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Chemical cross-linking: A feasible approach to prolong doxylamine/pyridoxine release from spray-dried chitosan microspheres

Plamen Katsarov[a,d,*], Bissera Pilicheva[a,d], Yordanka Uzunova[b], Georgi Gergov[c], Margarita Kassarova[a,d]

[a] Department of Pharmaceutical Sciences, Faculty of Pharmacy, Medical University – Plovdiv, Bulgaria
[b] Department of Chemistry and Biochemistry, Faculty of Pharmacy, Medical University – Plovdiv, Bulgaria
[c] Department of Chemistry, Faculty of Pharmacy, Medical University – Sofia, Bulgaria
[d] High-technological Center for Emergency Medicine, Plovdiv, Bulgaria

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ABSTRACT

Spray-dried chitosan microparticles have been widely exploited as vehicles for mucosal drug delivery. Despite their advantages as pharmaceutical formulations, one of the major challenges is achieving sustained drug release, which would diminish toxicity and dosage frequency. The aim of this study was to formulate mucoadhesive glutaraldehyde cross-linked chitosan microparticles loaded with doxylamine succinate and pyridoxine hydrochloride as potential nasal drug delivery systems with sustained release. Microparticle models were formulated *via* spray-drying technique, using glutaraldehyde in different concentrations (0.1–1.0 mg/mL) as a cross-linking agent for chitosan. The obtained particles were with spherical shape, smooth surface and median diameter of 4 μm. The drug entrapment efficiency was high (80.47%–94.25%), indicating a tendency to decrease at higher glutaraldehyde concentrations. FTIR data demonstrated that there were no chemical interactions between glutaraldehyde and the drugs. The *in vitro* studies showed that the cross-linking process substantially limited particles swelling. The cross-linked particles exhibited sustained drug release characteristics at pH 6.8 over a period of 5 h with an initial burst-effect in the first 30 min. Drug release followed Korsmeyer-Peppas kinetics. Although a decrease of the particles mucoadhesive properties was observed after modification, all cross-linked formulations demonstrated high *in vitro* adsorption of mucin. The proposed models of mucoadhesive microsphere with sustained drug release are a perspective ground for further development of a novel delivery system for nasal administration of doxylamine and pyridoxine.

1. Introduction

Over the past few decades polymer microparticles have been considered as promising systems for nasal drug delivery. Due to their light weight they easily adhere on the nasal tissue and reach vast mucosal surface areas, which result in a high drug uptake (Deutel et al. 2015). Moreover, various mucoadhesive polymers have been used in order to prolong the contact of the formulation with the mucus membrane. In particular, chitosan is one of the most extensively investigated mucoadhesive polymers and has been widely employed in the preparation of drug systems for mucosal administration (Casettari and Illum 2014). Due to their biodegradability, low toxicity, biocompatibility and excellent mucoadhesive properties, chitosan nano- and microparticles have been considered as appropriate vehicles for drug delivery (Mohammed et al. 2017; Ali and Ahmed 2018).

Despite the advantages of chitosan microparticles as pharmaceutical formulations, one major challenge persists. Achieving sustained drug release especially when incorporating water-soluble drugs is of significant importance and could offer diminished drug toxicity and dosage frequency. Chitosan matrix swells quickly in water and often releases rapidly the incorporated drugs, unable to provide sustained drug delivery. A possible approach to make the polymer structure more rigid and suited for controlled drug release is to modify the chitosan structure by cross-linking of its chains. Depending on the nature of the cross-linker, the interactions forming the cross-linked structure are mainly ionic or covalent bonds. In the ionic (physical) cross-linking process, a network of ionic bridges between negatively charged components and the positively charged chitosan chains is formed. Covalent (chemical)

* Corresponding author at: Department of Pharmaceutical Sciences, Faculty of Pharmacy, Medical University – Plovdiv, 15A, Vassil Aprilov Blvd., 4002 Plovdiv, Bulgaria.
E-mail addresses: plamen.katsarov@yahoo.com (P. Katsarov), bisserapi@gbg.bg (B. Pilicheva).

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cross-linking on the other hand can more effectively affect the physicochemical stability of the chitosan matrix since the cross-linking process is irreversible. The higher stability of such modified chitosan is based mainly on the covalent bonds, but other interactions (hydrogen or hydrophobic bonds) are also possible (Szymańska and Winnicka 2015). To date, one of the most common chemical cross-linkers of chitosan is glutaraldehyde. The primary amine ($-NH_2$) groups of chitosan provide a reaction site for covalent interaction with the aldehyde molecules, thus allowing polymer modification and achieving a stable cross-linked network (Desai and Park 2005; Gonçalves et al. 2005; Gupta and Jabrail 2006; Kulkarni et al. 2007; Ramachandran et al. 2011).

Although cross-linking of chitosan has been proposed by many authors as a possible approach to modify the drug release pattern, it could negatively influence the mucoadhesive characteristics of the polymer. The degree of cross-linking significantly influences polymer chain mobility, which is crucial for interpenetration and entanglement with the mucus gel. It is known that the more flexible the chains, the better interdiffusion and penetration of the polymer within the mucus network. As cross-linking density increases, polymer chain mobility decreases and hence the mucoadhesive strength is reduced (Andrews et al. 2009). Therefore, the amount of the cross-linker should be optimized and its influence on both release kinetics and mucoadhesion should be evaluated.

The aim of this study was to formulate mucoadhesive glutaraldehyde cross-linked chitosan microparticles as sustained release drug delivery systems *via* spray-drying technique and to investigate how the process of cross-linking affects their physical and chemical characteristics.

Another challenging aspect of this work was the simultaneous incorporation of two APIs in the microparticles. Doxylamine succinate (DOX) and pyridoxine hydrochloride (PYR) were used as model drugs. The combination has been approved on the pharmaceutical market for the treatment of nausea and vomiting with proven efficacy and safety during pregnancy (Slaughter et al. 2014). So far, this drug combination is available only in various oral dosage forms with modified drug release. However, the oral administration is associated with certain constraints and is not particularly suited when treating nausea and vomiting. The nasal cavity, on the other hand, is nowadays being dynamically explored for systemic delivery of various therapeutic agents including antiemetics such as metoclopramide (Gavini et al. 2008) and ondasetrone (Gungor et al. 2010).

The design of polymeric microcarriers with optimal bioadhesive and biopharmaceutical properties is a technological challenge; its successful realization would be a prerequisite for the formulation of a novel and convenient drug delivery system for nasal administration of doxylamine and pyridoxine.

2. Material and methods

2.1. Materials

Chitosan from shrimp shells (low-viscosity, degree of deacetylation > 70%), doxylamine succinate, pyridoxine hydrochloride, glutaraldehyde (50% aqueous solution), mucin (porcine stomach, type II) and Bradford reagent were purchased from Sigma Aldrich, USA. All solvents and reagents were of analytical grade.

2.2. Methods

2.2.1. Microspheres preparation

Microspheres were formulated by spray-drying technique. Solutions with concentration of chitosan 2% (w/v), doxylamine 1% (w/v) and pyridoxine 1% (w/v) were prepared in 2% (v/v) acetic acid. A chemical cross-linking reaction was carried out by adding glutaraldehyde (GA) dropwise into the resultant solutions under stirring (400 rpm) for 20 min (Gaspar et al. 2015). Three different concentrations of GA were used: 0.1, 0.5 and 1 mg/mL. The mixtures were spray-dried through a 0.7 mm nozzle using Büchi Mini Spray Dryer B-290 (Büchi Labortechnik AG, Flawil, Switzerland). The spray-drying parameters were previously optimized applying 3^2 factorial design and constant process settings were used: 140 °C inlet temperature, 160 mL/h pump rate, 600 L/h airflow rate and 95% aspiration (Katsarov et al. 2017a). Non-cross-linked microspheres were also prepared as a basis for comparison.

2.2.2. Scanning electron microscopy (SEM)

Scanning electron photographs of the formulated particle were taken using Philips SEM 515 (Philips, Eindhoven, The Netherlands). Small amounts of microspheres were fixed with double-sided adhesive on holders and were coated with a thin layer of gold in SC7620 Mini Sputter Coater (Quorum Technologies Ltd., UK). SEM was performed at 25 kV acceleration voltage and 5000× magnification.

2.2.3. Evaluation of production yield, size and particle size distribution

Particle production yield was calculated as the ratio of the weight of the obtained microspheres (W_1) to the weight of the spray-dried polymer and drugs (W_2) according to the following equation: *Yield* (%) = (W_1 / W_2) × 100. Particles size distribution and median diameter were determined by laser diffraction technique using LS 13320 analyzer (Beckman Coulter, USA). Dry samples of 100 mg particles were analyzed with a Tornado system for powdered samples. All measurements were repeated three times.

2.2.4. UV spectrophotometric analysis

Simultaneous spectrophotometric quantitative determination of doxylamine and pyridoxine was carried out using Evolution 300 UV–visible spectrophotometer (Thermo Fisher Scientific, USA). The absorption spectra of the sample solutions were recorded in the wavelength range of 200–400 nm at 1.0 nm intervals, using VisionPRO™ software (Thermo Fisher Scientific). In order to separate the overlapping drug spectra, a multivariate chemometric method on the principle of partial least squares (PLS) was applied (Wold et al. 2001). A PLS2 model for the binary mixture DOX/PYR was constructed on the basis of 11 mixtures containing different concentrations of DOX and PYR (Katsarov et al. 2018). PLS2 calibration was carried out using the MVC1® MatLab toolbox (Olivieri et al. 2004).

2.2.5. Determination of drug content in the formulations

The amount of doxylamine and pyridoxine in the particles was extracted with phosphate buffered saline (PBS), pH 6.8 for 24 h at room temperature and was determined spectrophotometrically applying the developed PLS2 method. Drug loading (*DL*) (%) was calculated as the ratio of the determined amount of drug (mg) in the microspheres (W_d) to the weight (mg) of the microspheres (W_m) according to the following equation: *DL* (%) = (W_d / W_m) × 100. Drug entrapment efficiency (*DEE*) (%) was calculated on the basis of the determined drug loading and the estimated theoretical drug loading (Saigal et al. 2013). All experiments were performed in triplicate, and the means of the obtained values were reported.

2.2.6. Fourier transformed infrared spectroscopy (FTIR)

Infrared spectra of the cross-linked microspheres were recorded on a Nicolet iS 10 FTIR spectrometer (Thermo Fisher Scientific, Pittsburgh, PA, USA), equipped with a diamond attenuated total reflection (ATR) accessory. The analysis was performed under the following conditions: 64 scans, resolution of 4 nm, and spectral range 4000–650 cm^{-1}.

2.2.7. X-ray powder diffraction (XRPD)

XRPD analysis of the cross-linked microspheres was performed in order to characterize the physical state of the loaded drugs. Diffractograms were recorded on a D2 Phaser X-ray powder diffractometer (Bruker AXS GmbH, Germany). Ni-filtered Cu radiation at