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Development of Cetylpyridinium-Alginate Nanoparticles: A Binding and Formulation Study

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ABSTRACT

In this study the development of stable polyelectrolyte-surfactant complex nanoparticles composed of alginate and cetylpyridinium chloride (CPC), with and without $ZnCl_2$, for therapeutic use, is investigated. The mechanism of CPC binding by alginate was analyzed using a cetylpyridinium cation (CP^+) selective membrane electrode. The cooperative nature of the interaction between CP^+ and alginate was underlined by the sigmoidal shape of the binding isotherms. The presence of salts was shown to weaken interactions and, moreover, $ZnCl_2$ reduced the cooperativity of binding. The CP^+ cations in the form of micellar associates acted as multivalent crosslinkers of the alginate chains where stable dispersions of CP-alginate nanoparticles were formed in water at CP^+/alginate charge ratios from 0.2 to 0.8. Characterization of the nanoparticles showed hydrodynamic diameters from 140 to 200 nm, a polydispersity index below 0.2, a negative zeta potential and spherical morphology. The entrapment efficiency of CPC was ~94%, the loading capacity more than 50% and prolonged release over 7 days were shown. The formulations with noted charge ratios resulted in stable CP-alginate nanoparticles with a potential of treating periodontal disease.

1. Introduction

Periodontal disease is initiated by a pathogenic infection on a tooth's surface in the form of a microbial biofilm, which gradually leads to chronic inflammation of the periodontal tissues. This results in loss of their attachment and progressive destruction of the ligament and alveolar bone (Jain et al., 2008; Joshi et al., 2016; Zupančič et al., 2015b). According to the World Health Organization, 15-20% of the adult population is suffering from a severe periodontal disease, so search for an effective treatment is important (Petersen et al., 2005). Conventional treatment consists of mechanical removal of the dental plaque, followed by adjuvant therapy with antiseptics or antibiotics. Systemic administration of antibiotics is associated with adverse side effects and the development of bacterial resistance. Local drug delivery systems (DDS) have therefore been developed, unfortunately with some drawbacks, such as short residence time, need for removal of non-biodegradable carriers, inconvenient application and, consequently, poor patient compliance (Joshi et al., 2016).

To overcome these shortcomings research has been carried out on new DDS for local delivery, one example being in situ formation of implants (Do et al., 2015a; Do et al., 2015b) and, on the nanoscale DDS such as liposomes, lipid and polymeric nanoparticles (NPs) and nanofibers (Bertoncelj et al., 2014; Zupančič et al., 2015a; Zupančič et al., 2015b; Zupančič et al., 2016). Polyelectrolyte complex NPs, prepared by crosslinking of the polyelectrolyte with divalent ions or by mixing oppositely charged polyelectrolytes, have drawn a lot of attention in recent years due to the simplicity of formation and formulation, good biocompatibility and relatively high encapsulation yield compared to hydrophobic polymer or lipid based nanocarriers (Umerska and Tajber, 2015). Important aspects in the development of DDS are entrapment efficiency and the loading capacity of the active agent in the nanocarriers. Both parameters are related directly to the structure and the physico-

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chemical parameters of both active agent and matrix (Grabnar et al., 2003).

In this study the focus was on developing polymeric NPs, using alginate, as a bioadhesive and biodegradable polymer, to enable prolonged retention in the periodontal pockets and controlled release of the drug, both without the need for later mechanical removal. Alginate is a natural polysaccharide and an anionic polyelectrolyte that forms NPs in the presence of polyvalent cations by a spontaneous method called ionic crosslinking (Grabnar and Kristl, 2011). As the crosslinking agent, divalent Ca^{2+} ions are very well known while, more recently, Zn^{2+} ions have also been investigated for the preparation of gels and micro- and nanoparticles (Jonassen et al., 2013; Pistone et al., 2015; Rajaonarivony et al., 1993). Zn^{2+} ions were chosen as the crosslinkers in this study also for their inherent antimicrobial properties that lead to reduced plaque formation (Gu et al., 2012; Pistone et al., 2015). However, loading Zn-alginate NPs with a drug has not been studied yet.

CPC is a cationic pyridinium compound and an effective antiplaque agent recognized by the Food and Drug Administration. It is already used in oral rinses due to its antiplaque and antibacterial effects in the early stages of periodontal disease (Haps et al., 2008) and has also been proved to be effective against biofilm formation (Pandit et al., 2015). CPC forms micelles in aqueous media due to its amphiphilic structure and because of its positive charge strong interaction with negatively charged alginate chains is expected. Interactions between polyelectrolytes and oppositely charged surfactants in dilute aqueous solutions have frequently been investigated (Bračič et al., 2015; Jain et al., 2004; Kogej, 2010). Surfactant molecules have been shown to self-assemble into micelle-like aggregates threaded on a polymer chain like pearls on a string (Chavanpatil et al., 2007; Pippa et al., 2013; Prelesnik et al., 2011; Sitar et al., 2012).

Although CPC has been shown to be effective in treating periodontal disease, an appropriate nanodelivery system based on this substance has not been described. Our special interest was to investigate the possibility of preparing cetylpyridinium alginate NPs with and without Zn^{2+} as a crosslinker. CPC binding by alginate was investigated using a CP^+ selective membrane electrode in the presence and absence of added salts (NaCl and $ZnCl_2$) in order to establish the mechanism of successful nanoparticle formation. The overall aim of this study was, therefore, to develop alginate NPs loaded with CPC that could be administered into the periodontal pocket, where prolonged release of CPC would take place at the site of infection.

2. Materials and methods

2.1. Materials

Sodium alginate (molecular weight (Mw) = 1.38×10^5 g/mol, Protanal LF 10/60) was kindly provided by FMC BioPolymer (Norway) with a content of α-L-guluronate (G, monomer unit molecular weight (M_m) = 198 g/mol) of 65% – 75%. The content of β-D-mannuronate (M, M_m = 198 g/mol) was 25% – 35%, as stated by the manufacturer. Before use, alginate was purified by dialysis and freeze-drying, as described earlier (Pistone et al., 2015). Zinc chloride (purity ≥ 98.0%, M_w = 136.3 g/mol) was purchased from Sigma Aldrich (Germany) and sodium chloride (purity ≥ 99.5%, M_w = 58.44 g/mol) from Merck (Denmark). CPC monohydrate (CID 31239, M_w = 358.0 g/mol, CMC (water, 25 °C) = 6.3×10^{-4} M (Skerjanc et al., 1988)) was provided by Sigma Aldrich (USA). Water was purified with a Milli-Q system with a 0.22 μm Millipak® 40 filter (Millipore ™, Ireland).

2.2. Preparation of nanoparticles

Alginate NPs were prepared by mixing $ZnCl_2$ and/or CPC solutions with dilute alginate solutions of various concentrations, in the same manner as reported (Jonassen et al., 2013; Pistone et al., 2015). All starting solutions were prepared with MilliQ water and filtered through 0.80 μm Millex®AA syringe filters (MilliporeTM, Ireland). Using a peristaltic pump with fitted silicone hose (inner diameter 1 mm), the whole amount of crosslinker was added dropwise (9.3 ml/min), over 10 minutes, to the alginate solution (in 100 mL borosilicate vials) under continuous stirring (cylindrical magnetic stir bar 20 × 6 mm) at 550 rpm. Prior to characterization, samples were stored overnight at 25 ± 1 °C.

2.3. Experimental design for nanoparticle preparation

Zn-alginate nanoparticles with loaded CPC

Compositions of the prepared formulations are listed in Table 1. Concentrations of alginate (0.06 and 0.07% w/w) and CPC (0.005% w/w) were kept constant. The amount of Zn^{2+} was varied and expressed as the ratio of weight of $ZnCl_2$ to weight of alginate (w:w). Charge ratios, C^+/P^-, where C^+ denotes cations (CP^+ and Zn^{2+}) and P^- denotes negative charges on alginate, were calculated using molar masses of CPC and $ZnCl_2$ and molar mass of the repeat monomer unit of sodium alginate (G or M) with one carboxyl group. These formulations are termed CP-Zn-alginate NPs.

Cetylpyridinium-alginate nanoparticles

Two series of NPs were prepared with alginate and CPC only (no $ZnCl_2$). In the first series, the concentration of alginate was varied and that of CPC kept constant, and conversely in the second series (Table 2). The samples are termed CP-alginate NPs. Cetylpyridinium cation (CP^+) to alginate polyion (P^-) charge ratios (C^+/P^-) were calculated in the same manner as above. A 0.0085% w/w alginate solution without added CPC ($C^+/P^- = 0$) was used as a control sample.

2.4. Characterization of the nanoparticle dispersions

Measurements of pH

The pH of the NP dispersion was measured at 25 °C using the InLab® Expert Pro-ISM pH electrode (Mettler Toledo) calibrated with three standards pH 4.01, 7.01 and 9.00.

Hydrodynamic diameter and zeta potential

A Zetasizer Nano NZ (Malvern Instruments Ltd., UK) was used for hydrodynamic diameter (D_h) and zeta potential (ζ) measurements. All measurements were carried out at 25 °C, using the He-Ne laser (λ = 633 nm) with backscatter detection (scattering angle of 173°). The autocorrelation functions, from which the diffusion coefficient (D) was calculated, were obtained by measuring the intensity fluctuations of the scattered light. Employing the Stokes-Einstein equation ($D = kT/6\pi\eta R_h$, where T is 298 K and η is the viscosity of water) the particle's hydrodynamic radius (R_h) was calculated. The data are reported as D_h (=2 × R_h). Software (version 6.20) provided the average D_h and the polydispersity index (PDI). When the PDI > 0.3, the samples are so polydisperse that the measurements cease to be valid. Nine measurements were

Table 1
Compositions of CP-Zn-alginate formulations containing 0.06–0.07% w/w alginate and 0.005% w/w CPC.

$ZnCl_2$: alginate w:w	C^+/P^- ratio
1:99	0.07
3:97	0.13
5:95	0.20
10:90	0.37
15:85	0.56