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Development of niacinamide loaded elastic liposome as a potential transepidermal delivery system

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ABSTRACT

Niacinamide (NA), one of the most frequently utilized materials in cosmeceutical areas, possesses a drawback in its ability to penetrate the skin layers. To overcome this, the present study developed and characterized novel NA-loaded elastic liposomes (EL) to enhance the NA transepidermal penetration. The EL were prepared by reverse phase evaporation technique with different NA content of 100, 150, and 200 mg. The developed NA-loaded EL demonstrated mean sizes of ~300 nm, polydispersity indexes of 0.2–0.3, zeta potentials of ~-20 mV, and moderate entrapment efficiencies of up to 40%. The liposome formation was confirmed by a Maltese cross-like structure, observed under polarized light microscopy, with a high elasticity property. Interestingly, the NA-loaded EL showed 100 times greater *in-vitro* permeation-enhancing effect than the NA cream counterpart. Conclusively, the novel NA-loaded EL have much potential in the transepidermal delivery of hydrophilic molecules, such as NA, across the skin.

INTRODUCTION

Niacinamide, (NA, nicotinamide, 3-pyridinecarboxamide), a derivative of niacin (vitamin B3) that acts as the precursor of the cofactors NA adenosine dinucleotide phosphate and NA adenosine dinucleotide (NAD), and possesses numerous functions in more than 35 cellular biochemical reactions in the skin. In fact, NA has been topically utilized in various medical and cosmeceutical applications including antioxidant [1], photo-immunosuppression prevention [2], acne post-inflammation treatment [3], collagen production enhancement [4], sebum production reduction [4], pigment reduction [5], andintercellular lipid synthesis increment [6]. NA is chemically stable to oxidation and photolysis, which is beneficial for its formula developments [7]. Moreover, compared to another biologically active form of niacin, nicotinic acid, NA does not activate skin flushing and variation in blood pressure and body temperature [8]. Therefore, NA is one of the best-investigated ingredients in cosmeceutical areas, especially for the skin anti-aging and whitening action.

Nevertheless, due to its inherent hydrophilicity, topical application of NA is limited, with poor skin penetration. The stratum corneum, the outermost layer of the skin, mainly consists of corneocytes and intercellular hydrophobic lipid lamellae, which prevent harmful substances from entering the body [9]. Unfortunately, these hydrophobic lipids also significantly limit the skin penetration of hydrophilic compounds [10]. In fact, the NA skin absorption is generally <3% of the total applied dose of 4 µg/cm^2 after 24 hours [11]. Consequently, although proving effectiveness on the enhanced NAD levels in skin cells, NA needs to be used in high dose, and thus, potentially causes side effects to the skin [12]. Therefore, a novel approach is necessary to improve the skin penetration of NA.

To this end, one potential approach is nanocarriers, since they have been progressively employed as delivery

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systems for numerous pharmaceutical and cosmetics compounds [13–17]. Among various types of nanocarriers, liposomes show much benefits for transepidermal delivery of NA, since they could effectively encapsulate both hydrophobic and hydrophilic compounds [18]. However, owing to the skin barrier, conventional liposomes are limited to lipophilic drugs with respective molecular weights of <500 Da. Thus, a more deformable type of liposome, called elastic liposomes (EL) has been formulated to increase the transepidermal delivery of hydrophilic drugs [19]. EL exhibits lipid bilayers, similar to the conventional liposomes, but with the inclusion of a compound known as edge activator to enhance the vesicle deformability. Thus, they are considered as a safe delivery system that can penetrate the skin integrity, while maintaining their intact vesicles via the hydrophilic channel in the epidermis lipid lamellar regions and shunt pathways [20]. Therefore, due to their excellent biocompatibility and deformability, EL could be a promising strategy to enhance transepidermal delivery of NA. To the best of our knowledge, only one study has been reported on this issue, however, the total NA cumulative amount permeated through the skin over 24 hours of the presented NA-loaded EL was only ~2.5 times higher than that of the 2% NA solution [9]. This value might not be sufficient to enhance NA skin absorption. Thus, novel systems are necessary.

Hence, this study developed and characterized novel NA-loaded EL, with high skin permeability as a potential transepidermal delivery system for hydrophilic compounds. In addition, the effects of different types of semi-permeable membrane used in *in-vitro* permeation studies were evaluated. The particles were developed using the reverse phase evaporation technique and physico-chemically characterized in terms of phase transition, mean particle size, zeta potential, drug entrapment efficiency (EE%), and particle deformability. Finally, *in-vitro* skin permeations of the formulations were conducted on both the porcine ear epidermis skin and synthetic polycarbonate artificial skin, using Franz diffusion cell.

MATERIALS AND METHODS

Materials

NA was bought from Phitsanuchemical (Phitsanulok, Thailand). Cholesterol (CH) was imported from Sigma Chemical (Steinheim, Germany). Lipoid S100-3 (Hydrogenated phosphatidylcholine, HPC) was imported from Lipoid GmbH Co., Ltd. (Ludwigshafen, Germany). Chloroform and ethanol were purchased from RCI Labscan (Bangkok, Thailand). All other utilized chemicals and solvents were analytical grade or higher. Polycarbonate membranes, diameter of 19 mm, with 0.4- and 0.1-µm pore size (Nuclepore™, Whatman®), were bought from Whatman International Ltd. (New York, USA).

Preparation of the blank EL

The blank EL (unloaded EL) was prepared by the reverse phase evaporation technique. To this end, the organic phase containing HPC, CH, and Span 80 (Table 1), was dissolved in an ethanol-chloroform mixture (5.3:1 v/v), followed by stirring (750 rpm) at 60ºC to form a clear solution. Then, deionized (DI) water (the aqueous phase) was added to the prepared organic phase to a final volume of 20 ml and stirred (750 rpm) at 60ºC. Finally, the organic solvent was removed using a rotavapor (R153, Buchi, Switzerland) and the particles were extruded 10 times through a 0.45-µm membrane to ensure uniform particle sizes and consistency of the final obtained EL product [19]. The particle size was then measured following "Physico-chemical characterizations", and the formula possessing the smallest size was considered optimal and selected for the preparation of NA-loaded EL.

Table 1. Effect of HPC and CH content on the mean sizes of the blank EL.

Formulation factor	HPC:CH:Span 80 (w/w/w)	Particle size (nm)
	1:1:1	653 ± 30[a]
HPC content	1.5:1:1	646 ± 32[a]
	2:1:1	736 ± 36[b]
	1.5:1:1	646 ± 32[a]
CH content	1.5:1.5:1	316 ± 15[c]
	1.5:2:1	378 ± 18[c]

Different letters (a, b, c) denote significant differences between particle sizes.

Preparation of NA-loaded EL

The optimal blank EL formulation was utilized to fabricate NA-loaded EL, following the same method. Briefly, the aqueous phase containing different NA amounts of 100, 150, and 200 mg, were respectively mixed with the organic phase. Then, the EL was obtained by evaporating the organic solvents, followed by extrusion (10 times) through a 0.45-µm membrane. Finally, the formulations were stored at 4ºC for further experiments.

Physico-chemical characterizations

Size, zeta potential, and morphology

The EL mean size (hydrodynamic diameter) and polydispersity index (PDI) were determined by dynamic light scattering (DLS) using the ZetaPALS® analyzer (Brookhaven Instrument Corporation, Holtsville, USA) equipped with a 35-mW Helium-Neon laser diode (632.8 nm) and a goniometer (BI-200SM) coupled with a digital correlator (BI-9010AT). Samples were dispersed in DI water and measured for five cycles by the auto-measuring mode.

The zeta potential was determined by the phase analysis light scattering method with the ZetaPALS®. Measurements were conducted at room temperature and an angle of 14.8º to the incident light. Samples were dispersed in DI water and measured for 10 cycles.

The EL shapes were observed using polarized optical microscopy (Olympus, USA), with a microscope attached to a 530-nm full wavelength retardation wave plate.

Drug EE%

The ability to encapsulate the NA of the EL was determined by the drug EE% values using the indirect method. For this, 10 mg of the freeze-dried NA-loaded EL was weighed and dispersed in 1 ml of DI water, followed by centrifugation

at 17,000 rpm for 30 minutes. The supernatant containing the non-entrapped NA was collected, and diluted with methanol, and the non-entrapped NA amount was determined using UV-Vis spectroscopy at 262 nm (standard curve equation of y = 0.0268x – 0.0002 (R^2 = 0.999)). The EE% of the encapsulated NA was then calculated using equation (1).

$$EE\% = [1 - (\text{amount of non-entrapped NA})/(\text{initial amount of NA})] \times 100\% \quad (1)$$

Deformability measurement

The elasticity of the EL liposomal membrane was measured based on the alteration of the vesicle size while passing through the 100-nm polycarbonate membrane, using a manual mini-extruder (Avanti Polar Lipid Inc., Alabaster, AL) [21]. The EL was subjected into the syringe, followed by the extrusion process (manually by hand). The EL initial mean sizes and their respective sizes after extrusion were determined by the DLS method. The EL elasticity, demonstrating by the deformability percentage, was calculated following equation (2).

$$\text{Deformability (\%)} = [\text{Initial EL size (nm)} - \text{EL size after extrusion (nm)}] \times 100/[\text{Initial EL size (nm)}] \quad (2)$$

In-vitro permeation study

To investigate the EL ability to deliver NA transepidermally, *in-vitro* permeation study was performed using a Franz diffusion cell (PermeGear, Hellertown, USA). For comparison purposes, 2 different kinds of membranes were utilized, namely natural porcine ear epidermis and synthetic 0.2-µm polycarbonate membrane (Nucleopore®, Whatman, Costar GmbH, Bo-denim, Germany). To get the porcine ear epidermis, pig ear skin was obtained from the slaughterhouse, subcutaneous-fat-tissue removed, immersed in hot water at 60ºC for 2 minutes, and the epidermis was separated from the dermis using a heat separation technique. Then, the trans-epidermal water loss (TEWL) value of the separated epidermis was measured by a tewameter (Model TM 300, Courage and Khazaka electronic GmbH, Germany). A TEWL value of less than 15 g/m^{2}h indicates the undamaged epidermis [14]. The experiments were conducted with the ethical approval of the Naresuan University Animal Ethics Committee, Phitsanulok, Thailand (NU-AEE610504).

For the *in-vitro* permeation test, both membranes (diffusion area of 2.46 cm^2) were individually fitted between the Franz cell donor and receptor chambers, followed by stabilization for 30 minutes. The receptor chamber contained 6 ml of DI water as a permeation medium, and the medium was maintained at a temperature of 32ºC ± 0.5ºC, simulating the skin normal temperature. The samples (freshly prepared following the protocol described in "Preparation of NA loaded EL") were evenly subjected and spread on the membrane. These samples were not subjected to centrifugation, thus, both the non-entrapped NA and the encapsulated NA inside the EL were present. The tested samples included NA loaded EL 1%, 1.5%, and 2%, which contained 3, 4.5, and 6 mg NA, respectively. The 2% NA cream was used as a control (6 mg NA mixed with a cream base to get a 300 mg sample). At each pre-determined time interval of 30, 60, 120, 240, and 480 minutes, 500 µl of the medium in the receptor chamber was withdrawn and fresh-medium replaced. The withdrawal samples were then measured by UV-Vis spectroscopy (Genesys-10 Series, Thermo Fisher Scientific Inc., USA) at 262 nm (standard curve equation of y = 0.0268x – 0.0002 (R^2 = 0.999)) to determine the permeated NA amount.

Statistical analysis

The experiments were carried out in triplicate and demonstrated in terms of mean ± standard deviation (SD). The Student's *t*-test and ANOVA were utilized to denote the differences between samples, with 95% confidence intervals and a significant level of $p < 0.05$. Tukey's *post-hoc* test was used to address multiple comparisons, where necessary. Tukey's test is appropriate for identifying significant differences between groups while controlling for type-I errors.

RESULTS AND DISCUSSION

Preparation of the blank EL

A report on the NA-loaded EL for transepidermal delivery has been published in the literature, nevertheless, the total NA amount permeated through the skin of that study was only ~2.5 times higher than that of the 2% NA solution [9]. Herein, we aimed to develop novel EL to significantly enhance the NA transepidermal delivery, as well as to compare the effect of membrane choice (i.e., natural *vs.* synthetic type) on the *in-vitro* drug penetration. To this end, we first preliminary formulated the unloaded/blank EL with different amounts of HPC and CH, to obtain the optimal formula.

Regarding the EL preparation method, the reverse phase evaporation technique was utilized due to the fact that this method produces EL with relatively small particle sizes that are suitable for topical administration. More importantly, the EL formulated by the reverse phase evaporation technique attained higher drug EE% (39.51% ± 3.38%) for hydrophilic drugs (i.e., NA) compared to the conventional method (30.0% ± 3.8%) such as the film hydration technique [22]. Nevertheless, this EE% value was not optimal. Further improvements may be achieved through optimizations of (1) preparation methods (the rotary evaporation-sonication method could be more effective than the vortexing-sonication method [23]), (2) lipid compositions and drugs/lipids ratios (HPC and CH show superior properties compared to other lipids [19]), (3) edge activator types and concentrations (the effectiveness of edge activator follows Span 85 > Span 80 > Sodium cholate > Sodium deoxycholate > Tween 80 [23]), and (4) other formulation factors such as charge-inducing agents and sonication addition.

Table 1 shows that the amount of HPC and CH significantly affected the EL sizes. The particle sizes tended to increase as the amount of HPC increased. On the other hand, when the CH amount increased, the mean vesicle sizes decreased. This could be because HPC, a phospholipid, is the main backbone of the EL outer membranes, while CH influences the membrane's mechanical properties by increasing their

mechanical strength and enhancing the lipids packing density [24,25]. Thus, an increase of CH enhances the EL rigidity, consequently decreases their particle sizes. Generally, liposomes with a size of >600 nm cannot penetrate deeply through the skin and stuck in the stratum corneum, while vesicles with a size of ≤300 nm are ideal for transepidermal delivery [21]. Moreover, span 80 acts as a non-ionic surfactant and an edge activator that enhances vesicle deformability. The effect of its concentration on the liposome properties has been investigated elsewhere, and thus, was fixed in this study [13,14]. Conclusively, formulation with a HPC:CH:Span 80 ratio of 1.5:1.5:1 w/w/w (formulation 4), which possessed the smallest size of 316 ± 15 nm, was selected to encapsulate the NA.

Preparation and characterizations of the NA-loaded EL

The optimal formulation was used to encapsulate NA at 3 different amounts of 100, 150, and 200 mg, corresponding to the percentage of NA content of 1%, 1.5%, and 2% w/w, respectively. The particle properties are shown in Table 2 and Figure 1. Obviously, all 3 formulas demonstrated a mean particle size of ~300 nm, similar to that of the blank EL, indicating that the NA encapsulation did not affect the EL size. Additionally, a low PDI of less than 0.3 was observed, suggesting a narrow size distribution between EL particles [19]. It is worth to notice that although the EL was extruded 10 times through a 0.45-µm membrane, the PDI was still higher than 0.2, possibly due to the particle interactions and system behaviors. The system negatively surface charge of ~-20 mV, which comes from HPC, could enhance the EL stability [26]. Moreover, under polarized light microscope, all formulations proved the liposome lamellarity by illustrating Maltese cross-like structures, which confirms the success of EL fabrication (Fig. 1) [27].

Interestingly, the NA initial amount was a parameter influencing the EE% (Table 2). As the initial amount of NA increased from 100 to 200 mg, the EE% significantly decreased from ~39.5% to ~30%. Correspondingly, the particle NA content (i.e., the amount of NA entrapped in the EL) increased from ~40 to ~50 mg. Noticeably, no significant difference was found between the 150- and 200-mg formulations, in terms of EE% and NA content, indicating the EL vesicles have reached their encapsulation limit for NA at the initial loading amount of 150 mg. Noticeably, the low EE% of NA in EL can be attributed to several factors, including the lipid compositions of EL, the inherent partitioning behavior of the particles, the EL sizes, and, especially, the hydrophilic nature of NA. Nevertheless, in this study, the EL formulated by the reverse phase evaporation technique attained higher NA EE% compared to the conventional method such as the film hydration technique [22].

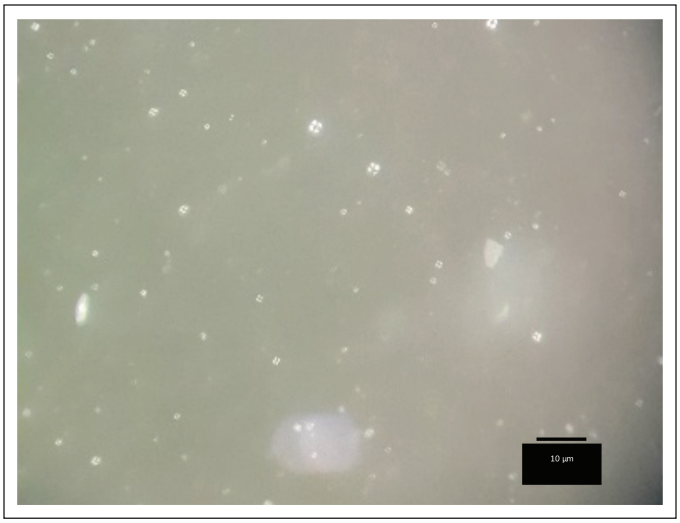

Figure 1. Optical micrographs of NA loaded EL composed of HPC:CH:Span 80 at the ratio of 1.5:1.5:1 w/w/w. Scale bar: 10 µm.

The NA-loaded EL elasticity was revealed in terms of the deformability percentage. For this, the particle deformability significantly increased from 5.77% to 6.67% as the initial NA amount increased from 100 to 200 mg. A high deformability value (> 10%) indicates large differences in the liposome sizes prior and after extruding through a 100-nm membrane, possibly due to breaking and fragmentation into smaller particles. Thus, low deformability percentages, as shown in our results, are considered great elastic property, and appropriate for EL. Additionally, our data demonstrate that EL with higher NA contents possessed higher deformability index. This might be explained that the NA had some interactions with the EL lipid components, consequently making the membranes less elastic. Last but not least, the particles still preserved their physicochemical properties (i.e., size, PDI, zeta potential, EE%, and deformability) for 1-month storage at room temperature, indicating appropriate short-term stability.

***In-vitro* permeation study**

Since NA is a hydrophilic compound [10] with inadequate skin permeation and absorption [11], we developed novel NA-loaded EL to potentially enhance NA skin permeability. To this end, three NA-loaded EL formulations,

Table 2. Physico-chemical characteristics of NA loaded EL composed of HPC:CH:Span 80 at the weight ratio of 1.5:1.5:1, with different NA content of 1%, 1.5%, and 2%. Data are shown as mean ± SD.

% NA content	Particle size (nm)	PDI	ZP (mV)	EE (%)	NA content (mg)	Deformability (%)
1.0	331 ± 22	0.23 ± 0.04	-23.2 ± 2.1	39.51 ± 3.38^{a}	39.51 ± 3.38^{c}	5.77 ± 0.01^{e}
1.5	358 ± 15	0.22 ± 0.08	-18.6 ± 3.4	31.51 ± 3.43^{b}	47.27 ± 5.15^{d}	6.30 ± 0.02^{f}
2.0	328 ± 37	0.22 ± 0.03	-19.8 ± 2.6	28.22 ± 4.22^{b}	56.44 ± 8.44^{d}	6.67 ± 0.01^{g}

PDI: polydispersity index; ZP: zeta potential; EE: entrapment efficiency.

Different letters (a–g) denote significant differences ($p < 0.05$) between samples.

with different NA content, 1%, 1.5%, and 2%, were tested, in comparison with the control, 2% NA cream.

Two kinds of *in-vitro* permeation-test membranes, a synthetic 0.2-µm polycarbonate membrane and a natural porcine ear epidermis, were utilized and compared. The porcine tissues were selected because they have been confirmed to be similar with human skin [28]. For instance, the stratum corneum of both tissues composes of orthokeratotic stratified squamous epithelium with similar thickness of ~20 µm; the epidermis layers consist of basal keratinocytes with a thickness of ~72 µm; and the subcutis layers contain fat cells. Moreover, the hair follicles, sweat glands, and sebaceous glands are also very comparable between the porcine and human tissues [28]. This information suggests the study reliability and suitability as a model for human skin permeation test.

In both membranes, no significant burst release and permeation was observed, possibly due to the fact that NA has been incorporated into the EL, whereas the NA cream could not penetrate well into the skin tissue. For the synthetic membrane (Fig. 2A), the 1% NA-loaded EL formulation possessed a less NA content at the receptor chamber compared to the 1.5% and 2% NA-loaded EL, as well as the 2% NA cream. The results suggested a simple passive diffusion transport of NA through the membrane, which commonly depend on the drug concentration. The EL systems, with mean sizes of ~300 nm, could not squeeze through the 200-nm synthetic membrane. Thus, the detected NA was the NA released from the particles and passed through the membrane, but not the intact NA that was residing inside the EL. Consequently, the permeation data using the synthetic membrane were not reliable. Our data further contributed to the literature, which has demonstrated that the silicone synthetic membrane is not a predictive material of skin permeation in mammalian tissues [29].

Interestingly, when using the natural porcine epidermis, the 2% NA cream demonstrated almost no NA permeation (Fig. 2B), indicating that the free NA could not penetrate these layers

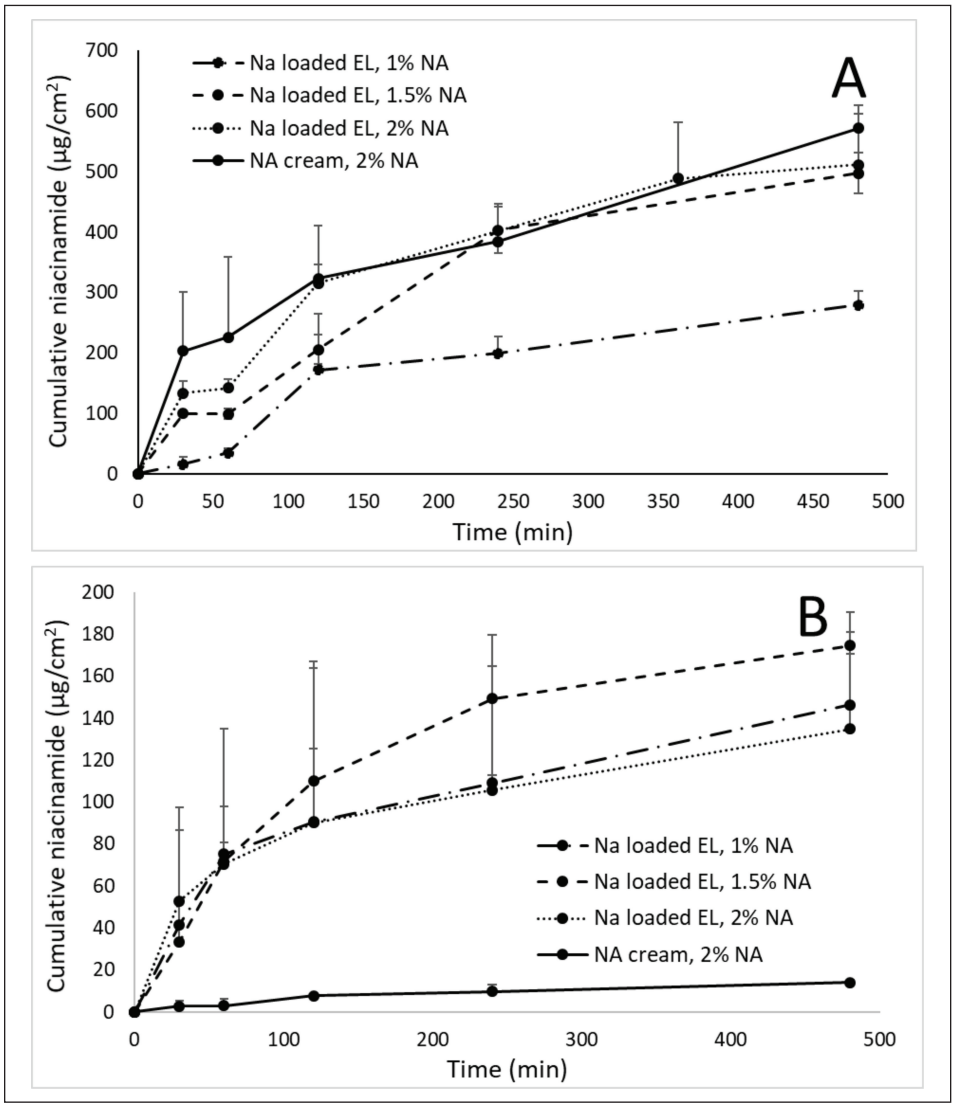

Figure 2. *In-vitro* permeation profiles of 2% NA cream and NA loaded EL at different NA content of 1%, 1.5%, and 2%, using (A) synthetic 0.2-µm polycarbonate membrane and (B) natural porcine ear epidermis. Error bars show SD for $n = 3$.

Table 3. Fitted data on various models of the NA release and permeability on the porcine ear epidermis from NA cream and NA loaded EL samples.

Model	R^2 value			
	2% NA cream	NA loaded EL 1%	NA loaded EL 1.5%	NA loaded EL 2%
Zero-order	0.9060	0.7980	0.8062	0.7451
First-order	0.9066	0.8151	0.8164	0.7534
Higuchi	**0.9714**	**0.9658**	**0.9604**	**0.9489**
Hixson-Crowell	0.8977	0.8084	0.8122	0.7481
Korsmeyer-Peppas	0.8561	0.7240	0.8222	0.6395

The highest R^2 values are bold.

due to its inherent hydrophilicity. On the other hand, all NA-loaded EL formulations showed >100-fold higher NA permeation than the 2% NA cream (the cumulative permeated NA of 2% NA cream and NA loaded EL 1%, 1.5%, and 2%, was averaged at 14.11, 146.25, 174.59, and 134.76 µg/cm^2, respectively). For comparison, only one study has been reported on this issue, however, in that study, the total cumulative NA amount from NA-loaded EL permeated through the skin over 24 hours was only ~2.5 times higher than that of the 2% NA solution [9]. Hence, our formulation could better enhance the NA skin permeation and absorption. The result confirmed that 300-nm NA-loaded EL could be transported through the porcine epidermis, despites the fact that this epidermis having a pore-size of 0.4–100 nm [30]. The transepidermal penetration of EL could be explained by two possible pathways, the intercellular and the shunt pathway. The EL could squeeze intercellularly through the hydrophilic channels existed in the epidermis layers due to their high elasticity and deformability [31,32]. Moreover, owing to their nano-size, the increased drug transepidermal penetration could also be attributed to the delivery of intact NA-loaded EL via sweat glands and hair follicles that known as the shunt pathway [33,34].

Moreover, when fitted with the release and permeation of EL samples with various common models of zero-order, first-order, Higuchi, Hixson-Crowell, and Korsmeyer-Peppas (Table 3), the Higuchi model showed the highest R^2 values of more than 0.9. A linear fit to the Higuchi model implies that the drug release is diffusion-controlled, which is often the case in topical formulations like creams and emulsions, as in our samples. Additionally, the steady-state flux (Js, calculated as Js = Qr/At, where Qr is the amount of permeated NA at steady state, A is the membrane area, and t is the permeation time at steady state) of the 2% NA cream, NA loaded EL 1%, 1.5%, and 2% was 1.760, 75.242, 71.744, and 70.520 µg/cm^{2}h. The permeability coefficient (Kp, calculated as $Kp = Js/C_0$, where C_0 is the initial total NA concentration at the donor site) of these formulas was 0.088×10^{-3}, 7.524×10^{-3}, 4.783×10^{-3}, and 3.526×10^{-3} cm/h, respectively. Indeed, both the Js and Kp of all EL formulas surpassed those of 2% NA cream, indicating the effective permeability of the particles across the epidermis layer. Interestingly, when compared between EL formulas with different NA amount (1%, 1.5%, and 2%), the Kp was independent with the drug concentration, while Js showed similar value. This could be explained by vesicle penetration via intercellular and shunt pathways may become saturated.

Study limitations

The present study, although demonstrating interesting results, still has some limitations. First, the EE% values observed for NA (28%–39.5%) were relatively low. NA is a hydrophilic compound, thus, low lipid solubility inherently limits its entrapment within the lipid bilayers. Further improvements may be achieved through optimizations of (1) preparation methods, (2) lipid compositions and drugs/lipids ratios, (3) edge activator types and concentrations, and (4) other formulation factors such as charge-inducing agents and sonication addition. Second, stability is a key factor in the development of effective pharmaceutical formulations, especially for vesicular systems. Future research could incorporate long-term stability testing to assess the impact of various storage conditions, including different temperatures (i.e., 4ºC, 25ºC, and 40ºC) and freeze-thaw cycles, on vesicle integrity, EE%, and drug release/permeability characteristics. Third, although the porcine skin tissue closely mimics human skin morphology and permeability, its adsorption characteristics may still differ from human skin. Therefore, further validation with human skin models will be necessary for more accurate predictions of in-vivo performance. Finally, clinical studies on human skin and safety testing (i.e., irritability test, biocompatibility test) could be considered in future works, with required ethical clearance from an institutional review board and the collection of informed consent from all participants, in accordance with the Declaration of Helsinki.

CONCLUSION

In this study, novel NA-loaded EL were successfully prepared, physico-chemically characterized, and *in-vitro* permeability investigated. The optimal formulation possessed spherical shape particles with a mean size of ~300 nm, a zeta potential of ~-20 mV, an EE% of ~40%, and a deformability value of < 10%. Regarding the membrane suitability, the natural porcine ear epidermis closely resembled the human skin and was an ideal choice for the permeability test, whereas the synthetic 0.2-µm polycarbonate membrane was not appropriate. The NA-loaded EL showed more than 100-fold enhancement of NA *in-vitro* transepidermal delivery compared to the NA cream, at the same NA concentration. Conclusively, the newly developed EL demonstrate much potential in delivery hydrophilic molecules across skin.

AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

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CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

ETHICAL APPROVALS

Ethical approvals detials are given in 'Materials and Methods' section.

DATA AVAILABILITY

All data generated and analyzed are included in this research article.

PUBLISHER'S NOTE

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USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declares that they have not used artificial intelligence (AI)-tools for writing and editing of the manuscript, and no images were manipulated using AI.

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