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Fluoride loaded polymeric nanoparticles for dental delivery

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ABSTRACT

The overall aim of the present paper was to develop fluoride loaded nanoparticles based on the biopolymers chitosan, pectin, and alginate, for use in dental delivery. First, the preparation of nanoparticles in the presence of sodium fluoride (NaF) as the active ingredient by ionic gelation was investigated followed by an evaluation of their drug entrapment and release properties. Chitosan formed stable, spherical, and monodisperse nanoparticles in the presence of NaF and tripolyphoshate as the crosslinker, whereas alginate and pectin were not able to form any definite nanostructures in similar conditions. The fluoride loading capacity was found to be 33–113 ppm, and the entrapment efficiency 3.6–6.2% for chitosan nanoparticles prepared in 0.2–0.4% (*w*/w) NaF, respectively. A steady increase in the fluoride release was observed for chitosan nanoparticles prepared in 0.2% NaF both in pH 5 and 7 until it reached a maximum at time point 4 h and maintained at this level for at least 24 h. Similar profiles were observed for formulations prepared in 0.4% NaF; however the fluoride was released at a higher level at pH 5. The low concentration, but continuous delivery of fluoride from the chitosan nanoparticles, with possible expedited release in acidic environment, makes these formulations highly promising as dental delivery systems in the protection against caries development.

1. Introduction

Fluoride is now recognized to be the most efficient agent to control dental caries mainly through topical effect by inhibiting demineralization and enhancing remineralization of the dental hard tissues (Buzalaf et al., 2011). Despite its well-established caries-protective role, conventional self-care products of fluoride, such as tooth pastes and mouth rinses, is associated with short oral residence time due to the constant cleansing action of saliva. This unfavorable, but physiologically important function of saliva makes it difficult to maintain cariostatic concentrations of fluoride in the oral fluids leading to suboptimal fluoride regimens particularly in patients at elevated risk of developing caries (Fontana and Zero, 2006). A study on the remineralization effect of low-concentration fluoride rinse by Chow et al. concluded that the effectiveness of a fluoride regimen depends less on the dose and more on the ability of the treatment to utilize fluoride efficiently for remineralization (Chow et al., 2002). In addressing the inefficient use of fluoride, improved methods of fluoride delivery to the teeth are sorely needed.

Fluoridated gels and varnishes have been introduced to the commercial market to prolong the contact time between fluoride and tooth surfaces (Pessan et al., 2011b). However, these modalities often involve professional application and contain a much higher concentration of fluoride carrying the risk of excessive fluoride intake. Other fluoride-releasing formats are bioadhesive tablets which have been developed to release fluoride in a sustained manner (Bottenberg et al., 1998; Owens et al., 2005). Being applied to the oral mucosa, these dosage forms can cause local irritation and are often poorly tolerated by the patients. Materials for dental restorations have also been incorporated with fluoride in order to protect against recurrent caries following a restoration placement (Wiegand et al., 2007). Despite acting conveniently as fluoride reservoirs, the insertion of these materials requires professional intervention and is limited to patients at risk for restoration failure. More recently, advanced systems, such as microparticles, have been investigated for the controlled delivery of fluoride. *In vitro* studies showed that biocompatible polymeric microparticles could be used as drug delivery systems to enhance fluoride retention in the oral cavity and promote its time-dependent release from potentially different oral care products (de Francisco et al., 2013; Keegan et al., 2012). Apparently, the physical size of the systems plays a key role in oral cavity retention because mouthfeel and the tendency of being sensed are critical factors for patient acceptability and compliance. With this in mind, advancing into the nanoscaled size range would offer even more benefits based on the unique size-dependent properties of particles, including high surface area to volume ratio, and the ability to penetrate biofilm (Allaker, 2010; Wang et al., 2015) and biomimic oral processes

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(Hannig and Hannig, 2010; Hannig and Hannig, 2012). Constructing nanoparticles with bioadhesive polymers would allow more efficient use of fluoride by increasing its local concentration at the dental hard tissues while evading rapid salivary clearance and excess consumption of the mineral, thus, minimizing systemic effects.

Several methods have been developed to prepare polymeric nanoparticles (Reis et al., 2006). One of the simplest and mildest procedures is based on ionic gelation involving electrostatic complexation between the polymer and an oppositely charged species, often of low molecular weight such as $CaCl_2$, $ZnCl_2$, and the polyanion tripolyphosphate (TPP) (Grenha, 2012; Jonassen et al., 2013; Paques et al., 2014). Due to the crosslinking effect when the two aqueous solutions are mixed, the polymer precipitates and forms nanosized particles. The characteristics of the structures prepared by this method are dependent on several formulation variables, including polymer molecular weight and concentration, and crosslinker to polymer ratio. Recent studies have reported that more compact and smaller nanoparticles with narrower size distributions were produced in the presence of moderate amount of salt due to the salt-induced screening effect (Huang and Lapitsky, 2011; Jonassen et al., 2012; Jonassen et al., 2013; Pistone et al., 2015). This shows in fact that the ionic strength of the solvent is also an important parameter to address in the preparation of biopolymeric nanoparticles by ionic gelation. Giving this background, the most common fluoride compound used in caries prophylaxis is sodium fluoride. In terms of drug properties, this is an unconventional drug molecule to be entrapped in a hydrophilic carrier having very low molecular weight (41.99 g/mol) and high water solubility (4.3 g/100ml at 25 °C). Being also a monovalent salt itself, the drug will affect the ionic strength of the solution and thereby the formation of the nanoparticles. With the overall aim of developing fluoride loaded nanoparticles for use in dental delivery, this study will first investigate the preparation of polymeric nanoparticles in the presence of NaF by ionic gelation followed by an evaluation of their function as fluoride delivery systems in terms of drug entrapment and drug release. Three biopolymers, *i.e.* chitosan, pectin, and alginate, were chosen for the present investigation due to their recognized bioadhesivity, biocompatibility, biodegradability, and low toxicity (Liu et al., 2008).

2. Materials & methods

2.1. Materials

Amidated pectin (Genu pectin LM-102 AS) was obtained from CPKelco (Lille Skensved, Denmark), and ultrapure chitosan chloride (Protasan® UP CL 213, Novamatrix) and sodium alginate (Protanal® LF 10/60) were both obtained from FMC Biopolymer (Norway). Characteristics of the polymers are shown in Table 1. Sodium alginate and amidated pectin (AM-pectin) were purified in-house by dialysis using Spectra/Por 6 membranes (Spectrum Laboratories Inc., CA, USA) with MWCO 8 kDa, then freeze-dried and stored in the refrigerator until further use. Tripolyphosphate (TPP, sodium triphosphate pentabasic), $ZnCl_2$, and NaF were supplied from Sigma-Aldrich (Germany), sodium phosphate monohydrate ($NaH_2PO4{\cdot}H_2O$) and disodium phosphate dihydrate ($Na_2HPO4{\cdot}2H_2O$) from Merck (Darmstadt, Germany), and NaCl (BDH Prolabo®) from VWR Chemicals (Leuven, Belgium). All chemicals were of analytical grade and used as received. All solutions were prepared with Milli-Q purified (Millipore, Molsheim, France) and filtered (0.22 μm, Millipak® 40, Millipore) water.

2.2. Preparation of fluoride loaded nanoparticles

Fluoride loaded polymeric nanoparticles were prepared by ionic gelation in Milli-Q water by means of a peristaltic pump (Watson-Marlow 520S IP3, Cornwall, UK). First, 15 g of NaF solution was added dropwise at 25 rpm pumping speed to 45 g polymeric solution under magnetic stirring, followed by 15 g of crosslinker solution added in the same manner. Total stirring time for each mixing was 10 min. The polymer solutions were filtered 0.8 μm, and the NaF and crosslinker solutions were filtered 0.22 μm with syringe filters prior to mixing. In the preliminary experiments, the polymer were first dissolved in different solvents (Table 2) before adding the crosslinker solution to the polymer solution in the same manner as previously described. All formulations were allowed to stabilize in room temperature (~20 °C) overnight prior to characterization.

2.3. Characterization of nanoparticles

2.3.1. Size and size-related parameters

The hydrodynamic mean diameter and the polydispersity index (PDI) of the nanoparticles together with the derived count rate were determined by dynamic light scattering (DLS) using a Zetasizer Nano ZS (Malvern Instruments Ltd., Worcestershire, UK). The derived count rate, corresponding to the number of photons detected per second, was used as an expression for the intensity of scattered light (Huang and Lapitsky, 2012; Pistone et al., 2015). All measurements were performed at 25 °C with backscatter detection and scattering angle of 173°. The refractive index and the viscosity of pure water at 25 °C were used as calculation parameters. Each sample was measured in triplicate using analysis model for general purpose.

2.3.2. Imaging

Atomic force microscopy (AFM) imaging of the nanoparticles was performed using a NanoWizard® AFM system (JPK Instruments AG, Germany) with NSC35/AlBS Ultrasharp Silicon Cantilevers (MikroMasch, Spain) through intermittent contact mode in air. The system was set up on an Eclipse TE2000-S inverted optical microscope (Nikon Instruments Inc., Japan), mounted on an anti-vibration table (Halcyonics MOD-1 M plus, Accurion GmbH, Germany) inside a JPK acoustic enclosure. 10 μl of the nanoparticle suspension was pipetted onto freshly cleaved mica. After 30 s adsorption time, excess liquid was removed with a filter paper applied at the edge of the mica. Excess nanoparticles deposited on the mica were removed by rinsing the samples three times with 10 μl of Milli-Q water. The samples were allowed to air-dry at room temperature overnight prior to imaging.

2.4. In vitro stability

The fluoride containing nanoparticles were stored as suspensions in the refrigerator and their *in vitro* stability was studied for one month. At specific time points, the nanoformulations were characterized in terms of particle size and PDI described in the preceding section.

2.5. Fluoride content

2.5.1. Drug loading

10 ml of the sample suspensions were transferred to pre-washed Spin-X® UF centrifugal concentrators (Corning®, NY, USA) with 30 k MWCO PES membrane for ultrafiltration and centrifuged for 10 min at 5000 rcf (20 °C). This step was repeated until the volume of collected filtrate was sufficient for analysis using a fluoride ion selective electrode (ISE, Orion ionplus 9609, Thermo Fisher Scientific, MA, USA) coupled with an ion meter (Orion 720A, MA, USA). In order to provide a constant background ionic strength, 0.5 ml of Total Ionic Strength Adjustment Buffer (TISAB III, EMD Millipore, Darmstadt, Germany) was added to 5 ml of each sample and standard (based on prepared NaF solutions). Prior to measurements, the electrode operation (slope) was checked according to the manufacturer's instructions. All measurements were performed in plastic beakers under magnetic stirring at 900 rpm (~20 °C). Samples were measured in triplicate and the fluoride concentration (in ppm) was found employing a calibration curve in the appropriate concentration range. The amount of fluoride entrapped in the formulation, *i.e.* the drug loading (DL), was calculated