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RESEARCH PAPER

# Poly(Lactide-co-Glycolide) Nanocapsules Containing Benzocaine: Influence of the Composition of the Oily Nucleus on Physico-Chemical Properties and Anesthetic Activity

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## ABSTRACT

***Purpose*** The aim of this work was to investigate the influence of the oily nucleus composition on physico-chemical properties and anesthetic activity of poly (lactide-co-glycolide) nanocapsules with benzocaine.

***Methods*** Nanocapsules containing benzocaine were prepared with three different oily nucleus composition and characterized by mean diameter, polydispersivity, zeta potential, pH and stability were investigated as a function of time. *In vitro* release kinetics were performed in a system with two compartments separated by a cellulose membrane. Intensity and duration of analgesia were evaluated in rats by sciatic nerve blockade.

***Results*** The greatest stability, slower release profile and improvement in the local anesthetic activity of BZC were obtained with the formulation using USP mineral oil as component.

***Conclusions*** Results from our study provide useful perspectives on selection of the primary materials needed to produce suspensions of polymeric nanocapsules able to act as carriers of BZC, with potential future application in the treatment of pain.

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**KEY WORDS** benzocaine · local anesthetic · nanocapsule · oily nucleus · PLGA

## INTRODUCTION

Polymeric nanoparticles (PN) are defined as solid colloidal particles, with a size range of 5 to 1,000 nm, which can be synthesized using either polymerization processes or pre-formed polymers. Nanoparticles can be used to increase the therapeutic value of low solubility pharmaceuticals, by improving the solubility due to the drug incorporation in these particles. PN can be classified as nanospheres (NS) or nanocapsules (NC), with the physical form being dependent on the preparation technique and the materials used. NC consist of an involucre of polymers and an internal nucleus (usually oily), and are also known as nano-vesicular systems, since they present a core-shell type structure in which the pharmaceutical (which normally has hydrophobic characteristics) is located in the oily interior, adsorbed on the wall of the polymeric shell, or dissolved in the core. NS consist of a polymer matrix without any oily inclusions, where the pharmaceutical is either adsorbed or dispersed throughout the matrix (1–7).

Polymeric NC can be prepared using biodegradable polymers, such as the polyesters poly-ε-caprolactone (PCL), poly(lactide) (PLA) or poly(lactide-co-glycolide) (PLGA). PLGA is a copolymer often used to produce nanocapsules able to be loaded with pharmaceuticals, because its hydrolysis releases monomers that are intermediates in human metabolism and that therefore present low or zero toxicity (8,9).

Local anesthetics (LA) are amphiphilic molecules whose primary pharmacological activity involves the reversible blocking of neuronal transmission (10–13). Benzocaine

(BZC) is an ester-type local anesthetic frequently used in topical formulations. Its parenteral administration is limited by the fact that it has low solubility in aqueous media (14,15). The anesthetic action of BZC is characterized by rapid onset, but short duration relative to the duration of pain. The literature reports toxic effects caused by BZC, such as methemoglobinemia or allergic reactions resulting from unintentional systemic absorption (16–19).

Ideally, a local anesthetic that has long-lasting activity is selective for sensory rather than motor blocking and presents low systemic toxicity (11,15,20,21). One way of promoting such effects is through modified release of the chemicals using carrier systems, such as polymeric nanoparticles (22–25).

Modified release formulations for local anesthetics have been much studied in the literature, where the carrier systems investigated have included cyclodextrins (12,26–34), liposomes (35–43), or polymeric micro- and nanoparticulates (44–48). NC have been used as carriers for local anesthetics, since many of the pharmaceuticals (such as BZC) belonging to this class of chemicals possess low solubility (amongst other characteristics), which can be mitigated by encapsulation in the particles (3).

The oils used in the NC should show characteristics including absence of toxicity, good physico-chemical stability, no ability to dissolve the polymer, and high affinity for the pharmaceutical. The oils most commonly used are medium- or long-chain triacylglycerols, such as the commercially available Miglyol® or Myritol® (49,50).

The literature reports that the type of oil present in NC formulations influenced the size and the efficiency of encapsulation of metipranolol (51). In earlier work, our group prepared and characterized the encapsulation of the local anesthetics bupivacaine (52) and BZC (44–46), in polymeric NC containing an oily nucleus of Miglyol®. However, up to now there have been no studies of the influence of different types of oils on nanocapsule performance, considering the characteristics of these colloidal systems (size, polydispersity, zeta potential) and the encapsulation efficiency of local anesthetics.

In the present work, three nanocapsule formulations were prepared, using the biodegradable polymer poly(D, L-lactide-co-glycolide) (PLGA 50:50) with different oils (USP mineral oil, isopropyl myristate, decyl oleate Cetiol®V), in order to assess the influence of the composition of the oily nucleus on the encapsulation efficiency of BZC, the stability of the colloidal suspension (using measurements of size, polydispersity and zeta potential) and the physico-chemical stability of the polymer (using pH analysis), as well as the release profiles of the formulations and their mechanisms. The goal was to obtain formulations with potential for improving the therapeutic action of BZC.

## MATERIALS AND METHODS

### Materials

The following materials were used: benzocaine (Sigma Aldrich Chem. Co.), poly-D,L-lactide-co-glycolide 50:50 (PLGA, 45,000 g/mol) (Sigma Aldrich Chem. Co.), sorbitan monostearate (Span60®) (Sigma Aldrich Chem. Co.), polysorbate 80 (Tween80®) (LabSynth, Brazil), USP mineral oil (Cognis, Brazil), isopropyl myristate (Cognis, Brazil), decyl oleate (Cetiol®V) (Cognis, Brazil), and analytical grade acetone (LabSynth, Brazil). The solvents used in chromatographic analyses were HPLC grade methanol (JT Baker®) and Milli-Q water.

### Methodology

#### *Preparation of the PLGA Nanocapsules with Different Oils*

The PLGA nanocapsules were prepared by the solvent displacement (interfacial deposition) method (53). Two phases, one organic and the other aqueous, were employed. The organic phase comprised the PLGA polymer (100 mg), acetone (30 mL), benzocaine (40 mg), sorbitan monostearate (40 mg) and oil (200 mg). The oils tested were USP mineral oil (formulation A), isopropyl myristate (formulation B) and Cetiol® V (formulation C). The aqueous phase was prepared from Polysorbate 80 (60 mg) and deionized water (30 mL). After solution preparation, the organic phase was slowly added under the aqueous phase using a small funnel, with magnetic stirring. The resulting suspension was stirred for 10 min to remove the organic solvent, then concentrated under low pressure using a rotary evaporator, until a final BZC concentration of 4 mg/mL was achieved (44).

#### *Determination of the Partition Coefficients of Benzocaine in the Different Oils Used in the Formulations*

The partition coefficients ($P$) of benzocaine in the oils were determined using a system composed of two phases, one aqueous (containing a known initial quantity of BZC), and the other organic (containing each of the oils used in the formulations). The benzocaine was dissolved in 1 mL of deionized water, to which 1 mL of the test oil was added, followed by vigorous agitation for 10 min at ambient temperature (25°C). After this step, the system was allowed to rest for 24 h to ensure complete distribution of the anesthetic between the phases. The samples were then centrifuged, and the benzocaine present in the aqueous phase determined by UV–vis spectroscopy (54). The value of the partition coefficient ($P_{oil/water}$) was determined from the ratio of the BZC concentrations in the oily and aqueous

**Table I** Mean Diameters (nm), Polydispersity, Zeta Potentials (mV), Encapsulation Efficiencies, Drug Contents, pH and $t_{50}$ of Different Suspensions of PLGA Nanocapsules Containing Benzocaine

| Parameter | Formulation A | Formulation B | Formulation C |
|---|---|---|---|
| Mean diameter (nm) | 138.1 ± 0.3 | 149.4 ± 2.6 | 169.4 ± 1.4 |
| Polydispersity | 0.108 ± 0.008 | 0.260 ± 0.014 | 0.161 ± 0.002 |
| Encapsulation efficiency (%) | 72.4 ± 1.2 | 73.3 ± 0.8 | 67.5 ± 0.9 |
| Drug content (% *w/w*) | 6.58 ± 0.77 | 6.67 ± 0.48 | 6.13 ± 0.65 |
| Zeta potential (mV) | −21.8 ± 4.1 | −21.2 ± 0.2 | −17.9 ± 1.7 |
| pH | 3.76 | 3.75 | 3.73 |
| $t_{50}$ (min) | 130 | 99 | 88 |

phases (19,55). All measurements were performed in triplicate.

### *Size, Polydispersity and Zeta Potential Measurements*

The photon correlation spectroscopy technique was used to determine the average size (hydrodynamic diameter) and polydispersity of the various formulations. The analyses were performed after diluting the nanocapsule suspensions with Milli-Q water (1/100, *v/v*), with a ZS-90 (ZetaSizer, Malvern Instruments, UK) particle analyzer, at a fixed angle of 90° and temperature of 25°C. The size distribution and polydispersity were measured as a function of time (0, 30, 60 and 90 days), and expressed as the average of three determinations (22,25,46).

The values of the zeta potential (in mV) were also determined using the ZS-90 instrument, with the same dilutions and time periods as above. Results were expressed as the average of six determinations.

### *Chemical Stability Measurements*

The chemical stability of the polymer was investigated using pH analyses of the PLGA nanocapsule suspensions with different oils containing BZC. The measurements were made with a Corning pH electrode connected to a Tecnal potentiometer, previously calibrated using pH 7.0 and 4.0 buffer solutions, as a function of time (0, 30, 60 and 90 days), and expressed as the average of three determinations. The pH measurements were performed at 25°C using a volume of 10 mL of each colloidal suspension (preparede with different oils) containing BZC (concentration of 4 mg/mL).

**Table II** Values of the Partition Coefficient ($P_{o/w}$) of Benzocaine Using Different Oils

| System | *log P* |
|---|---|
| (A) USP mineral oil/water | 1.133 |
| (B) Isopropyl myristate/water | 1.392 |
| (C) Cetiol®V/water | 0.995 |

### *Measurements of the Encapsulation Efficiency and Drug Content of Benzocaine in the PLGA Nanocapsules*

The encapsulation efficiency was determined using the ultrafiltration/centrifugation technique. The nanocapsule samples were centrifuged in ultrafiltration vessels composed of a regenerated cellulose membrane with molecular exclusion pore size of 30 kDa (Microcon, Millipore). After this step, BZC was quantified in the filtrate using high performance liquid chromatography (HPLC). The BZC encapsulation efficiency (EE%) of BZC associated with the nanocapsules was calculated from the difference between the total and free BZC concentrations, measured in the dispersion and the ultrafiltrate (Eq. 1) (3,56,57).

$$\mathrm{EE}\,(\%) = \frac{W_s}{W_{total}} \times 100\% \qquad (1)$$

where $W_s$ is the amount of BZC in PLGA nanocapsules and $W_{total}$ is the amount of BZC used in formulation.

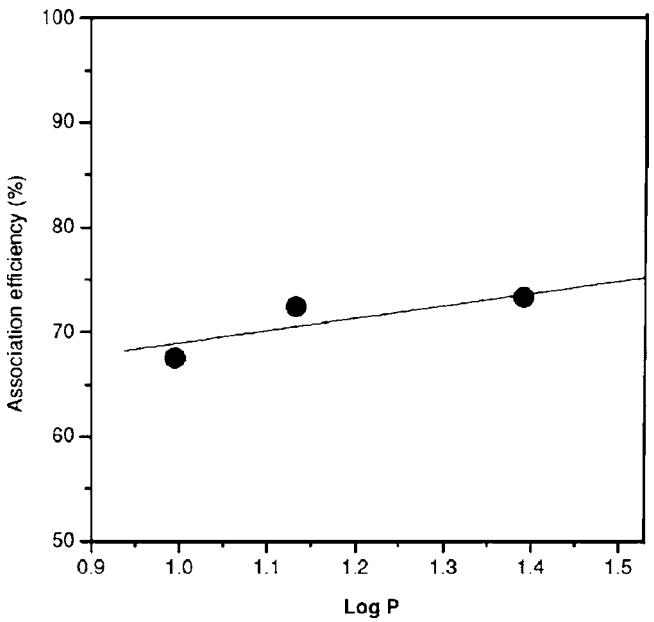


**Fig. 1** Correlation between *log P* and the encapsulation efficiency of benzocaine with PLGA nanocapsules.

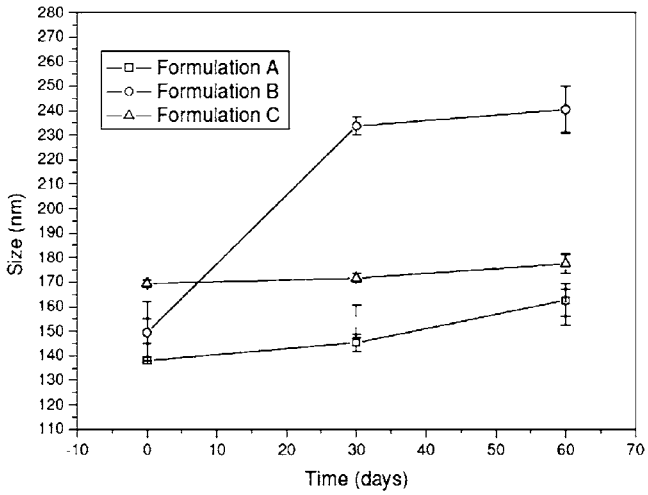


**Fig. 2** Mean particle diameter (nm) as a function of time (0, 30 and 60 days), for the formulations of PLGA nanocapsules with BZC.

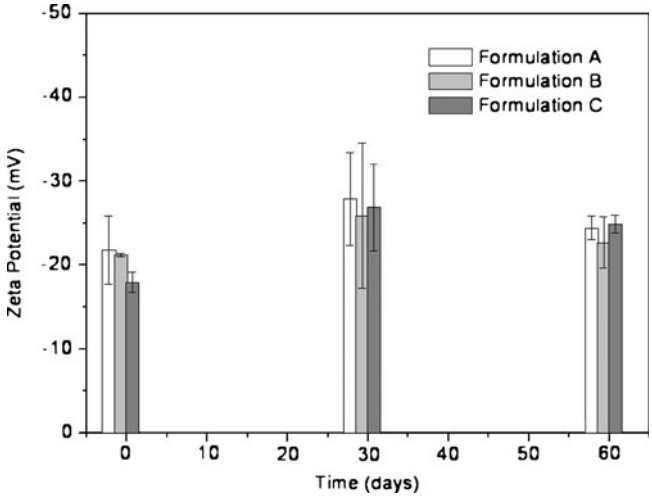


**Fig. 4** Zeta potential (mV) of the particles as a function of time (0, 30 and 60 days), for the formulations of PLGA nanocapsules with BZC.

The drug content (DC%) was calculated as the ratio between the amount of the drug encapsulated by the nanocapsules and the final weight of the nanocapsules (Eq. 2) (23,58)

$$\mathrm{DC}\,(\%) = \frac{W_s}{W_{P+S}} \times 100\% \qquad (2)$$

where $W_s$ is the amount of BZC in PLGA nanocapsules and $W_{P+S}$ is the final weight of nanocapsules (polymer + oil + surfactant + BZC).

The BZC was assayed by high-performance liquid chromatography. The system consisted of a Varian Microsorb $C_{18}$ column, a Varian ProStar 410 Autosampler, a Varian ProStar 210 pump, a Varian ProStar 210 UV detector. The mobile phase consisted of methanol/water (60:40%, *v*/*v*), at a flow rate of 0.8 mL/min. The total sample volume injected was 20 µL, and BZC detection was at 285 nm at an approximate retention time of 5 min. All samples were previously filtered through Millipore 0.22 µm polyethersulfone membranes. Quantification of BZC employed a validated calibration curve ($Y = -0.02554 + 2.21708\ X$, $r = 0.99944$, LOD = 2.38 µg/mL, LOQ = 7.93 µg/mL) (45).

### *In Vitro Benzocaine Release Experiment*

Release of benzocaine, either free or associated with the nanocapsules, was measured using a system composed of two compartments, a 1 mL donor compartment (containing free drug or nanocapsule suspension in a concentration of 4 mg/L) and a 200 mL receptor compartment containing pH 7.4 HEPES buffer, separated by a cellulose membrane

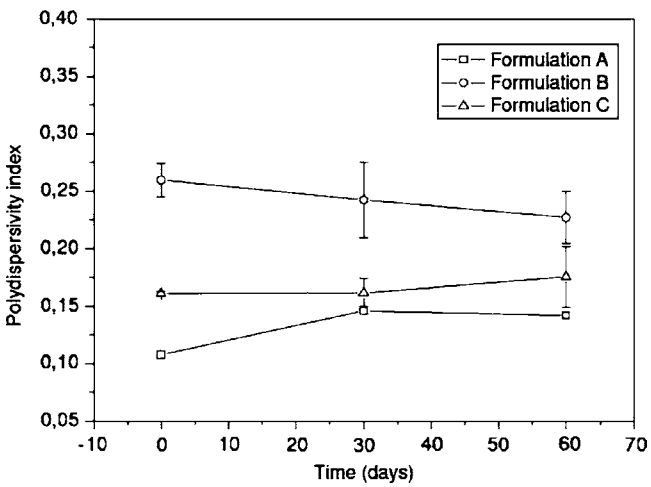


**Fig. 3** Polydispersity index of the particles as a function of time (0, 30 and 60 days), for the formulations of PLGA nanocapsules with BZC.

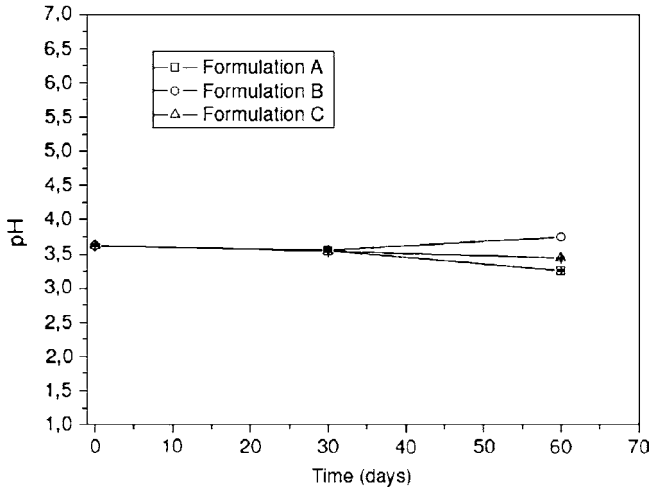


**Fig. 5** pH values of the nanocapsule formulations as a function of storage time (0 and 60 days) at ambient temperature (25°C) ($n = 3$).

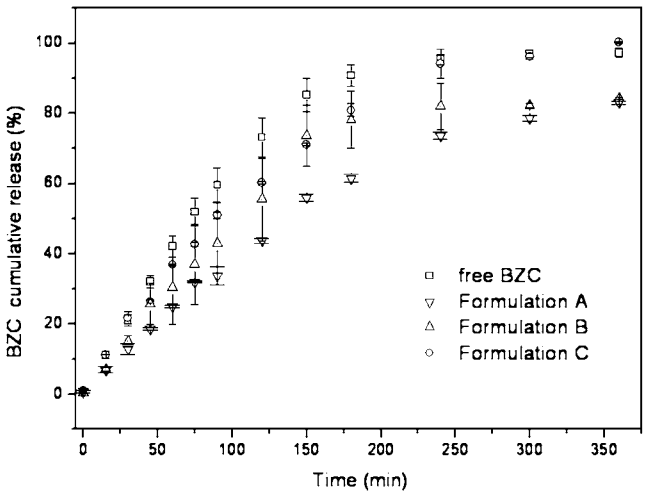


**Fig. 6** Cumulative release of BZC (%) in solution and in suspensions of PLGA nanocapsules ($n = 3$).

with a molecular exclusion pore size of 1,000 Da, maintained under agitation and sink conditions (59). Two mL aliquots were withdrawn from the receptor compartment at intervals of 15, 30 or 60 min, over a total period of 360 min, and analyzed using the UV–vis procedure (the drug was detected at 285 nm). After measurement, the aliquots were returned to the receptor compartment in order to maintain a constant volume. The absorbance values were converted to % of BZC released, using a standard of free BZC in buffer solution. The measurements were made in triplicate.

### *Mathematical Modeling*

The following theoretical models were used to analyze the behavior of BZC release from the PLGA nanocapsules prepared with different oils: zero order, first order, Higuchi and Korsmeyer-Peppas (52,60–63).

### *Pharmacological Assays: Sciatic Nerve Blockade*

Male adults *Swiss* mice (30–35 g) were obtained from CEMIB-UNICAMP (Centro de Bioterismo, State University of Campinas (UNICAMP), Campinas, São Paulo). Experimental protocol was approved by the UNICAMP Institutional Animal Care and Use Committee, which follows the recommendations of the Guide for the Care and Use of Laboratory Animals. Mice, divided in groups of 6 animals each, were randomly selected for the pharmacological assays and treated by injection into the popliteal space (0.1 mL) with 4 $mg.mL^{-1}$ BZC free and formulations A (USP mineral oil), B (isopropyl myristate) or C (Cetiol® V) containing BZC at the same concentration.

Before the experiment, animals were selected according to their ability to walk normally with four limbs on both the top and inverted side of a wire mesh screen (1 mm diameter wire, 5 mm mesh). All formulations were injected inserting a needle into the popliteal space on the posterior surface of the knee, in the area of the sciatic nerve. Motor blockade intensity was evaluated by the loss of motor function in the injected limb according to the scores: 0 (normal movement), 1 (unable to flex the limb completely) and 2 (total paralysis) The efficacy of motor blockade was evaluated every minute, from 1 to 5 min, and thereafter every 10 min up to at least 1 h following the injection. Latency (time between injection and the loss of motor function), time to reach the maximum score (Tmax), time for motor function recovery (Time for recovery) and total local anesthetic effect (area under the effect curve *vs.* time, expressed as score/h) were evaluated (64–66).

Sensory blockade evaluation was performed by the paw pressure test (73) using an analgesymeter (Ugo Basile, Varese, Italy). The withdrawal reflex was considered representative of the pain threshold or Paw Withdrawal Threshold to Pressure (PWTP), considering the registered force (in grams) on the injected paw. The baseline of the

**Table III** Parameter Values Obtained After Application of Mathematical Models to the Release Curves of Benzocaine Associated with Four Different Formulations of PLGA Nanocapsules

| | Zero order[a] | First order | Higuchi | Korsmeyer- Peppas |
|---|---|---|---|---|
| BZC:Formulation A (USP Mineral Oil) | | | | |
| | | | | $n = 0.73$ |
| Release constant (k) | 0.23%.$min^{-1}$ | 0.007 $min^{-1}$ | 5.75 $min^{-1/2}$ | 0.001 $min^{-n}$ |
| Correlation coefficient (r) | 0.710 | 0.966 | 0.995 | 0.988 |
| BZC:Formulation B (Isopropyl Myristate) | | | | |
| | | | | $n = 0.91$ |
| Release constant (k) | 0.24%.$min^{-1}$ | 0.009 $min^{-1}$ | 7.18 $min^{-1/2}$ | 0.005 $min^{-n}$ |
| Correlation coefficient (r) | 0.656 | 0.961 | 0.987 | 0.980 |
| BZC:Formulation C (Cetiol® V) | | | | |
| | | | | $n = 0.85$ |
| Release constant (k) | 0.28%.$min^{-1}$ | 0.008 $min^{-1}$ | 7.36 $min^{-1/2}$ | 0.006 $min^{-n}$ |
| Correlation coefficient (r) | 0.735 | 0.967 | 0.998 | 0.985 |

[a] % relative to [(mg drug released/mg drug total)*100]

PWTP test was measured before nanoparticles or local anesthetic injection, in order to determine the pain threshold of the animal. Baseline values of 30–50 g were selected as the pain threshold, and animals that presented lower or higher values than that baseline were excluded. The established antinociception cut-off value was 150 g, considered to be representative of the anesthetic state (12,39,40). After treatment, measurements were carried out at intervals of 15 min during the first hour, 30 min in the second and third hour and finally 60 min up to 5 h after treatment.

### *Statistical Analysis*

Motor function evaluation data (latency, Tmax, time for recovery and AUC) were analyzed by the Kruskall-Wallis test and expressed as medians (minimum and maximum limits), while sensory function data were analyzed by one-way analysis of variance (One-way ANOVA), with Tukey-Kramer as a post hoc test. Statistical significance was defined as $p<0.05$. Data were analyzed using Origin 6.0 Software (Microcal TM Software Inc., USA) and Graph Pad Instat (Graph Pad Software Inc., USA) programs.

## RESULTS AND DISCUSSION

Benzocaine has a low aqueous solubility, of 0.66 mg/mL (19,46), and carrier systems such as polymeric nanocapsules can be a valuable means of assisting its transport. One of the most important steps in the preparation of nanocapsule formulations is the selection of the oil that will form the oily nucleus of the capsules. Previous studies of PLA (46) and PLGA (44) nanocapsule formulations with a mixture of capric/caprylic acid triglycerides and BZC showed that the oil used was highly effective for both encapsulation of the anesthetic and colloid stability. The present work employed three different commercial oils (USP mineral oil, isopropyl myristate and Cetiol® V) in alternative formulations to those described in the literature. These oils were chosen because they are non-toxic and widely used to aid solubilization in a variety of pharmaceutical formulations.

The parameters, mean diameter, polydispersity index (which indicates the size distribution of the nanocapsules, with values below 0.2 normally considered satisfactory) (5), zeta potential (which reflects the surface charge of the nanocapsules), pH (an indicator of the physico-chemical stability of the polymer) and encapsulation efficiency (affinity of the pharmaceutical with the system), are important since they provide essential information on the natures of the colloidal systems under investigation. The results (Table I) indicated that for the different NC suspensions containing BZC studied, the values of these parameters were compatible with those for colloidal suspensions described previously (67).

With respect to size, formulation B presented initial polydispersity values greater than 0.2, indicative of an

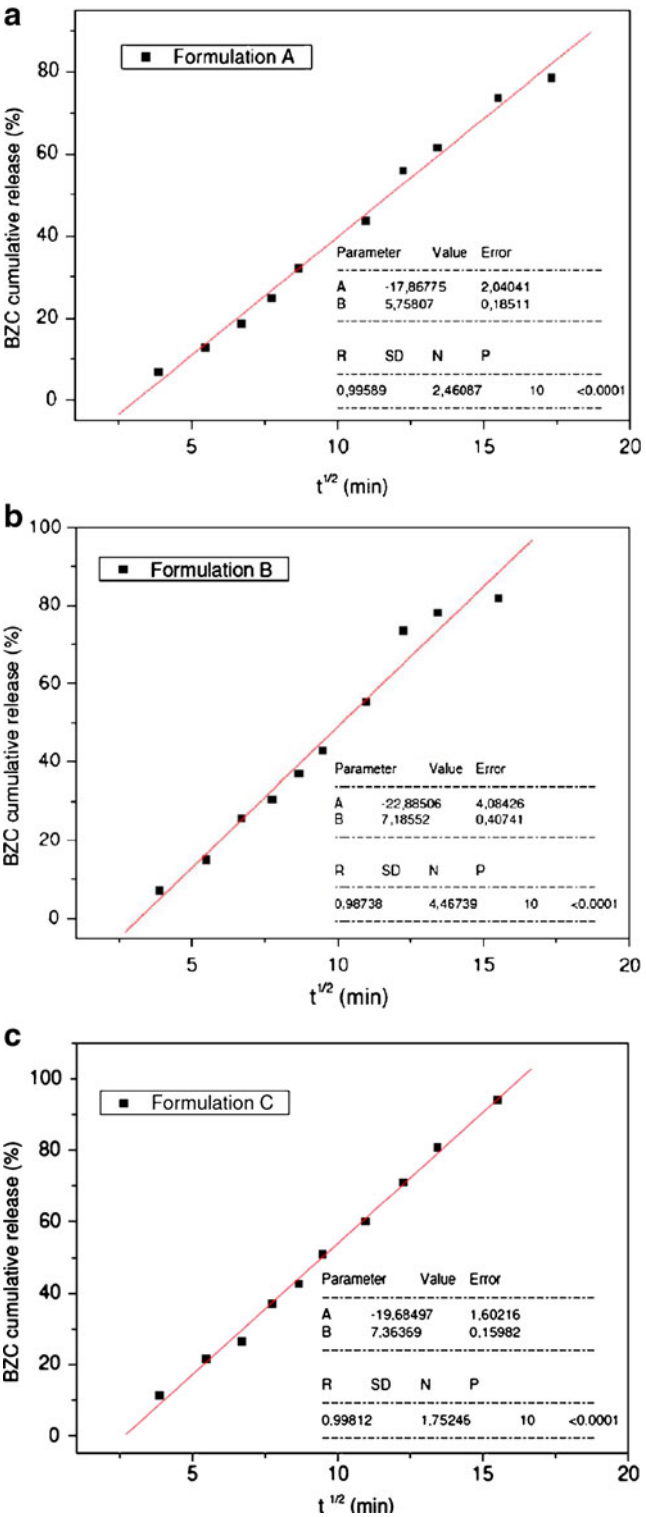


**Fig. 7** Results obtained using the Higuchi mathematical model. Formulations using: **a** USP mineral oil; **b** isopropyl myristate; **c** Cetiol® V.

unsatisfactory size distribution. Formulation A presented the most negative zeta potential, reflecting good colloid stability due to electrostatic repulsion between the nanoparticles (5,7).

In previous work, the encapsulation efficiency of BZC in PLGA nanocapsules with an oily nucleus of Miglyol® 810 was found to be around 73% (44,46). Various factors can influence the encapsulation efficiency of pharmaceuticals in nanocapsules produced by the nanoprecipitation method, notably the polarity of the compound concerned. The more non-polar pharmaceuticals usually present encapsulation efficiencies greater than 70% (68,69). In the present work, the highest values obtained were 72.4 and 73.3%, for formulations A and B, corresponding to drug content values of 6.58 and 6.67, respectively, which is compatible with the value of *P* obtained. This high encapsulation efficiency and drug content could be explained by the greater solubility of BZC in the oily component of these formulations, in agreement with previous reports that the degree of encapsulation and drug content values are related to solubility in the oily nucleus. This fact could be due to the carbon chain and hence greater lipophilicity which reduced the loss of drug into the aqueous phase (23,58,70,71).

Confirmation of the degree of interaction of BZC with the different oils used here was achieved by determination of the oil/water partition coefficients (Table II). These showed that the affinity of BZC for the oils decreased in the order isopropyl myristate > USP mineral oil > Cetiol® V, which correlated well ($r^2$>0.99) with the encapsulation efficiencies of BZC using the different oils (Fig. 1). This confirms that the pharmaceutical/oil interaction is critical in encapsulation of BZC with the hydrophobic interiors of nanocapsules (7). However, it is noteworthy that besides the interaction of the drug with the different oils showing a good correlation with the partition coefficient, it should be noted that the drug, such as BZC, can interact with the polymer chains of PLGA, as described the interaction by drugs with polymers (such as PLA, PLGA) (72).

The stability of the formulations A, B and C was determined by measuring the mean diameter, polydispersity, zeta potential and pH of the dispersions after storage times (at ambient temperature 25°C) of 0, 30 and 60 days following preparation.

In the case of formulations A and C, the mean particle diameter remained almost constant throughout the period (Fig. 2), indicating that the formulations were stable with respect to size (for at least 60 days) and that there was no aggregate formation (which would have been evidenced by an increase of particle size). In contrast, there was a large increase in the mean diameter of formulation B particles, especially up to 30 days, indicative of aggregate formation. Isopropyl myristate was therefore unsuitable for production of stable dispersions.

Polydispersity values less than 0.2 are normally used to indicate the existence of a narrow particle size range. Large fluctuations with time could indicate aggregate formation, hence increasing the nanoparticle size range (5). There were small changes with time of the polydispersity values of all the formulations (Fig. 3), while values greater than 0.2 for formulation B, throughout the period, were indicative of inadequate stability.

The zeta potential can be influenced by the composition of the particles and the dispersant medium, pH, and ionic strength of the medium. All formulations showed changes in zeta potential during the period (Fig. 4). As a rule, the higher the potential, the more stable the NC suspension. Hence, similar results were found for all formulations, indicating their stability.

Analysis of pH changes with time can provide an indication of possible degradation of the polymer, due to hydrolysis and release of some of its components (3). The formulations showed initial pH around 3.7, and this low pH may be due to ionization of acidic groups in the end of polymer chain of PLGA. The values of pH measured in this work are in agreement with pH values described in literature for PLA nanocapsules formulations (67).

Shifts in pH were observed in all cases (Fig. 5), with a tendency towards stabilization with time. The sharpest decreases occurred for formulations A and C, possibly due to polymer degradation producing free lactic acid (67,72).

For the release profile determination assays, a two-compartment experimental system used allowed separation of BZC from the nanocapsules by passage through a membrane so that the encapsulation between BZC and the particles could be assessed by the measured transfer

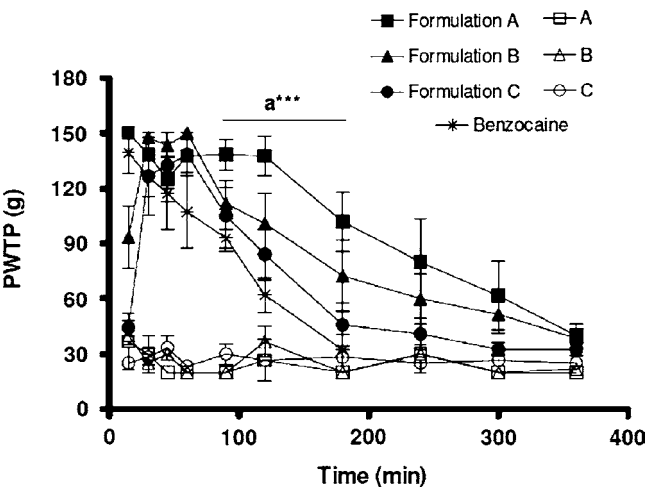


**Fig. 8** Time-course (min) on the PWTP test showing vehicles (A for USP mineral oil, B for isopropyl myristate and C for Cetiol® V $BZC_{free}$ nanocapsules), 4 $mg.mL^{-1}$ $BZC_{plain}$ and formulations A, B or C. Data are expressed as mean ± SD ($n$ = 6–7 per group). a- Formulation A *vs.* Formulation B, C and $BZC_{plain}$, ***$p$ < 0.001.

**Table IV** Latency, $E_{max}$, Time For Recovery and Total Effect of Motor Blockade (AUC) for Plain and Polymeric Nanocapsules Formulations Containing Benzocaine at 4 $mg.mL^{-1}$

| Groups | Latency (sec) | $E_{max}$ (score) | Time for recovery (min) | AUC (score/h) |
|---|---|---|---|---|
| BZC | 1 (−) | 1 (1–2) | 25.0 (15.0–30.0) | 43.0 (33.5–44.0) |
| Formulation A | 1 (−) | 1 (1–2) | 60.0 (50.0–60.0)*** | 53.5 (53.5–64.0)*** |
| Formulation B | 1 (−) | 1 (1–2) | 60.0 (50.0–60.0)*** | 59.0 (59.0–66.5)*** |
| Formulation C | 1 (−) | 1 (1–2) | 50.0 (50.0–60.0)*** | 53.5 (53.5–64.0)*** |

Data are expressed as median (minimum–maximum) ($n = 6$–7). a formulation A, B or C and benzocaine (BZC) at 4 $mg.mL^{-1}$ (0.4%). ***$p < 0.001$ (Kruskall-Wallis test). *BZC* benzocaine; *Emax* maximum effect in score value; *AUC* area under the curve; Formulation A = USP mineral oil nanoparticles containing BZC; Formulation B = isopropyl myristate nanoparticles containing BZC; Formulation C = Cetiol® V nanoparticles containing BZC

rate. The profiles obtained (Fig. 6) showed that complete (100%) transfer (release) of free BZC occurred after 250 min, with a 50% release time ($t_{50\%}$) of 70 min. The three formulations showed modified release profiles (Table II), with the largest change for formulations A (containing USP mineral oil, Table III), whose greater encapsulation efficiency resulted in slower release (73).

Formulations A and B showed an inversion of their behavior compared to their encapsulation efficiencies (the $t_{50\%}$ of A was higher than that of B, while the encapsulation efficiency of B was higher than that of A). This could be due to differences in interactions between the components of the formulation (BZC, surfactant, polymer, oil).

The results clearly demonstrate the important influences on the BZC release profiles of the BZC:oil interaction (evidenced by partition coefficient values) and the BZC: nanocapsule interaction (encapsulation efficiency), amongst other physico-chemical phenomena (62). A variety of models can be applied to the release profiles in order to explore the mechanisms involved, which could also include desorption of BZC from the particle surface, diffusion through the pores of the matrix or polymeric wall, and particle disintegration or erosion (3,24,62).

Results of application of the zero order, first order, Higuchi and Korsemeyer-Peppas models to the release curves are provided in Table III, where the correlation coefficients (r) and release constants were calculated using linear regressions. For all formulations the release mechanism followed the Higuchi diffusion model, as shown graphically in Fig. 7. This model describes the release of a solute when the process follows Fick's Law of diffusion (62,63). Analysis of the release curves obtained after application of the Higuchi treatment provided release rate constants, $k$, for formulations A, B, C of 5.75, 7.18, 7.63 $min^{-1/2}$, respectively, indicating that the release was considerably slower using formulation A (containing USP mineral oil) (Table III).

In spite of BZC is not being clinically used by parenteral route, sciatic nerve blockade was used as an experimental model, providing us information about the intensity and duration of motor and sensory blockade evoked by all formulations when compared to the plain solution (Fig. 8). In addition, BZC concentration (0.4%) tested, in this study, WAS based on those experimentally and clinically used for parenteral well-described local anesthetics, such as bupivacaine (0.0625 and 0.5%) (74,75).

The injection of the vehicles (nanocapsules BZC-free) did not induce any effect on motor blockade. However, the overall motor function was significantly impaired after injection of $BZC_{plain}$ or formulations A, B and C, as observed on latency, maximum effect ($E_{max}$), time for recovery normal motor function and total effect of the local anesthetic (area under the curve, AUC) for each experimental group. Inter-groups comparisons revealed statistical differences on time for recovery and AUC for each formulation (A, B, and C, $p < 0.001$) when compared to $BZC_{plain}$, as shown in Table IV. In this manner, the treatment with the three different formulations did not enhance the intensity but increased the duration of motor blockade.

The sensory blockade results showed that the BZC formulations (A, B and C) significantly increased ($p < 0.001$) the duration and intensity of the antinociceptive effect,

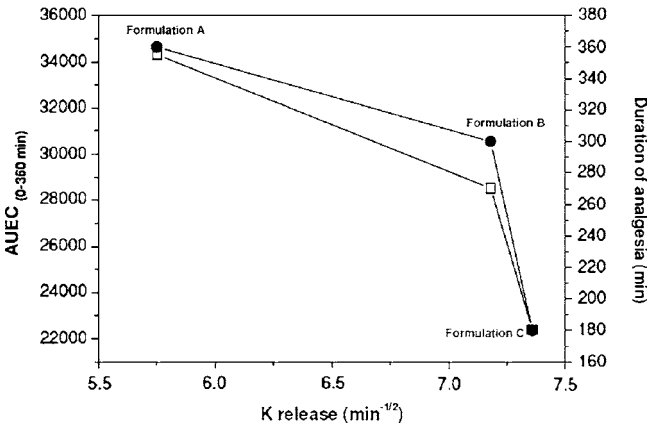


**Fig. 9** Relationship between the release constant (K release), duration of analgesia and area under the effect curve ($AUEC_{0-360\ min.}$) for three different formulations containing benzocaine. Empty squares and full circles refer to $AUEC_{0-360\ min.}$ and duration of analgesia, respectively.

when compared to their plain solution. Assessing individual time-values, formulation A was different from $BZC_{PLAIN}$, formulations B and C at the interval from 90 up to 180 min ($p<0.001$), increasing the intensity (2.2-fold at 120 min) and providing analgesia until 300 min.

In the development of drug delivery system for local anesthetic some aspects must be considered, such as the drug must be sufficiently encapsulated to maintain the therapeutic concentration and its diffusion and/or uptake by nerve fibers must be slow, determining the latency, spread, intensity and duration of nervous blockade (36). For this reason, the release profile of BZC from the nanocapsules was an important consideration from the perspective of therapeutic efficiency (3). Here, BZC release was measured in the absence and presence of the PLGA nanocapsules prepared using the three different oils. Comparisons among the three formulations revealed a relationship between K release and pharmacodynamic parameters (Fig. 9) such as duration of analgesia and total local anesthetic effect ($AUEC_{0–360}$). Both the duration of analgesia and total local anesthetic effect (including intensity) increased with the lowest K release values, evidencing the modified release profile of the formulation A.

## CONCLUSIONS

Different formulations of nanocapsules containing benzocaine were prepared and characterized. This study showed the preparations and characterization of three different oily-nucleous (USP mineral oil, isopropyl myristate and Cetiol® V) PLGA-nanoparticles containing a hydrophobic local anesthetic agent, benzocaine. The formulation containing isopropyl myristate failed on stability as assessed using size and polydispersity values as parameters. However, local anesthetic activity assays showed an improvement on intensity and duration of analgesia only provided by the treatment with the formulation containing USP mineral oil as component. This work provides useful perspectives on selection of the primary materials needed to produce suspensions of polymeric nanocapsules able to act as carriers of BZC, with potential future application in the treatment of pain.

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