Skin permeation of hydrophobic drugs loaded into polymeric nanoparticles

V.A. Feitosa; T.S. Balogh; K.L. Guimarães; M.H.A. Zanin; A.M. Oliveira; N.N.P. Cerize

Laboratory of Chemical Processes and Particle Technology, BioNanoManufacturing Center, Institute for Technological Rescarch (LPP/Bionano/IPT), São Paulo, Brazil
ncerize@ipt.br

ABSTRACT

Polymeric nanoparticles have been employed for topical pharmaceutical applications due to its capacity to carry drugs across the skin. Biocompatible polymers are extensively applied as coating nanoparticles allowing them to present controlled physical and chemical characteristics. The proposal of this work was to evaluate the skin permeation of polymeric nanoparticles loading hydrophobic drugs. The emulsion/diffusion process was applied to produce nanocapsules composed by poly(ε-caprolactone, PCL) and PEO-PPO-PEO triblock copolymers (Pluronic®), this process promotes the nanoencapsulation of drugs into oil core from pre-formed polymer precipitation. The organic phase was comprised of polymers (Pluronic F127 and PCL), oil, Nile red fluorophore, and hydrocortisone acetate as hydrophobic drug model, solubilized in ethyl acetate. The aqueous phase was comprised of distilled water saturated with ethyl acetate and Tween 20 surfactant. The organic phase was transferred into the aqueous phase under ultrahigh agitation (7,000 rpm), the emulsion formed was immediately transferred to a stirred reactor, the dilution phase (water and Tween® 20) was added and then the solvent was extracted under vacuum pressure. The *in vitro* skin permeation of nanoparticles containing the fluorophore Nile red was evaluated by a vertical diffusion cell employing pig ear skin. The study covered the steps of preparation of animal membrane; monitoring the permeation into vertical diffusion vessel (Franz cell) up to 8 hours; preparation of histological sections into cryomicrotome; and visualization of fluorescence by confocal laser microscopy. It was possible to verify the location of polymeric nanoparticles containing the Nile red fluorophore encapsulated in presence or absence of hydrocortisone drug. It was observed that after two hours, the permeation of the particles was very low. However, for a more prolonged exposure (8 hours) was observed the deposition of the particles in the outer layers of the skin (stratum corneum and epidermis) as well as in hair follicles. These data show that the produced nanoparticles perform the delivery of the drug in the superficial skin layers, fulfilling the role of topical application.

Keywords: Nanotechnology, drug delivery, nanocapsules, topical administration.

1 BACKGROUND

The skin is an alternative route for delivering drugs [1]. Topical and transdermal drug delivery systems are gaining increasing popularity and several drugs have been successfully delivered into skin for local and/or systemic action. [2]. The skin drug delivery is advantageous in that it can not only concentrate drug molecules in a specific skin area but also can reduce unexpected side effects [1].

However, one the major limitations of skin drug administration is the penetration barrier provided by the protective stratum corneum, composed of keratin-rich corneocytes and an intercellular matrix of a unique composition of lipids [3].

The increasing progress of nanotechnology in cosmetic and pharmaceutical fields allow the development of several formulations designed to overcome stratum corneum barrier [4]. Nanocarrier systems can improve dermal penetration, including vesicular delivery systems (*e.g.* liposomes, polymersomes, transfersomes, ethosomes, and niosomes), nanostructured lipid carriers (*e.g.* solid lipid nanoparticles, lipid nanocapsules, nanoemulsions, and solid lipid nanoparticles), and polymeric-based particles (*e.g.* nanospheres and nanocapsules) [1,3,5].

Synthetic, semi-synthetic and natural polymers have been explored for nanoparticle production. Increased attention has been paid to biocompatible and biodegradable polymers, including poly(lactide acid, PLA, poly(lactide-co-glycolide acid, PLGA), poly(ε-caprolactone, PCL) as well as biocompatible amphiphilic triblock copolymers poloxamers, composed of poly(ethylene oxide, PEO) and poly(propylene oxide, PPO), PEO-PPO-PEO, commercially available under the trade name Pluronic®.

In order to determine the drug delivery of polymeric nanostructures into skin, we investigate the skin penetration of hydrophobics molecules loaded with colloidal nanoparticles based on PCL and Pluronic polymers. Employing confocal laser scanning microscopy, we demonstrated that a colloidal polymeric nanocarrier system can significantly improve the specific topical delivery of hydrophobic compounds only through the stratum corneum and epidermis, but not into dermal skin layer.

2 EXPERIMENTAL

Polymeric nanoparticles (NP) were prepared by the emulsion/diffusion/extraction solvent technic, previously described [6] with some modifications. An organic phase (10 mL) containing ethyl acetate, 2% PCL 10 kDa, 2% Pluronic®F127, 5% oil (Miglyol®810, caprylic capric triglycerides TGCC; or Dhaytan IDB®, isobutyl benzoate), 0.5% hydrocortisone acetate drug, and 0.25 % Nile red was homogenized under ultrahigh agitation (7,000 rpm) into an aqueous solution (40 mL) saturated with ethyl acetate and 1% Tween®20 surfactant. The aqueous phase immediately turned milky due to the PCL precipitation. The emulsion formed was immediately transferred to a stirred reactor was added the dilution phase (water and 1% Tween®20). The ethyl acetate was then extracted under reduced pressure at 50 °C for approximately 45 min.

Dynamic Light Scattering (DLS) was employed to measure particles size and polydispersity (PdI) distribution. Scanning Electron Microscopy (SEM) was used to obtain particle morphology.

The *in vitro* skin permeation of nanoparticles containing the fluorophore Nile red was evaluated by a vertical diffusion cell employing pig ear skin. The formulations as centrifuged (10.000 rpm, 30 min) before use to remove unloaded Nile red. The study covered the steps of (*i*) preparation of animal skin (contact area of 1,54 cm^2); (*ii*) monitoring the permeation into vertical diffusion vessel (Franz cell) up to 8 hours, at 37 ºC, using Phosphate Buffered Saline (PBS) as acceptor solution; (*iii*) preparation of histological sections (10-20 µm) into a cryomicrotome at -20 ºC ; (*iv*) and visualization of fluorescence by Confocal Laser Scanning Microscopy (CLSM), with excitation at 488 nm and emission at 555 nm.

3 RESULTS

The polymeric nanoparticles showed uniform and spherical morphology (Figure 1), the size of 315 and 465 nm and Pdi of 0,27 and 0,53, for Nile red- and Nile red+hydrocortisone acetate-loaded NP, respectively.

According to the skin permeation study (Figure 2A), was found the Nile red unencapsulated showed sharp penetration through the skin, probably because of its high interaction with the skin lipids [7].

For Nile red-loaded NP, it was observed that after two hours, the skin permeation of the particles was very low (Figure 2B). However, for a more prolonged exposure time (8 hours) was observed the deposition of the nanoparticles in the outer layers of the skin (stratum corneum and epidermis) as well as in hair follicles (Figure 2C).

There was no change in the permeation profile of formulations with or without hydrocortisone acetate (Figure 2D and E), showing that the presence of drug did not influence the performance of nanoparticles.

In conclusion, the colloidal polymeric nanoparticles fulfilling the role of topical application, therefore, suitable for delivery of drugs acting on the epidermis, for example, anti-inflammatory drugs for skin infections treatment.

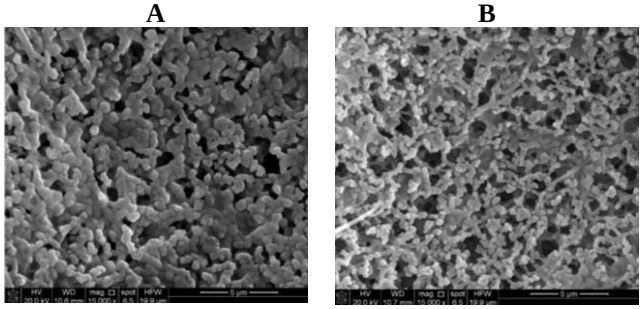

Figure 1. SEM morphology of colloidal nanoparticles (NP). **A.** Nile red-loaded NP (prepared with TGCC oil). **B.** The Nile red+hydrocortisone acetate-loaded NP (prepared with IDB oil).

ACKNOWLEDGMENT

The authors thank the FINEP (Financiadora de Estudos e Projetos), Brazil and CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico), Brazil, for research funding.

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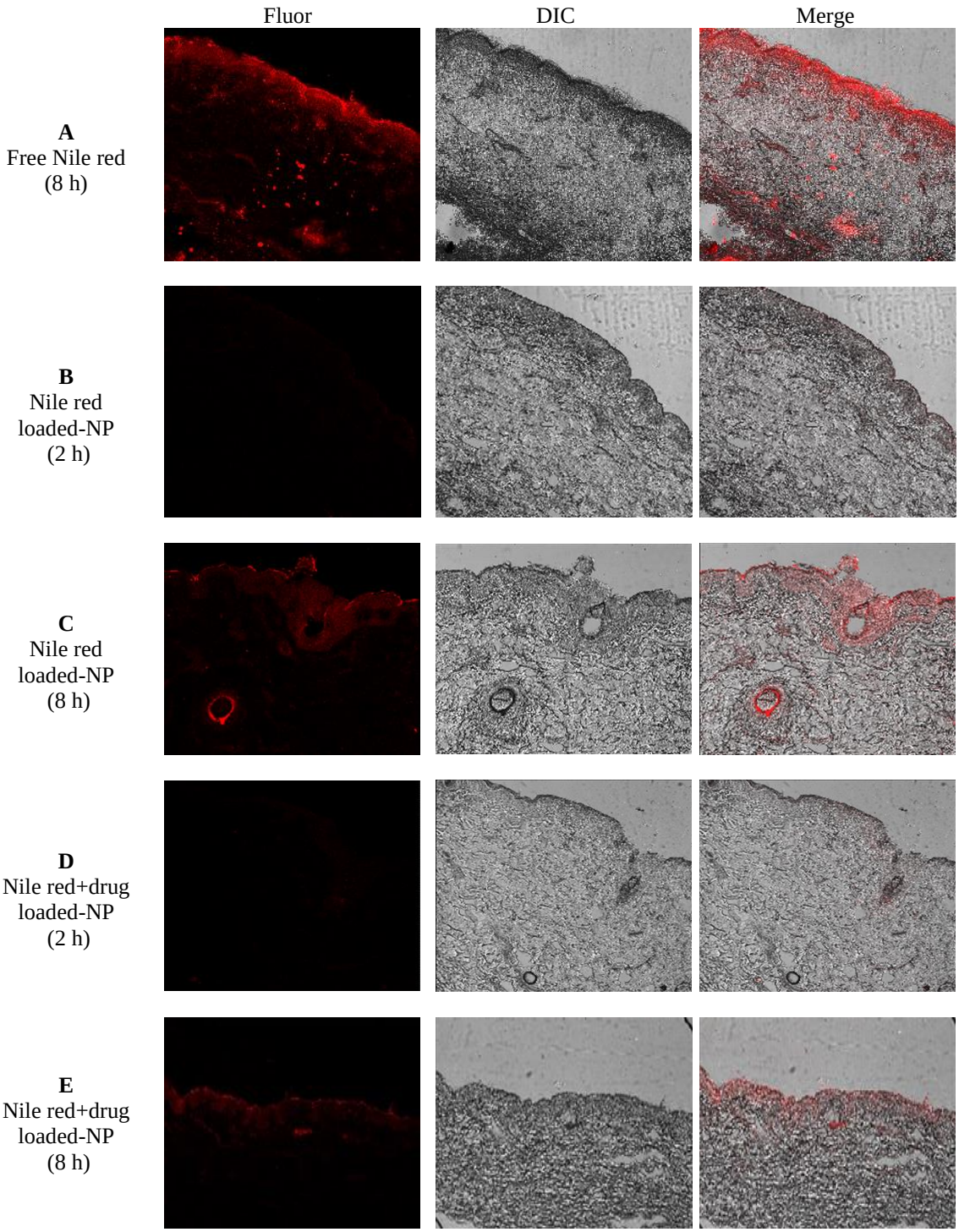

Figure 2. Confocal laser scanning microscopy of free Nile red and polymeric nanoparticles permeation across pig skin at 2 or 8 hours. Fluor: fluorescence, DIC: differential interference contrast. NP: nanoparticles, Drug: hydrocortisone acetate. Free Nile red was prepared by direct dilution into propylene glycol.