International Journal of Pharmaceutics 507 (2016) 12–20

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Skin penetration and dermal tolerability of acrylic nanocapsules: Influence of the surface charge and a chitosan gel used as vehicle

R.V. Contri[a], L.A. Fiel[a], N. Alnasif[b], A.R. Pohlmann[a,c], S.S. Guterres[a], M. Schäfer-Korting[b,*]

[a] Programa de Pós-Graduação em Ciências Farmacêuticas, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil
[b] Institut für Pharmazie (Pharmakologie und Toxikologie), Freie Universität Berlin, Berlin, Germany
[c] Instituto de Química, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil

ARTICLE INFO

Article history:
Received 21 January 2016
Received in revised form 23 March 2016
Accepted 24 March 2016
Available online 26 April 2016

Keywords:
Chitosan
Cytotoxicity
Irritancy
Nanocapsules
Polymer
Skin penetration

ABSTRACT

For an improved understanding of the relevant particle features for cutaneous use, we studied the effect of the surface charge of acrylic nanocapsules (around 150 nm) and the effect of a chitosan gel vehicle on the particle penetration into normal and stripped human skin *ex vivo* as well as local tolerability (cytotoxicity and irritancy). Rhodamin-tagged nanocapsules penetrated and remained in the *stratum corneum*. Penetration of cationic nanocapsules exceeded the penetration of anionic nanocapsules. When applied on stripped skin, however, the fluorescence was also recorded in the viable epidermis and dermis. Cationic surface charge and embedding the particles into chitosan gel favored access to deeper skin. Keratinocytes took up the nanocapsules rapidly. Cytotoxicity (viability $< 80\%$), following exposure for ≥ 24 h, appears to be due to the surfactant polysorbate 80, used for nanocapsules' stabilization. Uptake by fibroblasts was low and no cytotoxicity was observed. No irritant reactions were detected in the HET-CAM test. In conclusion, the surface charge and chitosan vehicle, as well as the skin barrier integrity, influence the skin penetration of acrylic nanocapsules. Particle localization in the intact *stratum corneum* of normal skin and good tolerability make the nanocapsules candidates for topical use on the skin, provided that the polymer wall allows the release of the active encapsulated substance.

1. Introduction

Several advantages render nanoparticulate carrier systems into an interesting platform for cutaneous use. Nanoparticles can enhance the penetration of loaded agents into the skin (Korting and Schäfer-Korting, 2010; Neubert, 2011), decrease skin irritancy caused by a drug (Shah et al., 2007; Contri et al., 2014a) and reduce skin thinning induced by glucocorticoids (Fesq et al., 2003), e.g. by epidermal targeting (Santos Maia et al., 2002; Schlupp et al., 2012). Persistency of the active substance on the skin surface (Contri et al., 2014b), increased photostability of the loaded active (Perugini et al., 2002) and better sensorial properties of the formulation (Külkamp-Guerreiro et al., 2013) can be provided, too.

Nanocapsules are specific nanoparticles, which are composed by two distinct compartments, usually a polymeric wall surrounding an oily core (Mora-Huertas et al., 2010). The poly(ethylacrylate, methyl-methacrylate) copolymer containing ammonium quaternary groups (Eudragit RS100®) is used for the production of nanocapsules for cutaneous (Contri et al., 2014a; Contri et al., 2014b), ocular (Katzer et al., 2014) and vaginal (Frank et al., 2014) applications. The cationic charge favors the interaction with negative charged tissues and cell surfaces.

Yet the very low viscosity of aqueous nanocapsule suspensions impedes the dermal application and the applied product tends to run-off. The incorporation of nanocapsules into semisolid vehicles (Paese et al., 2009; Ourique et al., 2011; Külkamp-Guerreiro et al., 2012) improves spreadability and the time of contact between the particles and the skin. Eudragit RS 100® nanocapsules containing capsaicinoids and embedded into chitosan gel modulate skin penetration of the capsaicinoids while decreasing skin irritation in humans (Contri et al., 2014a; Contri et al., 2014b). Chitosan is reported to enhance skin penetration due to an interaction with epidermal tight junction proteins (Valenta and Auner, 2004), which form a penetration barrier in the *stratum granulosum*

Abbreviations: $NC_{Cationic}$, cationic nanocapsules; $NC_{Anionic}$, anionic nanocapsules; CH-$NC_{Cationic}$, chitosan gel with cationic nanocapsules; CH, chitosan gel; POL 80, polysorbate 80 solution.

* Corresponding author at: Institut für Pharmazie, Freie Universität Berlin, Königin-Luise-Strasse 2+4, D-14195 Berlin, Germany.
E-mail address: monika.schaefer-korting@fu-berlin.de (M. Schäfer-Korting).

http://dx.doi.org/10.1016/j.ijpharm.2016.03.046
0378-5173/

beneath the lipid barrier in the *stratum corneum* (Brandner et al., 2008).

Only recently adverse health effects caused by nanomaterials are systematically addressed (Elsaesser and Howard, 2012), e.g. surface charge and the size influence the cytotoxicity (Eidi et al., 2010). Having surmounted the *stratum corneum,* nanoparticles can interact with viable skin cells, e.g. disrupting membranes or enhancing the production of reactive oxygen species. Reaching the systemic circulation, the nanoparticles can also cause systemic adverse effects. Penetration is enhanced in barrier-disrupted skin (Alnasif et al., 2014; Davies et al., 2015), which is seen in many skin diseases.

Here we describe the skin penetration, cytotoxicity and irritation potential of cationic acrylic nanocapsules (polymer: Eudragit RS 100®) in aqueous suspension and when embedded in a chitosan gel. The effects are compared to anionic acrylic nanocapsules (polymer: Eudragit S 100®) in aqueous suspension. While the effect of the particle surface charge on the penetration of encapsulated substances has been studied (Wu et al., 2010), the present investigation focuses on the penetration of the particle itself and how the surface charge and the vehicle composed of chitosan may influence this process.

2. Materials and methods

2.1. Materials

Eudragit RS 100® and Eudragit S 100® were obtained from Degussa (Darmstadt, Germany). Polysorbate 80 and capric/caprylic triglycerides were purchased from Labsynth (São Paulo, Brazil) and Brasquim (Porto Alegre, Brazil), respectively. Chitosan (medium molecular weight, deacetylation degree 77%), Rhodamine B (molar mass = 479.02 g Mol^{-1}; log*P* = 1.95), 4-(*N*,*N*-dimethyl)aminopyridine and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride were purchased from Sigma-Aldrich (São Paulo, Brazil). Castor oil was obtained from Campestre (São Bernardo do Campo, Brazil). The other chemical substances were of highest quality.

2.2. Production of nanocapsule suspensions and embedding in chitosan gel

Suspensions of blank nanocapsules (cationic: $NC_{Cationic}$; anionic: $NC_{Anionic}$) were obtained by the nanoprecipitation method as described (Contri et al., 2014a; Contri et al., 2014b; Fiel et al., 2014). The polymer (100 mg), which composes the particle shell, and the oily component of the particle core (capric/caprylic triglycerides) were dissolved in 27 ml of acetone. Following mixing of such phase with the aqueous phase (76 mg polysorbate 80 dissolved in 53 ml of ultrapure water), the organic solvent and the excess of water was evaporated under vacuum for a final volume of 10 ml (Table 1).

For the synthesis of dye-labeled nanocapsules (Table 1), capric/caprylic triglycerides were in part replaced by the Rhodamine B-castor oil conjugate. The conjugate was obtained as previously described (Fiel et al., 2014). Briefly, the carboxyl group of the fluorescent probe was activated using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride in the presence of 4-(*N*,*N*-dimethyl)aminopyridine, and then the intermediate was coupled with the hydroxyl function of the vegetable oil. The fluorescent product was characterized by thin layer chromatography, Proton nuclear magnetic resonance ^{1}H MNR, Fourier transform infrared (FTIR) spectroscopy, UV–vis and fluorescence spectroscopy.

The chitosan gel containing nanocapsules (CH-$NC_{Cationic}$) was prepared by manual mixing of chitosan (2.5%) and nanocasule aqueous suspension at room temperature, in total substitution of the water. Then, lactic acid (1%) was added, followed by manual mixing until the gel was formed (Contri et al., 2014b). Based on a previous published work on the topic (Contri et al., 2014c), the concentration of nanocapsules in the gel formulation is 2.7×10^{13} nanocapsules per gram.

2.3. Characterization of nanocapsule suspension

The nanocapsule suspensions were characterized in terms of average diameter and polydispersity index (1:500 v/v in ultrapure water) by dynamic light scattering (Zetasizer Nano ZS, Malvern Instruments, Worcestershire, UK) and in terms of zeta potential (500 v/v in NaCl 10 mM solution) by electrophoretic mobility (Zetasizer Nano ZS, Malvern Instruments). In addition, the pH was measured by potentiometry (B474, Micronal, São Paulo, Brazil). Fluorescence reading of Rhodamine B-labelled nanocapsules was at 605 nm with excitation at 540 nm (Varian Cary® 100, Agilent, Santa Clara, USA).

To confirm the integrity of the nanocapsules under experimental conditions, possible release of the fluorescent oil was studied by dialysis. One milliliter of each sample (nanocapsule formulations or fluorescent oil solubilized in 5% polysorbate 80 solution) in dialysis bags (Sigma Aldrich, São Paulo, Brazil, cut off 12,000 to 14,000 gMol^{-1}) were dialyzed against 80 mL of 5% polysorbate 80 solution for 48 h under magnetic stirring at 32 °C. The fluorescence intensity inside the dialysis bag and the nanocapsule average diameter were measured, as described above for the nanocapsule suspension, before and after dialysis.

2.4. Evaluation of nanocapsule penetration into normal and stripped human skin

Penetration was studied using cryoconserved human abdominal skin (thickness, 1 mm) of female and male donors (with permission) using a validated approach (Schäfer-Korting et al., 2008) based on the OECD guideline 428. All comparisons were made using skin of the same donor. On the day of use, the skin was thawed. For the stripped skin, the *stratum corneum* was almost completely removed by 30 tape strips (Tesa® 4124, Hamburg, Germany). Efficiency of tape-stripping was proven by measuring the thickness of the remaining *stratum corneum* following hematoxylin-eosin staining (Alnasif et al., 2014). Punched normal and stripped skin was mounted to Franz cells (PermeGear, Bethlehem, PA, USA). The test area was of about 0.2 cm^2. The receptor fluid (12 mL) was composed of phosphate buffered saline

Table 1
Components of the final nanocapsule suspensions (per 10 mL).

Formulations/Components	$NC_{Cationic}$	$NC_{Anionic}$	$NC_{Cationic}$ (Fluorescent)	$NC_{Anionic}$ (Fluorescent)
Eudragit RS 100®	100 mg	–	100 mg	–
Eudragit S 100®	–	100 mg	–	100 mg
Capric/Caprylic Triglycerides	330 μL	165 μL	247.5 μL	82.5 μL
Fluorescent Oil	–	–	82.5 μL	82.5 μL
Polysorbate 80	76 mg	76 mg	76 mg	76 mg