

Amphiphilic poly{[α -maleic anhydride- ω -methoxy-poly(ethylene glycol)]-co-(ethyl cyanoacrylate)} graft copolymer nanoparticles as carriers for transdermal drug delivery

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Abstract: In this study, the transdermal drug delivery properties of D,L-tetrahydropalmitine (THP)-loaded amphiphilic poly{[α -maleic anhydride- ω -methoxy-poly(ethylene glycol)]-co-(ethyl cyanoacrylate)} (PEGECA) graft copolymer nanoparticles (PEGECAT NPs) were evaluated by skin penetration experiments *in vitro*. The transdermal permeation experiments *in vitro* were carried out in Franz diffusion cells using THP-loaded PEGECAT NPs as the donor system. Transmission electron microscopy and Fourier transform infrared spectroscopy were used to characterize the receptor fluid. The results indicate that the THP-loaded PEGECAT NPs are able to penetrate the rat skin. Fluorescent microscopy measurements demonstrate that THP-loaded PEGECAT NPs can penetrate the skin not only via appendage routes but also via epidermal routes. This nanotechnology has potential application in transdermal drug delivery.

Keywords: poly{[α -maleic anhydride- ω -methoxy-poly(ethylene glycol)]-co-(ethyl cyanoacrylate)}, nanoparticles, transdermal drug delivery, D,L-tetrahydropalmitine

Introduction

Transdermal drug delivery has many advantages such as high patient compliance, no first-pass metabolism of drugs, and controlled delivery over long time periods compared to oral delivery or injection. However, the outer layer of skin, called stratum corneum with 10–15 μ m multilayered wall-like structure, has impressive barrier properties and provides an excellent barrier between the external environment and internal body, which causes difficulties for drug delivery through the skin.^{1–3} Many methods have been developed to enhance percutaneous absorption of therapeutic agents.^{4–6} Chemical enhancers with high affinity with skin lipids that can change the structure of stratum corneum physically or chemically^{5,7} and delivery devices that can penetrate stratum corneum^{4,8} have been widely used. However, chemical enhancers and delivery devices usually cause skin damage and weaken the function of the skin barrier.

Delivery carriers such as micelles,^{9,10} liposomes,¹¹ solid lipid nanoparticles (NPs),^{12,13} polymer and dendrimer NPs^{14,15} and inorganic NPs⁶ have been developed to improve percutaneous absorption of therapeutic agents while alleviating the damage to the skin. Due to the flexible modification of polymer structure and easy production, polymer NPs have potential applications in improving the transport efficiency of drugs and biomacromolecules through the skin. Cui and Mumper prepared DNA-loaded chitosan NPs and applied them to shaved rat skin, concluding that chitosan-based NPs

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containing DNA resulted in both detectable and quantifiable levels of luciferase expression in rat skin 24 hours after topical application.¹⁶

Recently, amphiphilic copolymer NPs were studied for transdermal drug delivery. Shim and colleagues reported that the permeation of minoxidil incorporated in 40 nm NPs of poly(ϵ -caprolactone)-block-poly(ethylene glycol) (PCL-b-PEG) was 1.5-fold higher in the epidermal layer and 1.7-fold higher in the receptor solution than that of 130 nm NPs of PCL-b-PEG when applied onto the skin of hairy pigs.¹⁷ In our lab, transdermal drug delivery properties of methoxy poly(ethylene glycol)-block-poly(D,L-lactic acid) (PEG-b-PDLLA) NPs by using paclitaxel as a model drug have been carefully studied. The PEG-b-PDLLA NPs can penetrate rat skin as integrated particles and efficiently transport paclitaxel through the skin. Previous reports indicate that amphiphilic copolymer NPs are effective carriers for transdermal drug delivery.¹⁸ Previously, we reported that the preparation of poly{[α -maleic anhydride- ω -methoxy-poly(ethylene glycol)]-co-(ethyl cyanoacrylate)} (PEGECA) graft copolymer and *in vitro* release property of D,L-tetrahydropalmatine (THP)-loaded PEGECA graft copolymer NPs (PEGECAT NPs).^{19,20} THP can be encapsulated into the hydrophobic core of PEGECAT NPs and the accumulated release is lower than 40% after 48 hours. Whether PEGECAT NPs encapsulating drug can effectively deliver drug through the skin or not has not been investigated. In this study, we research the *in vitro* transdermal permeation behavior of THP-loaded PEGECAT NPs by using THP as a model drug.

Materials and methods

Materials

Poly{[α -maleic anhydride- ω -methoxy-poly(ethylene glycol)]-co-(ethyl cyanoacrylate)} (PEGECA) graft copolymer ($M_n = 11600$, $M_{nPECA} = 6600$, $M_{nPEG} = 5000$) was prepared as described previously.¹⁹ D,L-tetrahydropalmatine (THP) was supplied by Tongrentang Co. (Tianjin, China). Dichloromethane (DCM) and acetone were purchased from the Tianjin Chemical Reagent Co (Tianjin, China). All reagents were analytical grade and used as received.

Preparation of THP-loaded PEGECAT NPs

THP-loaded PEGECAT NPs were prepared by nanoprecipitation technique.²⁰ Briefly, the drug was dissolved in PEGECAT solution in acetone, ie, 3 mg THP dissolved in 2 ml PEGECAT acetone solution at 50 mg/ml concentration and then the mixture solution was added into 10 ml

distilled water under magnetic stirring at ambient temperature and acetone was allowed to evaporate completely under agitation. The obtained dispersion was then centrifuged by a centrifuge (LD5-2A, Beijing, China) at 3000 rpm for 30 min in order to eliminate the aggregated particles. The supernatant was PEGECAT NPs dispersion, which was directly used for transmission electron microscopy (TEM) and particle size distribution. The supernatant could also be frozen and lyophilized by a freeze-dryer system (LGJ-10, Beijing, China) to obtain PEGECAT NPs freeze-dried powder, which can disperse into water to form NPs dispersions.

Characterization of THP-loaded PEGECAT NPs

The TEM specimens for the THP-loaded PEGECAT NPs dispersions were observed under a JEM-100CX II instrument. The samples were prepared by adding a drop of the PEGECAT NPs dispersion on the Formvar-coated copper TEM grid, and then dyed by phosphotungstic acid. The size and distribution of PEGECAT NPs were determined by BI-90Plus laser particle size analyzer (LPSA; Brookhaven Instruments, Holtsville, NY, USA). For all cases, λ of measurement was 678 nm, the angle of measurement was 90°, and the temperature of measurement was 25 °C.

In vitro permeation studies

Skin samples were obtained from adult Kunming rat (Tianjin Institute of Pharmaceutical Research, Tianjin, China). The subcutaneous fat tissue was removed before experiments. *In vitro* release was carried out at 37 °C with Franz diffusion cells (1 cm in diameter and 6 ml in receptor cell volume; Shishin Technology Co. Ltd, China). The receptor compartments were filled with phosphate-buffered saline (PBS; pH = 7.4), containing 0.02% w/v of sodium azide to retard microbial growth, and the receptor phase was stirred with small magnetic beads to mix the contents uniformly. The skin pieces were carefully mounted onto the receiver compartment of the diffusion cells with the stratum corneum facing in the direction of the donor compartment. Subsequently, 750 μ l of donor solution was applied to the stratum corneum side in the donor compartment. Two kinds of donor phase used, each containing 6.19% THP, were supersaturated water solution of THP and THP-loaded PEGECAT NPs aqueous dispersion, respectively. After application of donor solution, 1 ml of sample was withdrawn from receptor compartments at the appropriate time intervals and the same amount of fresh PBS was supplemented into each receptor. Acetonitrile (1 ml) was added to the sample to dissolve both copolymer and THP fully

and the amount of THP in the sample was determined by high-pressure liquid chromatography (HPLC; Agilent1100, Agilent Co., USA) using a mobile phase of methanol, 7% acetic acid and 4% acetic acid ammonium (v/v/v, 40:31:29) at 1 ml/min with a Hypersil ODS-2 (250 × 4.6 mm) C18 column.²⁰ The receptor solution was collected after the *in vitro* experiments and freeze-dried. The lyophilized powder was pressed into KBr pellets and analyzed using Fourier transform infrared (FTIR) spectroscopy (FT3000, Bio-Rad Laboratories, Hercules, CA, USA). Morphological evaluation and size of the PEGECAT NPs in receptor solution were performed using TEM and LPSA, respectively.

Fluorescence microscopy

Pyrene-loaded PEGECAT NPs were also prepared by nanoprecipitation technique.²⁰ The *in vitro* diffusion experiment was carried out according to the method mentioned above using an aqueous dispersion of pyrene-loaded PEGECAT NPs as donor phase. After the application period of 24 hours, excess dispersion was washed from the skin surface and the skin was removed and washed carefully with distilled water. Each specimen was frozen in liquid nitrogen. The specimens were subsequently sectioned vertically using a freeze-microtome (CM 1850; Leica, Solms, Germany) and observed using fluorescence microscopy (DM RXA2; Leica).

Results and discussion

Characterization of THP-loaded PEGECAT NPs and receptor fluid

Before the transdermal permeation experiments, the particle size and morphology of THP-loaded PEGECAT NPs were

characterized using TEM and LPSA as shown in Figure 1. THP-loaded PEGECAT NPs present spherical morphology as shown in Figure 1a. Figure 1b shows that the mean particle size of the THP-loaded PEGECAT NPs before release is less than 100 nm. After the permeation experiment of THP-loaded PEGECAT NPs dispersion for 24 hours, the receptor fluid was directly detected using LPSA and TEM. The integrated particles observed in Figure 2a indicate that THP-loaded PEGECAT NPs exist in the receptor compartment. It can be seen from Figure 2b that the particle size of the THP-loaded PEGECAT NPs in the receptor solution is larger than that of those in the donor, which might be due to the adsorption of protein or lipid in the skin. The receptor solution of the permeation experiment of THP-loaded PEGECAT NPs dispersion was collected and lyophilized into powder for FTIR analyses. It can be found from FTIR spectrum as shown in Figure 3 that a methylene-characteristic band, a carbonyl band, a C–O stretching band, a C=N stretching band, and a C–O–C stretching band appear at 2929 cm⁻¹, 1674 cm⁻¹, 1128 cm⁻¹, 2365 cm⁻¹, and 1082 cm⁻¹, respectively. The FTIR result coupled with the TEM and LPSA results suggest that THP-loaded PEGECAT NPs can penetrate the rat skin.

In vitro permeation study

In order to evaluate the drug delivery ability of PEGECAT NPs, the skin permeation of THP from supersaturated water solution of THP and THP-loaded PEGECAT NPs aqueous dispersion was compared. No THP was detected in the receptor solution when supersaturated water solution of THP was used as the donor fluid within 50 hours. The result indicates

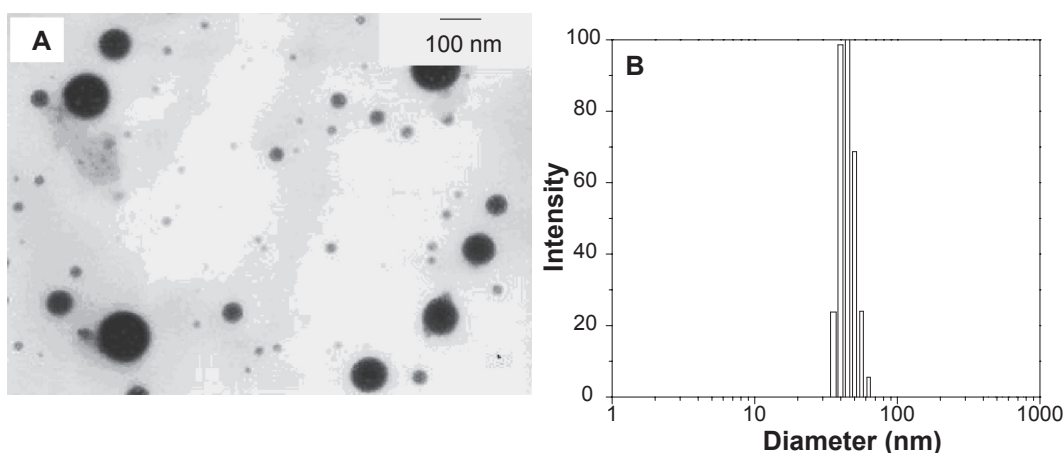


Figure 1 **A)** TEM image and **B)** size distribution of PEGECAT NPs loaded with 6.19% THP before permeation experiment *in vitro*.

Abbreviations: PEGECAT NPs, poly{[α -maleic anhydride- ω -methoxy-poly(ethylene glycol)]-co-(ethyl cyanoacrylate)} graft copolymer nanoparticles; TEM, transmission electron microscopy; THP, D,L-tetrahydropalmatine.

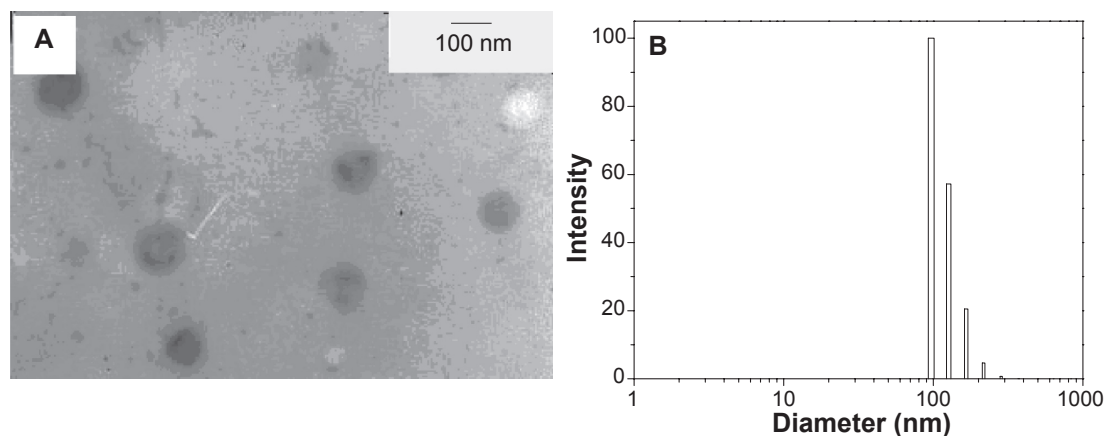


Figure 2 **A)** TEM image and **B)** size distribution of the particles in the receptor solution after 24 hours permeation experiment *in vitro*.

Abbreviation: TEM, transmission electron microscopy.

that THP itself is hard to permeate across the rat skin. However, THP was detected in the receptor solution when THP-loaded PEGECAT NPs aqueous dispersion was used as the donor fluid. It can be found from transdermal drug delivery kinetics of THP-loaded PEGECAT NPs as shown in Figure 4 that the rate of transdermal drug delivery is faster before 10 hours. The reason is that in the beginning stage NPs with small size can easily penetrate the skin. However, the stable channels for NPs with large size probably form after 10 hours due to the tardy interaction between NPs and stratum corneum.

Investigation on the permeation routes

To confirm the routes that PEGECAT NPs penetrate the skin, the lipophilic fluorescent probe pyrene-loaded NPs

were used to study skin penetration of PEGECAT NPs. It can be seen that the size of pyrene-loaded PEGECAT NPs is around 100 nm as shown in Figure 5a. An accumulation of fluorescence of pyrene-loaded PEGECAT NPs can be observed as shown in Figure 5b. Therefore, pyrene-loaded PEGECAT NPs can be used to investigate the permeation routes of PEGECAT NPs. Fluorescence micrographs presented in Figure 6 show the relative fluorescence intensity of the probe in the skin layers after applying an aqueous formulation of pyrene-loaded PEGECAT NPs to rat skin for 24 hours. An accumulation of fluorescence with even distribution can be clearly seen in both the upper layers of rat skin and deeper ones. Therefore, it can be concluded that PEGECAT NPs penetrate rat skin via appendage routes and epidermal routes.

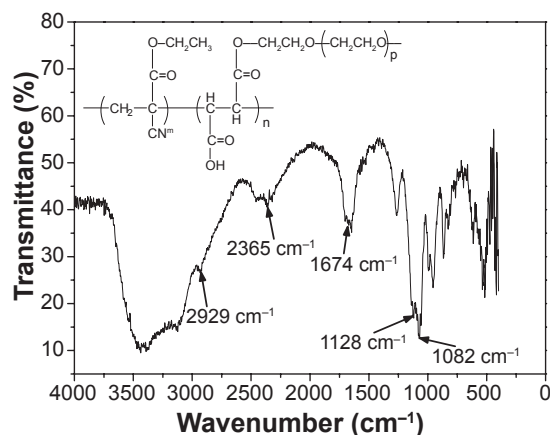


Figure 3 FTIR spectrum of lyophilized powder of the receptor fluid after *in vitro* skin permeation experiment applying PEGECAT NPs dispersion as the donor solution.

Abbreviations: FTIR, fourier transform infrared; PEGECAT NPs, poly{[α-maleic anhydride-ω-methoxy-poly(ethylene glycol)]-co-(ethyl cyanoacrylate)} graft copolymer nanoparticles.

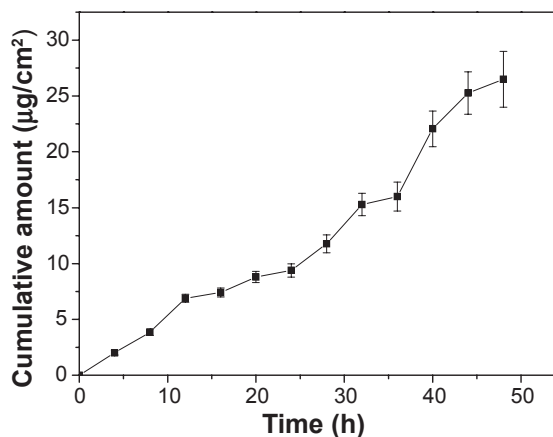


Figure 4 *In vitro* permeation profiles of THP from PEGECAT NPs dispersion through rat skin. Results are expressed as means ± SD of four different experiments.

Abbreviations: PEGECAT NPs, poly{[α-maleic anhydride-ω-methoxy-poly(ethylene glycol)]-co-(ethyl cyanoacrylate)} graft copolymer nanoparticles; SD, standard deviation; THP, D,L-tetrahydropalmatine.

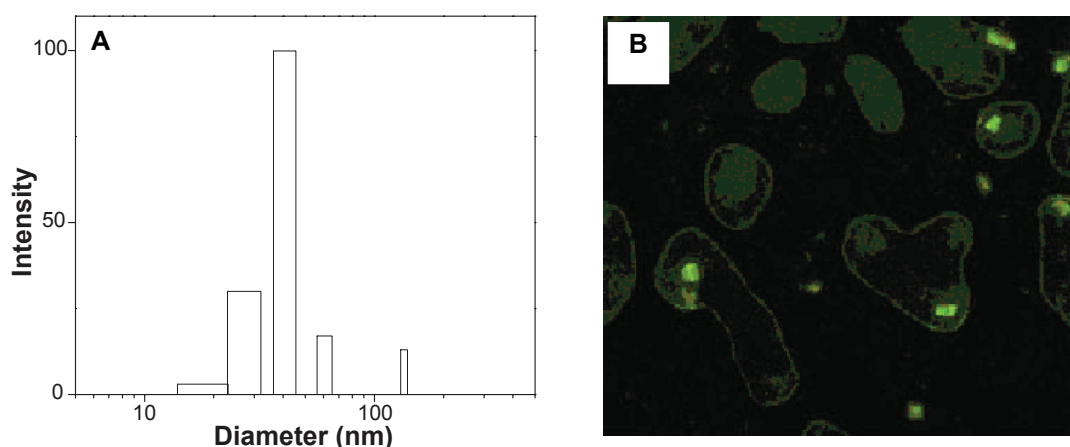


Figure 5 **A)** Particle size distribution and **B)** fluorescent micrograph of pyrene-entrapped NPs in aqueous dispersion.

Abbreviation: NPs, nanoparticles.

Conclusions

D,L-tetrahydropalmatine-loaded poly{[α -maleic anhydride- ω -methoxy-poly(ethylene glycol)]-co-(ethyl cyanoacrylate)} (PEGECA) graft copolymer nanoparticles (PEGECAT NPs) with the size of less than 100 nm were prepared. D,L-tetrahydropalmatine-loaded PEGECAT copolymer NPs can penetrate rat skin as integrated particles and efficiently deliver D,L-tetrahydropalmatine through the skin. The results of fluorescence microscopy demonstrate that the PEGECAT copolymer NPs penetrate the skin not only via appendage routes but also via epidermal routes.

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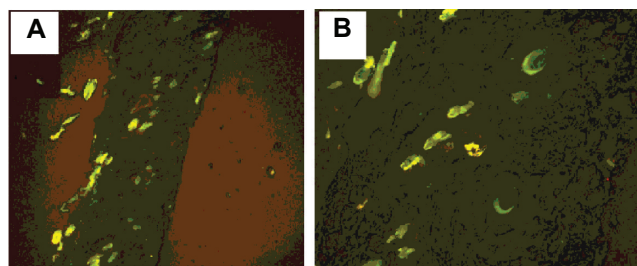


Figure 6 Fluorescence micrographs of vertical cross-sections of rat skin that was treated for 24 hours with pyrene-entrapped PEGECAT NPs dispersion (**a:** 50 $\times$, **b:** 100 $\times$).

Abbreviations: PEGECAT NPs, poly{[α -maleic anhydride- ω -methoxy-poly(ethylene glycol)]-co-(ethyl cyanoacrylate)} graft copolymer nanoparticles.

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