50.6°C[7]), increasing the chances of the formation of particle agglomerates. Despite the high melting point of carnauba wax, during the previous experiments in this work, large and irregular stacks were formed due to the rapid solidification of the wax (Figure 1). The factorial experimental design was valuable to optimize both formulation and process variables in order to obtain spherical particles.

The optimization of the formulation variables allowed the production of spherical microparticles with an average diameter varying from 80.92 µm (D_1) to 300.44 µm (C_1). Ketoprofen load (drug content) in the particles ranged from 3.82% (H_1) to 45.67% (B_1) (Table 1).

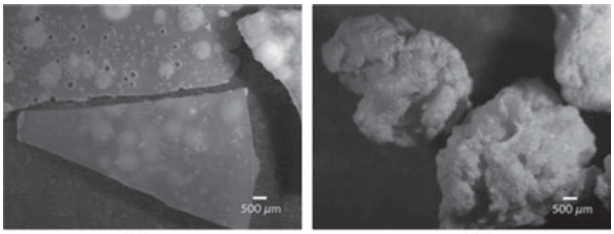

Figure 1. Results of preliminary tests for the preparation of carnauba wax particles by the emulsion congealing technique without (A) and with (B) ketoprofen in the wax phase.

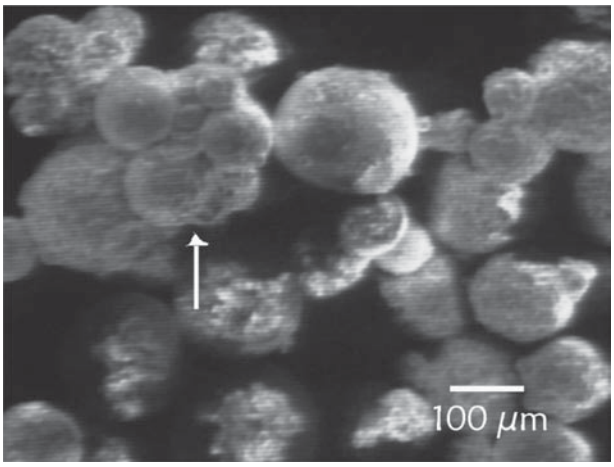

Figure 2. Photomicrograph (SEM) of carnauba wax particle agglomerates (arrow) from batch H1, prepared by the emulsion congealing technique.

Table 2. Results from a 2^2 experimental design with a central point for the preparation of ketoprofen entrapped carnauba wax microparticles. x_1 and x_2 represent the variables defined by the equations $x_1 = (Ag - 400)/200$ and $x_2 = (O - 6)/3$.

Particles	Ag (rpm)	O (g/200 mL)	x_1	x_2	Diameter (µm)	Ketoprofen load in particles (%, p/p)	Morphology
A_2	200	3	−1	−1	539.47	50.75	++
B_2	600	3	1	−1	163.99	49.27	+++
C_2	200	9	−1	1	568.29	48.78	++
D_2	600	9	1	1	195.46	40.42	+
E_2	400	6	0	0	285.14	47.74	++++
F_2	400	6	0	0	301.78	43.51	+++
G_2	400	6	0	0	336.53	51.11	+++

Ag: speed of agitation; O: total amount of ketoprofen and carnauba wax; Morphology (spherical) = +: bad, ++: regular, +++: good, ++++: very good. Other conditions: 0,5 % surfactant, 1/1 ketoprofen/wax ratio; cooling onset in 15 seconds.

Even though batch H1 was prepared with a lower drug:wax ratio (1:3 w/w), agglomerated particles were observed, also presenting more irregular surfaces (Figure 2). The literature reports that raising the drug content in the particles favors the formation of agglomerates due to a decrease in the melting point of the wax material, increasing the appearance of bridges between particles[51,52]. In this work, results demonstrated that this statement is not absolute, since other system variables can be modified, such as the cooling onset, avoiding the agglomeration of the microparticles.

The characteristics of the microparticles prepared according to two 2^2 experimental designs with a central point are presented in Tables 2 and 3. Microspheres were obtained with an average diameter varying from 152.22 to 568.29 μm and drug load from 19.55 to 60.55%. In Figure 3A, it can be observed that by increasing the speed of agitation of the emulsion the diameter of the particles was reduced. Smaller particles were also obtained when the ratios wax/water and drug/wax were reduced along with a longer time to initiate the cooling process (Figure 3B).

Results showed that increasing the amount of ketoprofen added to the melted wax lead to a higher drug loading in the microspheres (Figure 4A). Other variables had less significant influence regarding the drug load in the particles. A slight increase in drug loading occurred when the cooling of the system was initiated faster, stirring speed was lower and wax/aqueous phase ratio was smaller (Figure 4). The influence of the cooling onset time may be related to the excess of ketoprofen present in the melted wax phase that could be partially transferred to the aqueous phase. A more rapid cooling of the system (between 10 and 15 s after the emulsion was formed) was able to stop this process, preventing the loss of the drug from the wax particles.

As a result of the optimized conditions for both formulation and process, spherical particles with a high percentage of drug loading were obtained (Figure 5).

The drug loading efficiency observed in this work (up to 60%) is higher than the ones previously reported in the literature, demonstrating a successful application of this technique. Gowda and Shivakumar[51] were able to prepare microspheres containing lithium carbonate

Table 3. Results from a 2^2 experimental design with a central point for the preparation of ketoprofen entrapped carnauba wax microparticles. x_1 and x_2 represent the variables defined by the equations $x_1 = [(K/CW) - 1]/0,5$ and $x_2 = (t - 15)/10$.

Particles	K/CW	t	x_1	x_2	Diameter (μm)	Ketoprofen load in particles (%, p/p)	Morphology
A_3	1/2	5	−1	−1	208.86	29.47	+++
B_3	3/2	5	1	−1	216.22	60.55	++
C_3	1/2	25	−1	1	152.22	19.55	++++
D_3	3/2	25	1	1	189.00	50.61	+++
E_3	1/1	15	0	0	198.92	44.19	+++
F_3	1/1	15	0	0	196.42	46.41	+++
G_3	1/1	15	0	0	170.75	38.85	++++

K: ketoprofen; CW: carnauba wax; t: time (seconds); Morphology (spherical) = +: bad, ++: regular, +++: good, ++++: very good. Other conditions: 0,5% surfactant, speed of agitation: 600 rpm, wax/aqueous phase ratio: 6g/200 mL.

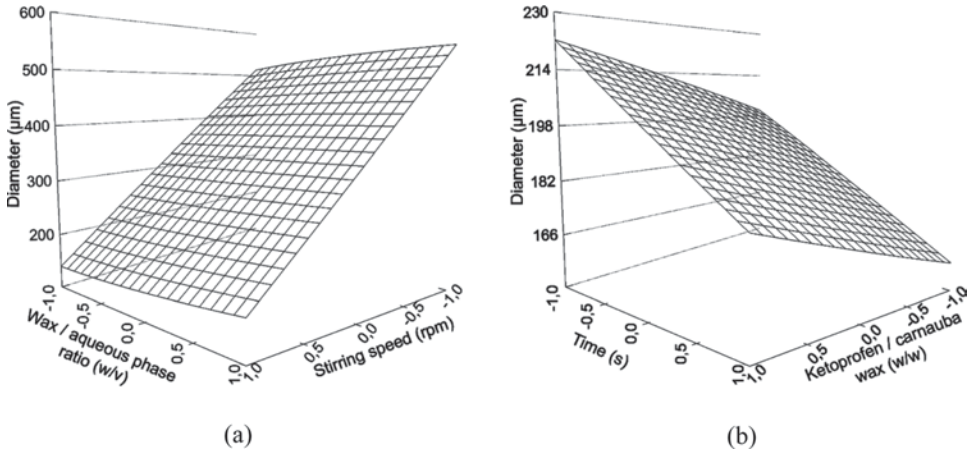

Figure 3. Diameter of ketoprofen entrapped carnauba wax microspheres prepared by emulsion congealing. (A) Response surface plane described by the equation $\hat{y} = 341.52 - 187.0775x_1 + 15.0725x_2$, where x_1 is stirring speed of the emulsion and x_2 is wax/aqueous phase ratio. (B) Response surface plane described by the equation $\hat{y} = 190.3386 + 11.0362x_1 - 20.9663x_2$, where x_1 is ketoprofen/carnauba wax ratio and x_2 is time of cooling onset.

with an entrapment efficiency of approximately 15% due to technical limitations when higher amounts of drug in relation to the wax/lipid phase were used. Hassan et al.[52], also working with ketoprofen encapsulated in beeswax and cotton seed oil particles, were not able to prepare spherical and reproducible pellets with a drug load higher than 30%.

Photomicrographs of particles from batch D3 allowed the observation of a distinct internal core, surrounded by a rigid layer (Figure 6). In these particles, the high drug load may have reduced the melting point of the carnauba wax in the interior of the microspheres. During preparation, ketoprofen present in the outer region of the particles could have migrated to the aqueous phase, originating an external layer with lower drug content, thus, more rigid. These microspheres also exhibited numerous orifices on their surface (Figure 6C). This apparent porosity may be a result of a combination of the several variables of the preparation technique, including the rapid cooling process[50], the stirring speed[46] and the previously mentioned migration of the drug from the outer layer of the microspheres to the aqueous phase. Other batches of particles did not exhibit these characteristics, but a uniform matrix structure.

Flow rate of the microparticles varied from 3.3 to 4.0 s/100g, and the angle of repose from 27 to 35°. Carr index was between 7 and 12%, while Hausner ratio was approximately 1.12. Particles from batch D3 presented flow rate of 3.6 s/100g, $\rho_B = 0.5\,g/cm^3$; $\rho_T = 0.54\,g/cm^3$

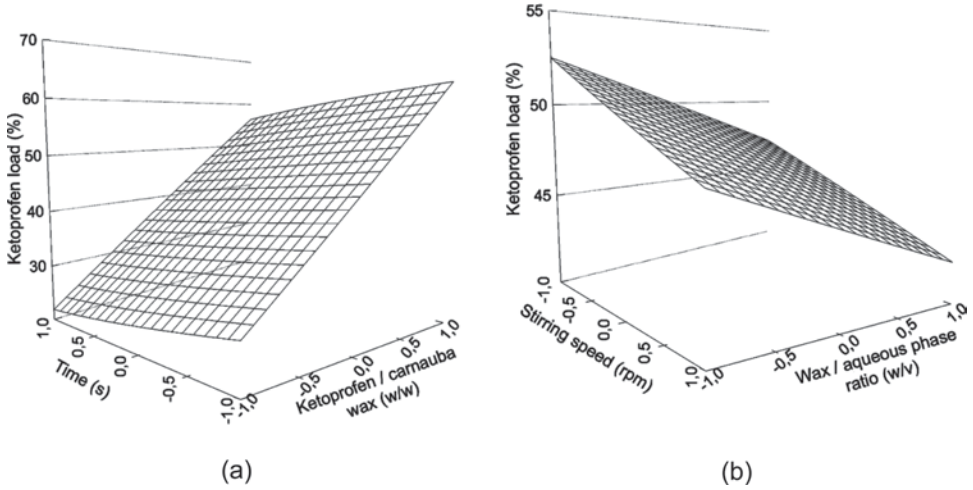

Figure 4. Efficiency of drug loading in carnauba wax microspheres prepared by emulsion congealing. (A) Response surface plane described by the equation $\hat{y} = 42.3571 + 17.2525x_1 - 3.2475x_2$, where x_1 is ketoprofen/carnauba wax ratio and x_2 is time of cooling onset. (B) Response surface plane described by the equation $\hat{y} = 47.3691 - 2.4605x_1 - 2{,}7038x_2$, where x_1 is stirring speed of the emulsion and x_2 is wax/aqueous phase ratio.

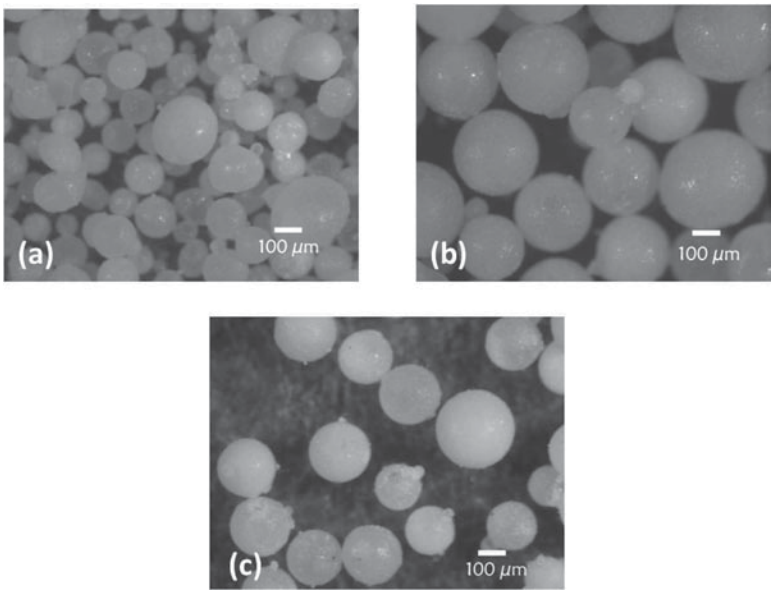

Figure 5. Carnauba wax microspheres from batches B2 (A), E2 (B) and D3 (C) prepared by emulsion congealing with ketoprofen loading efficiency of 49.7, 47.74 and 50.61%, respectively.

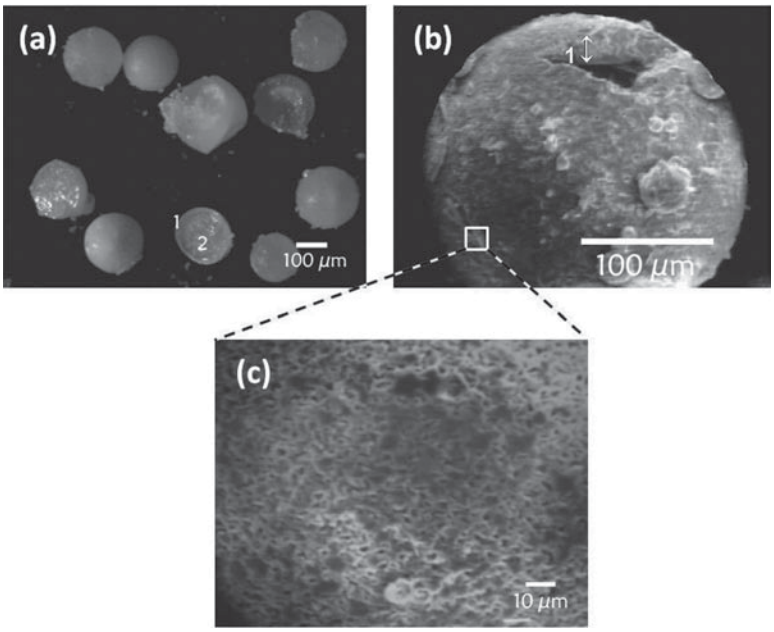

Figure 6. Ketoprofen loaded carnauba wax microspheres. (A) and (B) particles from batch D3 exhibiting a surrounding layer (1) and a core (2). (C) Close view of microparticles surface.

and angle of repose of 32.8°. For this batch, Carr index and Hausner ratio were 7.41 and 1.08%, respectively. According to these results, and following commonly accepted criteria for flow properties of solid particles[57], microspheres prepared in this work exhibited excellent flowability and did not demonstrate tendencies to cohesion or aggregation. These flow characteristics indicate a very good potential for the use of these particles in automated pharmaceutical processes for the preparation of tablets and capsules.

In vitro release assays of the microspheres resulted in dissolution percentages of ketoprofen from 15.5 to 76.4% after 24 h. When non-processed, free ketoprofen was tested, 100% dissolution was reached in approximately 1 h, demonstrating the efficacy of the carnauba wax particles in promoting a sustained release of ketoprofen (Figures 7 and 8).

Results from dissolution assays indicated that particles with smaller diameters and higher drug load exhibited a higher percentage of drug release within 24 h of the test. Data plotted in Figure 9 demonstrate that when evaluating the influence of the particles diameter in the dissolution rate (Figure 9a), ketoprofen load was maintained fairly constant (Figure 9c). Also, when ketoprofen load influence was being investigated (Figure 9d), the diameter of the particles was practically not altered (Figure 9b). This approach allowed for a more precise investigation of these individual parameters on the drug dissolution, also demonstrating the efficacy of the factorial experimental design in controlling both diameter and drug load of the particles.

Several mathematical models attempt to correlate dissolution profiles with the mechanisms of drug release from the drug delivery system[58–61]. In this work, zero order[58,60], first order[62], Higuchi[63] and Korsmeyer-Peppas[61,64,65] models were applied to analyze the dissolution profile of ketoprofen from carnauba wax microspheres. The release rates of each model were calculated by linear regression analysis. Coefficients of correlation (r^2) were used to evaluate the accuracy of the fit (Table 4).

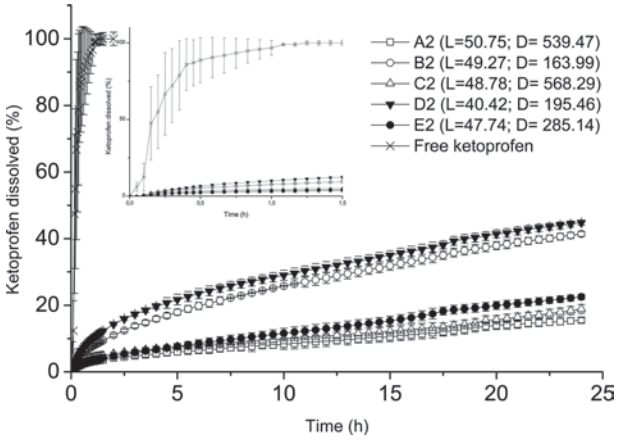

Figure 7. Dissolution profile of hard gelatin capsules containing 200 mg of ketoprofen in its free form or incorporated into carnauba wax microspheres. Microsphere batches were prepared according to Table 1. L = drug load (%); D = particle diameter (μm). Each dot represents the average of six determinations. Insert displays the initial 2 h of the dissolution assay.

According to Korsmeyer-Peppas model, ketoprofen release during the 24 h of the test was predominantly an anomalous transport ($0.43 < n < 0.85$), in which both Fickian diffusion and non-Fickian mechanisms occur[7] suggest that the most probable non-Fickian mechanism for wax matrices is erosion.

During the first 2 h of the dissolution, differences in the drug release were observed, varying from a

predominant diffusion ($n \approx 0{,}43$) to matrix erosion driven release ($n \approx 0{,}70$). During the following 22 h, D3 particles exhibited a Fickian diffusion release mechanism, whereas for the other batches drug release was an anomalous transport. Zero order and Higuchi models presented best fits for ketoprofen release from the microparticles formulations. The formulations did not have a good fit for the first order release mechanism, indicating that ketoprofen release from the microparticles was not dependent on the drug remaining in the carnauba wax matrix. The initial diffusion release profile and higher dissolution rate for the D3 particles may have been favored by the distinct structure of these particles (Figure 6), along with their small diameter, high drug content and porous surface.

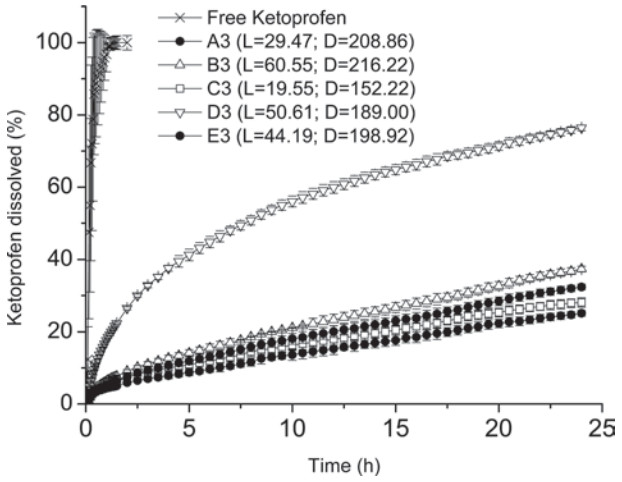

Figure 8. Dissolution profile of hard gelatin capsules containing 200 mg of ketoprofen in its free form or incorporated into carnauba wax microspheres. Microsphere batches were prepared according to Table 2. L = drug load (%); D = particle diameter (μm). Each dot represents the average of six determinations.

The literature reports that anomalous transport was also observed in particles prepared with other wax and lipid materials, exhibiting a better fit for the first order release kinetics[46,50,51]. Husson et al.[59] and Vergote et al.[66] prepared ketoprofen pellets that exhibited anomalous transport release mechanism utilizing different matrix materials; however, the release mechanism observed did not fit into the zero order model. In addition, microspheres prepared with other types of waxes and lipids[36,50,51] were not able to promote sustained and continuous drug release up to 24 h, without a significant initial burst release as observed in this work. These differences can be explained by differences in the nature of the carrier, particle size, aqueous solubility of the drug, physical state of the drug in the matrix, drug load and the presence of other formulation components, such as surfactants[43,67]. Cheboyina and Myandt[43] demonstrated that different release mechanisms can be obtained by altering some of these variables. Other parameters such as density, porosity, drug distribution in the particles and their integrity

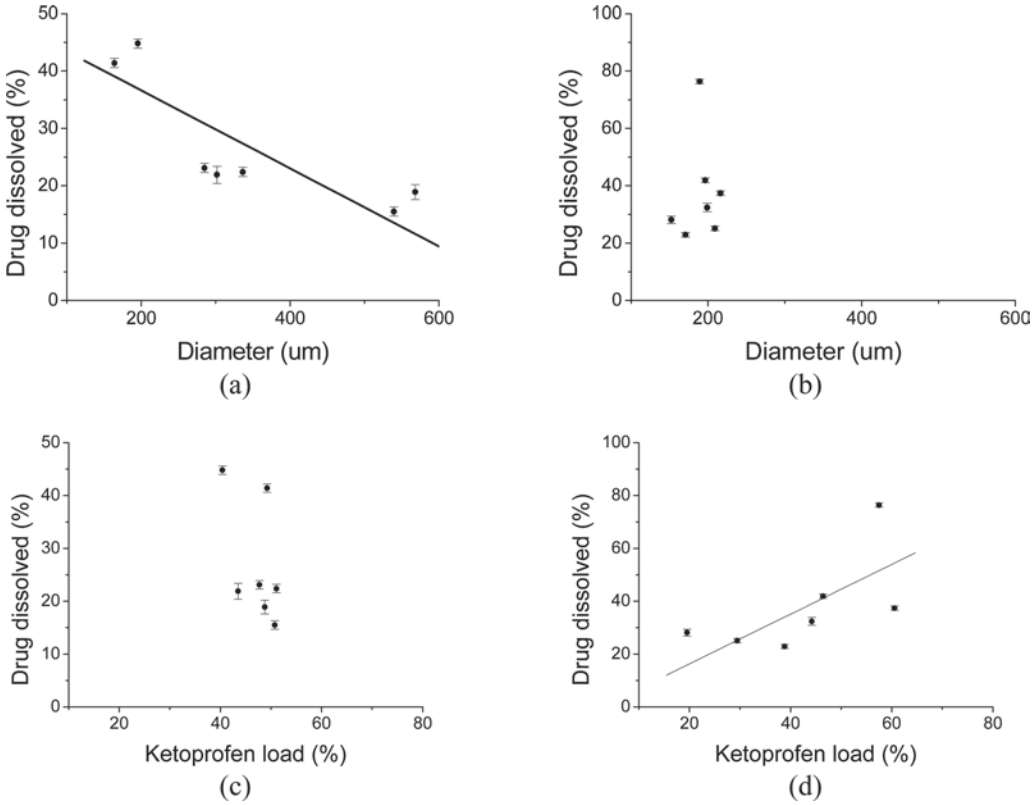

Figure 9. influence of drug load and microspheres diameter on the ketoprofen release after 24 h of the dissolution assay. (A) and (C) correspond to the experimental design in which stirring speed and wax/water phase ratio were modified. (B) and (D) correspond to the experimental design in which ketoprofen/carnauba wax ratio and emulsion cooling onset time were modified.

Table 4. Mathematical fit for zero order, first order, Higuchi and Korsmeyer-Peppas drug release models to ketoprofen dissolution profiles from carnauba wax microspheres.

Particle	Zero order			First order			Higuchi			Korsmeyer-Peppas					
	r^2			r^2			r^2			r^2			n		
	0–2 h	2–24 h	0–24 h	0–2 h	2–24 h	0–24 h	0–2 h	2–24 h	0–24 h	0–2 h	2–24 h	0–24 h	0–2 h	2–24h	0–24 h
$\mathbf{A_2}$	0.8440	0.9953	0.9744	0.7772	0.8520	0.8974	0.9066	0.9462	0.9785	0.9209	0.9786	0.9846	0.60	0.64	0.57
$\mathbf{B_2}$	0.9004	0.9861	0.9580	0.8254	0.8160	0.9150	0.9495	0.9953	0.9968	0.9660	0.9994	0.9992	0.60	0.54	0.54
$\mathbf{C_2}$	0.7752	0.9862	0.9835	0.4504	0.7568	0.8065	0.9143	0.9440	0.9694	0.9230	0.9560	0.9693	0.44	0.58	0.48
$\mathbf{D_2}$	0.9183	0.9871	0.9670	0.9098	0.6646	0.8670	0.9096	0.9941	0.9958	0.9621	0.9967	0.9961	0.70	0.47	0.49
$\mathbf{E_2}$	0.8621	0.9977	0.9879	0.7569	0.9420	0.9546	0.9366	0.9274	0.9683	0.9438	0.9922	0.9926	0.57	0.71	0.66
$\mathbf{A_3}$	0.8128	0.9980	0.9847	0.6329	0.9204	0.9339	0.9273	0.9483	0.9789	0.9239	0.9953	0.9919	0.51	0.67	0.61
$\mathbf{B_3}$	0.9052	0.9971	0.9740	0.8768	0.8967	0.9389	0.9175	0.9689	0.9865	0.9572	0.9950	0.9965	0.66	0.62	0.59
$\mathbf{C_3}$	0.7723	0.9971	0.9775	0.4621	0.8586	0.8893	0.9049	0.9724	0.9881	0.9127	0.9939	0.9901	0.44	0.60	0.54
$\mathbf{D_3}$	0.9400	0.9433	0.8940	0.9439	0.7515	0.9168	0.9208	0.9429	0.9862	0.9780	0.9957	0.9906	0.70	0.39	0.45
$\mathbf{E_3}$	0.8966	0.9973	0.9783	0.8485	0.9070	0.9456	0.9272	0.9613	0.9827	0.9557	0.9955	0.9969	0.64	0.64	0.61

r^2: correlation coefficient; n: release exponent

during the dissolution assay can also influence the drug release mechanism[50,68].

Conclusions

A modified emulsion congealing technique was used for the preparation of a novel multiparticulate system containing ketoprofen incorporated into carnauba wax microspheres. A fractional factorial experimental design allowed identification and control of the critical variables for both formulation and process that could influence the final characteristics of the particles. Microspheres exhibited optimal rheological properties, high percentage of drug load and were able to prolong and sustain drug release for more than 24 h. In light of their characteristics, carnauba wax microspheres prepared in this work are suitable for the preparation of sustained release pharmaceutical dosage forms such as tablets and capsules containing ketoprofen or other drug with similar physical-chemical properties.

Declaration of interest

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