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# Buccal delivery of low molecular weight heparin by cationic polymethacrylate nanoparticles

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ABSTRACT

Buccal delivery seems to be a very promising administration route for macromolecular drugs. Here, we explored the potential of cationic polymethacrylate nanoparticles (NPs) as a carrier system for the buccal delivery of low molecular weight heparin (LMWH). LMWH-loaded NPs were prepared by emulsification solvent diffusion method and the NPs were analyzed for their physiochemical properties, rheological evaluations and ex vivo transport studies across buccal mucosa. The prepared LMWH-loaded NPs showed a mean diameter between 400 and 500 nm with unimodal size distribution with negative surface charge. Viscosity measurements revealed a positive rheological synergism between the prepared NPs and mucin when mixed under physiological conditions. After 4 h, about 6.3 ± 0.9% of LMWH was released in case of using Eudragit® RS (ERS); while Eudragit® RL (ERL) NPs released only 3.0 ± 0.3 % of its LMWH content and this incomplete release was slightly ameliorated in the presence of mucin reaching to 7.2 ± 0.3 % and 4.8 ± 0.3 % for ERS and ERL, respectively. The ex-vivo permeability of heparin through the buccal mucosa was significantly increased after using polymetharylate NPs while no heparin permeation was detected from free heparin solution. Confocal laser scanning microscopy (CLSM) imaging indicated the mucoadhesive properties of the polymetharylate NPs where the drug-free NPs were detected in the superficial layers of buccal mucosa. LMWH-loaded NPs had less mucoadhesive properties showing significant deeper penetration of the mucosa. The results indicated that mucoadhesive cationic polymethacrylate NPs offer a possible approach for the buccal delivery of heparin.



## 1. Introduction

Recently, the buccal cavity has gained great attention as a route of administration for the systemic delivery of macromolecular drug cargos. It offers several advantages such as the circumvention of the gastrointestinal tract and the hepatic clearance, rapid cellular recovery, high vascularization, and the ease of administration. Unfortunately, buccal drug delivery has also some limitations related to the barrier properties of the buccal mucosa, salivary scavenging and the risk of accidental swallowing of the delivery system used (Patel et al., 2011; Sudhakar et al., 2006). In addition, issues such as low lipophilicity and large molecular size that limit the absorption of macromolecular drugs from the buccal mucosa have raised challenges hitherto unresolved. An ideal buccal drug delivery system would provide and maintain an intimate contact with the buccal mucosa for sufficient time periods with a permeation enhancing ability and stabilizing or protective properties for the delivered macromolecule. Several strategies have therefore been suggested to the design and development of new carrier systems or adjuvants to improve the buccal delivery of macromolecules. Examples include the use of chemical permeation enhancers (Aungst and Rogers, 1989; Sandri et al., 2005), enzyme inhibitors (Langoth et al., 2005), the use of mucoadhesive materials as adjuvants (Ayensu et al., 2012a, 2012b) and the design and development of the mucoadhesive nanoparticulate carriers with longer residence times and potential higher bioavailability (Venugopalan et al., 2001).

Polymethacrylates have been considered as one of these promising cationic polymeric carriers, which were widely investigated for their feasibility as a vehicle for buccal delivery. They have been used to sustain and control the release of drug from the administered dosage forms. Polymethacrylate has been also

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successfully uses as a film-forming rate-controlling mucoadhesive polymer (Wong et al., 1999; Morales et al., 2013).

Low molecular weight heparin is widely accepted as a potent anticoagulant in the treatment of postsurgical thrombosis (Haas and Blümel, 1989) and various thrombotic disorders (Freedman, 1991). Currently, its administration is limited to parenteral routes, given its low permeability to absorption barriers and its vulnerability to enzymatic degradation in the gastrointestinal tract (Goldberg and Gomez-Orellana, 2003). The invasiveness of the injection form is undoubtedly a disadvantage, particularly when long-term outpatient therapy is required. Therefore, alternative routes of administration of more convenient, efficient and economical nature are highly desirable.

Different kinds of particles loaded with heparin have been prepared for the enhancement of the oral delivery of heparin. Examples include chitosan nanoparticles and gelatin microparticles prepared by complex coacervation techniques (Paliwal et al., 2012; Sun et al., 2008; Lamprecht et al., 2007). In addition, poly(lactic-co-glycolic acid) (PLGA), poly-(ε-caprolactone) and polymethacrylate nanoparticles have been successfully used for heparin encapsulation and promising in vitro and in vivo results have been obtained (Jiao et al., 2002b; Hoffart et al., 2006). Microparticles (Jiao et al., 2002a; Viehof and Lamprecht, 2013) loaded with LMWH have shown also high potential to enhance the oral bioavailability of heparin.

Based on these encouraging results and the recently observed mucoadhesive characteristics (Lamprecht et al., 2006), polymethacrylate NPs seem to be promising as a biocompatible drug carrier for buccal delivery.

Here, polymethacrylate NPs were tested for their potential to deliver LMWH across the buccal epithelium and to obtain first insights in understanding the mechanism of heparin translocation across the buccal mucosa.

## 2. Materials and methods

### 2.1. Materials

Polymethacrylate (Eudragit® RS PO and Eudragit® RL PO) were kindly supplied by Evonik Röhm GmbH (Darmstadt, Germany). Ethyl cellulose (Ethocel standard 4 premium) was supplied by Colorcon (Dartford, England). Low molecular weight heparin (enoxaparin, 4000 IU anti-Xa/0.4 mL injectable solution) was purchased commercially from the respective marketed products. Lyophilized mucin from porcine stomach, N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride, Nile red and fluoresceinamine were purchased from Sigma Aldrich Chemie GmbH (Steinheim, Germany). All other chemicals were of analytical grade or equivalent purity.

### 2.2. Preparation of LMWH nanoparticles

The preparation of LMWH-loaded NPs was based on an emulsification-solvent diffusion technique. Briefly, 0.250 g of polymethacrylate was dissolved in 10 mL ethyl acetate; 4000 IU of LMWH solution (≈40 mg) was then added to the polymer solution and homogenized. To prepare fluorescently labeled NPs, 100 μL of Nile red solution in ethyl acetate (1 mg/mL) was added to the organic polymer solution prior to homogenization. The mixture was then emulsified with the aqueous phase (10 mL of 0.1%w/v PVA solution saturated with ethyl acetate) using the homogenizer (T 18 Ultra Turrax®, IKA®, Staufen, Germany) at 22,000 rpm for one minute, the resulting emulsion was poured quickly into another solution containing PVA 0.01% w/v to presolidify the emulsion droplets. After evaporation of ethyl acetate under reduced pressure in a Buchi Rotavapor RE 120 (Buchi, Flawil, Switzerland) with reducing the pressure stepwise down to 40 mbar by means of a diaphragm pump, the formulated NPs were recovered by centrifugation of the sample at 15,000 rpm for 30 min. Finally the NPs were washed with deionized water and dried by lyophilization. Drug-free polymethacrylate nanoparticles and ethyl cellulose NPs were fabricated by the same procedure omitting the LMWH solution.

### 2.3. Fluorescent labeling of LMWH molecules

Fluorescently labeled LMWH was prepared using fluoresceinamine in order to permit its detection by confocal microscope. The labeling protocol was adapted to a method previously described by (Lamprecht et al., 2006; Raman et al., 2013): In brief, an aqueous LMWH solution (1 mL, 0.023 mM, pH 5.6) was incubated with carbodiimide (300 μL, 0.034 mM) at room temperature for 4 h. The activated LMWH solution was then mixed with fluoresceinamine (300 μL, 0.086 mM), and incubated overnight. Free unreacting marker and linker were removed by dialysis (Spectrapor® 7, Spectrum Ltd., USA; membrane pore size: 1000 Da) against distilled water until no diffusion of the fluorescence was detected. Purified fluoresceinamine-LMWH solution was then lyophilized.

### 2.4. Scanning electron microscopy (SEM)

Scanning electron micrographs of the NPs were undertaken using SEM (S-2460N Hitachi, Japan). One drop of the NPs suspension was finely spread over a cover slip and allowed to dry overnight in a desiccator. Afterwards, the samples were coated with gold using a Polaron Sputter Coater (SC 7640, Quorum Technologies Ltd., UK) for 3 min and imaged with SEM. The samples were investigated at 20 kV acceleration voltages.

### 2.5. In vitro LMWH release

The in vitro release study was conducted on the lyophilized nanoparticles. The lyophilization of NPs suspension was performed using a lyophilizer (STERIS Lyovac GT2, Hürth, Germany). 5 mL of concentrated LMWH-loaded NPs suspension filled into a vial for injection and partially stoppered using parafilm. The freezing step was carried out in a shelf refrigerator at −30 °C for 24 h. The frozen suspension was then transferred to lyophilizer and subsequently dried by a standard procedure for at least 48 h.

Lyophilized NPs (50 mg) were suspended in 20 mL of PBS (pH 7.4). Specific amount of lyophilized mucin from porcine stomach was added to the dissolution medium to mimic the effect of mucin on the release of LMWH from the NPs. The NPs suspensions in presence or absence of mucin were gently stirred (250 rpm) at 37 °C in a water bath. At various time intervals, 1 mL samples were withdrawn and centrifuged for 30 min at 15,000 rpm. The supernatant was recovered and the biological activity of LMWH released was determined by using of ACTICHROME® Heparin (anti-Factor Xa) Kit from American diagnostic GmbH (Teien and Lie, 1977). Briefly, Antithrombin-III solution (200 μL) was mixed with 25 μL of heparin sample followed by 200 μL factor Xa and incubated for 1 min at 37 °C. 200 μL of chromogenic peptide substrate were then added and incubated for 5 min. The absorbance of the solution was measured spectrophotometrically at 405 nm. Results were analyzed using a standard calibration curve in the range of 0–0.6 IU/mL.

### 2.6. Determination of nanoparticle size, zeta potential and entrapment efficiency

The particles were analyzed for their particle size and size distribution in terms of the average volume diameters and