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Development of hydrophilic nanocarriers for the charged form of the local anesthetic articaine

Nathalie Ferreira Silva de Melo[a,b], Estefânia Vangelie Ramos Campos[a,b], Camila Morais Gonçalves[b], Eneida de Paula[b], Tatiane Pasquoto[c], Renata de Lima[c,d], André Henrique Rosa[a], Leonardo Fernandes Fraceto[a,b,*]

[a] Department of Environmental Engineering, São Paulo State University, Sorocaba, SP, Brazil
[b] Department of Biochemistry, State University of Campinas, Campinas, SP, Brazil
[c] Department of Biotechnology, University of Sorocaba, Sorocaba, SP, Brazil
[d] São Carlos Federal University, Sorocaba, SP, Brazil

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ABSTRACT

One of the current challenges in drug encapsulation concerns the development of carrier systems for hydrophilic compounds. Potential carriers include nanocapsules prepared with amphiphilic polymers, which consist of a polymeric coating surrounding an aqueous nucleus, or dense matrices such as nanospheres of alginate/chitosan, where the drug may be dispersed in the matrix or adsorbed on the surface. The development of new formulations of nanocarriers, for example the poly(ethylene glycol)-poly(ε-caprolactone) (PEG–PCL) nanocapsules and alginate/chitosan (AG/CS) nanospheres described in this work, is needed in the case of ionized drugs such as articaine. This amino amide local anesthetic is the drug of choice in dentistry for regional anesthesia as well as the relief of acute and chronic pain. Here, the physico-chemical properties of suspensions of the nanoparticles (considering diameter, polydispersion, and zeta potential) were determined as a function of time, in order to establish the stability of the systems. The formulations did not show any substantial changes in these parameters, and were stable for up to 120 days of storage at ambient temperature. Satisfactory encapsulation efficiencies were obtained for the PEG–PCL nanocapsules (60%) and the AG/CS nanospheres (45%). Cytotoxicity assays confirmed that the encapsulation of articaine reduced its toxicity, relative to the free drug. The most promising results were obtained using the vesicular system (PEG–PCL nanocapsules), which not only altered the release profile of the drug, but also resulted in the lowest toxicity. This carrier system therefore holds promise for use in future practical applications.

1. Introduction

Polymeric nanoparticles (NP) are used as carriers for drugs and other active molecules, and can be prepared in the form of matrix-based systems (nanospheres) or as vesicles (nanocapsules) [1–7]. An important consideration is that the nanoparticles should be manufactured using materials whose metabolites are nontoxic and can be easily degraded. Among the most widely used materials are natural biodegradable polymers such as alginate (AG), chitosan (CS), and poly(ε-caprolactone) (PCL) [6].

Alginates are polyanionic polymers extracted from brown algae. In the presence of certain divalent cations (such as Ca^{2+}) or cationic polymers (such as chitosan), these polymers undergo ionotropic gelification, with formation of inter-chain links that enable the incorporation of hydrophilic bioactive molecules by means of electrostatic and van der Waals' interactions, amongst others [8].

Alginate/chitosan (AG/CS) nanospheres can be produced by adding a solution of cations to a solution of alginate and chitosan, under agitation. The nanoparticles formed can then be used to encapsulate hydrophilic drugs [8–11]. AG/CS nanospheres are biocompatible, exhibit low toxicity, and are more effective for the modulation of drug release, compared to single component nanospheres composed of alginate or chitosan [10–15].

Poly(ε-caprolactone) is an aliphatic polyester that is widely used in drug release systems in the form of both nanospheres

* Corresponding author at: Corresponding author. State University of São Paulo, Environmental engineering, Avenida Três de Março, 511, Alto da Boa Vista, 18087-180 Sorocaba, SP, Brazil. Tel.: +55 15 32343414/+55 15 91191077.
E-mail address: leonardo@sorocaba.unesp.br (L.F. Fraceto).

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and nanocapsules [16,17]. Due to its hydrophobic nature, the incorporation of hydrophilic drugs in this polymer is low, and the drug is readily lost to the aqueous phase during nanoparticle formation. Alternative encapsulation methods are therefore required for these drugs [17,18]. One possibility is to modify the polymer with polyethylene glycol (PEG) in order to obtain nanocapsules with aqueous nuclei that can incorporate hydrophilic compounds.

Polyethylene glycol, a biocompatible hydrophilic polymer that has been approved by the United States Food and Drug Administration, can be added to PCL to produce a PEG–PCL diblock copolymer [19,20]. This copolymer is both more hydrophilic and more biodegradable than the PCL homopolymer. Studies conducted in vitro have confirmed its superior performance. In the form of nanocapsules with an aqueous nucleus, it has been used to develop release systems for hydrophilic drugs [20,21], with advantages including reduced drug toxicity and increased circulation time because the presence of PEG on the particle surface hinders recognition and opsonization by phagocytic cells [22]. The aqueous nucleus provides these nanocapsules with a high capacity for the incorporation of hydrophilic compounds, which are retained in the nucleus, protecting the tissues from irritation caused by drugs that are highly soluble in water, as well as diminishing the "burst effect" that is frequently observed with other types of polymeric nanoparticles [23].

Articaine hydrochloride (ATC) is an amino amide class local anesthetic, and in dental practice is often the drug of choice for control of acute and chronic pain. The compound is highly soluble in water (~5 g/L, p*K*a 7.8) [24,25]. ATC is unique in that it possesses a thiophene ring, rather than a benzene ring, which enhances both its potency and its ability to diffuse amongst the tissues [25–27]; this is the reason for its anesthetic effectiveness. However, a difficulty associated with the use of ATC concerns the risk of long-term or permanent paresthesia [27–29]. Another disadvantage is related to the high ATC concentration used (4%), because lesion of the mandibular nerves is concentration-dependent and the associated risks are therefore increased [25,30,31].

A possible strategy to help mitigate these adverse effects associated with the use of ATC is to deliver the drug using modified release systems, such as polymeric nanoparticles that can be loaded with hydrophilic compounds. The objective of this work was therefore to prepare, characterize, and compare two hydrophilic nanocarriers with different structures (AG/CS nanospheres and PEG–PCL nanocapsules), using the ionized form of articaine as a model drug, as well as to evaluate the stabilities and cytotoxicities of the systems. Up to now, there have been few studies concerning the development of nanocarriers for articaine in either its neutral or ionized forms [32,33].

2. Materials and methods

2.1. Materials

Articaine hydrochloride was donated by DFL Industries (Rio de Janeiro, Brazil). Sodium alginate, low molecular weight chitosan (50 kDa), poly(ε-caprolactone) (70–90 kDa), poly(ethylene glycol)-block-poly(ε-caprolactone) methyl ether (PEG ~5 kDa, PCL ~32 kDa), Tween 20, dextran 70, and triglycerides of capric and caprylic acid were purchased from Sigma-Aldrich. Calcium chloride was obtained from LabSynth. Acetonitrile (HPLC grade) was acquired from Tedia Chemicals. Dulbecco's Modified Eagle Medium (DMEM), colchicine, fetal bovine serum, penicillin, and streptomycin sulfate were purchased from Cultilab (Brazil). All other reagents used were either spectroscopic or analytical grade.

2.2. Preparation of the hydrophilic biodegradable nanocarriers

2.2.1. Alginate/chitosan nanospheres

The alginate/chitosan (AG/CS) nanospheres were prepared according to the ionotropic gelification method described by De & Robinson [10], with slight modifications. Briefly, ATC was added to 10 mL of a solution of sodium alginate (0.6 mg/mL), under magnetic agitation. Subsequently, 2 mL of calcium chloride solution (1.11 mg/mL) was added dropwise to the solution, under agitation, and the mixture was sonicated for 1 min (Unique sonicator, 500 W, 20 kHz). The alginate pre-gel was then maintained under magnetic agitation for 30 min. A solution of chitosan (2 mL, 0.65 mg/mL) was added dropwise to the pre-gel, after which the mixture was left under agitation overnight for stabilization of the nanospheres. The final concentration of ATC was 20 mg/mL [10,33].

2.2.2. PEG–PCL block copolymer nanocapsules

The PEG–PCL nanocapsules were prepared according to the interfacial coacervation method described by Ma et al. [34], with minor modifications. Firstly, 200 mg of the PEG–PCL copolymer and 100 mg of PCL were dissolved in 10 mL of acetone, followed by addition of 6 mL of an aqueous solution containing ATC (200 mg). The mixture was left under magnetic agitation for 30 min to form an emulsion. The emulsion was added (under magnetic agitation) to 20 mL of an aqueous phase containing Tween 20 (100 mg) and dextran 70 (200 mg), and the mixture was then kept under agitation for 10 min to obtain a suspension of nanocapsules containing ATC. This suspension was concentrated to a volume of 10 mL using a rotary evaporator, giving a final ATC concentration of 200 mg/mL [34].

2.2.3. Determination of nanoparticle morphology by transmission electron microscopy (TEM)

The morphologies of the AG/CS nanospheres and PEG–PCL nanocapsules were investigated using TEM. Uranyl acetate (2%) was added to the samples to provide contrast, after which aliquots were deposited onto copper grids coated with a carbon film and dried at ambient temperature. After drying, micrographs of the samples were obtained using a JEOL 1200 EXII microscope operated at 80 kV [35].

2.2.4. Characterization and stability of the particles

The chemical stability of the polymers was determined by measuring the pH of the suspensions using a potentiometer (Tecnal) that had been previously calibrated. The physicochemical stability of the suspensions of AG/CS nanospheres and PEG–PCL nanocapsules containing ATC was evaluated using measurements of average diameter, polydispersion, zeta potential, and pH, over a period of 120 days. The suspensions were stored in amber flasks at ambient temperature [36–38].

The measurements of average diameter and zeta potential were performed by dynamic light scattering (photon correlation spectroscopy, PCS) after dilution of the nanoparticle suspensions in deionized water (1:100, v/v), using a ZetaSizer Nano ZS 90 analyzer (Malvern Instruments) operated at 25 °C with a fixed angle of 90°. The polydispersion indices of the samples were also measured. The results were calculated as the average of three determinations [33,36] and the analyses were performed after 0, 15, 30, 60, and 120 days.

2.2.5. Analysis of the nanoparticles using ATR–FTIR and DSC

Infrared spectra were obtained for ATC, sodium alginate, chitosan, the PEG–PCL copolymer, the AG/CS nanospheres, and the PEG–PCL nanocapsules. Analyses of the nanospheres and nanocapsules were performed with and without the encapsulated drug, as well as using physical mixtures. These measurements employed a Varian 660 FTIR spectrometer equipped with an attenuated total