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Abstract

Introduction / Objectives: Peptide-based therapeutics offer promising biological activity for dermatological applications but face significant challenges in topical delivery due to their hydrophilicity and instability. This study aimed to develop a nanostructured lipid carrier (NLC) system using an ion pair strategy to improve the encapsulation efficiency and skin absorption of the positively charged peptide melanostatin.

Materials and Methods: Melanostatin was complexed with sodium dodecyl sulfate (SDS) to form an ion pair, with the optimal molar ratio determined through precipitation efficiency studies. Various liquid lipids and surfactants were screened to optimize the NLC formulation. The physicochemical properties, including particle size, polydispersity index (PDI), and zeta potential, were evaluated. Skin absorption studies were conducted comparing the optimized peptide-loaded NLC with a peptide solution of the same concentration.

Results: The optimal ion pair ratio was identified as 1:4 (peptide:SDS), achieving a precipitation efficiency of 99.9%. The NLC formulation using castor oil and Plantacare® 2000 exhibited small particle size (173.8 nm), low PDI (0.175), high encapsulation efficiency (99.9%), and stable zeta potential (-37.3 mV). In skin absorption studies, the peptide-loaded NLC significantly improved skin retention and transdermal permeation compared to the peptide solution, achieving a higher skin absorption efficiency (56.7% ± 12.5% vs. 43.7% ± 7.5%).

Conclusion: The ion pair-based NLC system effectively enhanced the encapsulation and skin delivery of melanostatin. This strategy shows strong potential for improving the stability and transdermal delivery of hydrophilic peptides, offering promising applications in dermatological and cosmetic formulations.

Key words: Nanostructured lipid carrier; Ion pair precipitation; Peptide delivery; Skin absorption

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1. Introduction

Peptide-based therapeutics have attracted substantial interest for dermatological and cosmetic applications due to their high specificity, potent biological activity, and relatively low toxicity (Morishita & Peppas, 2006). However, the direct encapsulation of peptides into lipid-based carriers presents significant challenges, primarily due to their hydrophilic nature, struc-

tural instability, and susceptibility to enzymatic degradation (Schroeder et al., 2009). To address these limitations, novel strategies have been explored to enhance peptide stability, encapsulation efficiency, and skin penetration.

Nanostructured lipid carriers (NLCs) represent a promising advancement in lipid-based delivery systems. By combining solid and liquid lipids into a stable nanoparticulate matrix, NLCs offer numerous benefits, including improved drug loading capacity, controlled release profiles, enhanced stability, and superior skin permeation (Muller et al., 2002). Recent progress in NLC technology has further optimized their physicochemical properties, making them suitable for the delivery of sensitive biomolecules such as peptides. Nevertheless, the intrinsic hydrophilicity and charge properties of peptides continue to pose barriers to efficient incorporation into lipid matrices.

Ion pair precipitation has emerged as an effective technique to overcome the solubility and stability issues associated with peptide delivery. This method involves the formation of a hydrophobic ion pair between the charged peptide and an oppositely charged surfactant or counterion, resulting in a complex that exhibits improved lipophilicity (Gao & Singh, 1998). The enhanced hydrophobicity facilitates the incorporation of peptides into lipid carriers without significant structural alterations, thereby preserving bioactivity and achieving high encapsulation efficiencies.

In this study, we applied the ion pair precipitation approach to enhance the encapsulation of melanostatin, a positively charged peptide, into NLCs. Sodium dodecyl sulfate (SDS), a negatively charged surfactant, was employed to form a stable ion pair with melanostatin, thereby improving its compatibility with the lipid matrix. After ion pair formation, the complex was incorporated into NLCs to evaluate the effectiveness of this strategy.

Comprehensive investigations were conducted to optimize the ion pair ratio, examine the influence of various liquid lipids and surfactants on encapsulation performance, and assess the impact on skin absorption. Our results demonstrated nearly 100% encapsulation efficiency and total recovery rate, highlighting the potential of this approach for efficient peptide delivery. Additionally, the study explored how formulation parameters affect the characteristics of the lipid carriers and their ability to enhance transdermal delivery, providing valuable insights for the future development of peptide-loaded NLCs.

2. Materials and Methods

2.1 Materials

The Melanostatin DM, a Nonapeptide for whiting, were bought from bio-race co.. Sodium dodecyl sulfate (SDS) were get from Merck used for ion pair sedimentation

The Cetyl palmitate(CP) is used for the solid lipid for peptide-loaded NLCs preparation. Four different liquid oils were used for NLCs evaluation including caprylic capric triglyceride、Isohexadecane、Isopropyl Myristate and castor oil. For the investigation of surfactnts effect. Four surfctants were used including Plantacsre ®2000、 Plantacare ® 810、 Poloxamer 188 and Tween 80. The trifluoroacetic acid (TFA) is get from thermo scitific.

2.2 Hydrophobic ion pairing (HIP)

Sodium dodecyl sulfate (SDS) was used as the anionic surfactant to form hydrophobic ion pairs with the peptide. Various molar ratios of SDS to peptide (1:2, 1:3, 1:3.5, 1:4, and 1:5) were tested. For each condition, 1 mL of the peptide (10000 ug in 0.25% TFA) mixed with 1 mL different SDS concentration in 0.25% TFA solution. the mixture was transferred into an Eppendorf tube and mixed thoroughly using a vortex mixer. The samples were then centrifuged at 10000 rpm for 5 minutes. The supernatant was collected, filtered, and analyzed by HPLC to determine the amount of unprecipitated peptide.

1.1 Preparation of NLC and serum

The formulations evaluated in this study were shown in table 1. Cetyl palmitate(CP) is used for the solid lipid for NLCs preparation. The SDS (60000 ug/mL) and peptide solution were first mixed in 1:1 ratio followed the liquid oil were added and stirred homogeniously. The CP then added and heated to 70 ^{0}C to melted the solid lipid and mixed with the liquid lipid. The surfactant is dissolved in water and heated to 80 ^{0}C in a separate beaker. The water phase is poured into oil phase slowly with stirring at 9500 rpm by a high-shear homogenizer. The resulting emulsion was then processed using ultrasonic probe sonicator (sonic vcx-130 , USA) at 130 W.

1.2 High-Performance Liquid Chromatography (HPLC) Analysis

The HPLC analysis was performed using an Agilent HC-C18 column (4.6 × 250 mm, 5 µm). The mobile phase consisted of deionized water containing 1% trifluoroacetic acid (solvent A) and acetonitrile containing 1% trifluoroacetic acid (solvent B). The gradient elution program was as follows: 0–5 minutes, 90% A and 10% B; 5–13 minutes, 70% A and 30% B; 13–14 minutes, 70% A and 30% B; and 14–15 minutes, 90% A and 10% B. The flow rate was maintained at 1.0 mL/min, and the column temperature was set at 25 °C. The injection volume was 100 µL. Detection was carried out using a UV detector at a wavelength of 280 nm.

1.3 Determination of particle size and zeta potential

The mean particle size (z-average) and zeta potential of the NLC were measured by photon correlation spectroscopy (Nano ZS90, Malvern, Worcestershire, UK) using a helium–neon laser with a wavelength of 633 nm. Photon correlations of spectroscopic measurements were carried out at a scattering angle of 178^0. A 1:200 dilution of the formulations was made using double-distilled water before the measurement.

1.4 Total recoveries and encapsulation effency evaluation

For the total recoveries of peptide evaluation, A 0.2 g NLC solution was dissolved in 5 mL hexane/ethanol(3:7, 0.3% TFA) solvent then filtered by a 0.45 um filter. The amount of peptide in solution was determined by HPLC.

To determine the encapsulation effency (E.E) , The 0.5 g NLC solution was poured into a Amicon Ultra-15 centrifugal filter then centrifuge at 10000 rpm for 60 minutes. The filtered solution was removed from the tube and diluted to 1 mL. The amount of unencapulated peptide in solution was determined by HPLC with UV detector. The encapsulation effency was calculated by the following equation:

E.E%= (total amount – unencapulated amount)/total amount X 100%

1.5 In vitro skin permeation study

The skin permeation of peptide was measured using a Franz diffusion assembly. The abdomenl skin of pig (3-6 month old) in a 350 um thickness was mounted between the donor and acceptor compartments. The donor medium consisted of 0.01g of vehicle containing peptide. The receptor medium (4.6 ml) was ethanol: 0.9% normal saline solution to maintain sink conditions. The available diffusion area between cells was 0.196 cm^2. The stirring rate and temperature were, respectively, kept at 600 rpm and 37 ^{0}C. the applied time is 8 hours.

At the end of applied time, the residue on the donor was removed and dilution 1 mL. the skin surface was wiped by a cotton swap then extracted by hexane/ethanol(6:4). The

amount of peptide in the above solution was considered as non-permeated peptide. The pig skin was immerged in 0.5 mL hexane/ethanol(6:4) and sonicated for 30 minutes then filtered by a 0.45 um filter. The peptide in donor solution was extracted by a 2 ml C-18 extraction column then eluted by hexane/ethanol (6:4).

3. Results and Discussions

3.1 Formulation design

To systematically investigate the influence of different liquid lipids and aqueous surfactants on the characteristics of the ion pair-melanostatin loaded nanostructured lipid carriers (NLCs), a series of formulations were prepared as summarized in Table 1. In all formulations, cetyl palmitate (CP) was selected as the solid lipid, while the liquid lipid varied among castor oil (CO), isohexadecane (IHD), isopropyl myristate (IPM), and caprylic/capric triglyceride (CCT). Sodium dodecyl sulfate (SDS) was used consistently to form ion pairs with melanostatin DM, facilitating its incorporation into the lipid matrix.

In the aqueous phase, four different surfactants were evaluated: Plantacare® 2000 (PL2000), Plantacare® 810 (P810), Poloxamer 188 (P188), and Tween 80 (T80). Each surfactant was paired with each liquid lipid, resulting in a total of sixteen formulations. This design allowed for the assessment of how the choice of liquid lipid and surfactant influenced key parameters such as encapsulation efficiency, particle size, polydispersity index, and formulation stability.

By systematically varying these two critical components, the study aimed to identify the optimal lipid and surfactant combination that would maximize the encapsulation efficiency of the melanostatin ion pair complex while maintaining desirable physicochemical properties for enhanced skin delivery.

Table 1 : Formulation of Peptide loaded NLCs

NO.	CP	CO	IHD	IPM	CCT	SDS	Peptide	PL2000	P810	P188	T80	Water
1-1	8	2	-	-	-	0.012	0.010	2	-	-	-	To 100
1-2	8	2	-	-	-	0.012	0.010	-	2	-	-	To 100
1-3	8	2	-	-	-	0.012	0.010	-	-	2	-	To 100
1-4	8	2	-	-	-	0.012	0.010	-	-	-	2	To 100
2-1	8	-	2	-	-	0.012	0.010	2	-	-	-	To 100
2-2	8	-	2	-	-	0.012	0.010	-	2	-	-	To 100
2-3	8	-	2	-	-	0.012	0.010	-	-	2	-	To 100
2-4	8	-	2	-	-	0.012	0.010	-	-	-	2	To 100
3-1	8	-	-	2	-	0.012	0.010	2	-	-	-	To 100
3-2	8	-	-	2	-	0.012	0.010	-	2	-	-	To 100
3-3	8	-	-	2	-	0.012	0.010	-	-	2	-	To 100
3-4	8	-	-	2	-	0.012	0.010	-	-	-	2	To 100
4-1	8	-	-	-	2	0.012	0.010	2	-	-	-	To 100
4-2	8	-	-	-	2	0.012	0.010	-	2	-	-	To 100

4-3	8	-	-	-	2	0.012	0.010	-	-	2	-	To 100
4-4	8	-	-	-	2	0.012	0.010	-	-	-	2	To 100

CP:Cetyl palmitate, IHD:Isohexadecane, IPM:Isopropyl Myristate, CCT:Caprylic Capric Triglyceride, CO : castol oil .SDS:Sodium dodecyl sulfate, Peptide:Melanostatin DM, Plantacare®2000 P810:Plantacare®810,P188:Poloxamer188, T80:Tween80,

3.2 Hydrophobic Ion Pair Screening

In the second part of the study, the effect of different molar ratios of sodium dodecyl sulfate (SDS) to peptide on ion pair formation was investigated. The precipitation efficiency of the peptide was evaluated at various SDS concentrations. As shown in Figure 1, the maximum precipitation efficiency (99.9%) was achieved at a 1:4 peptide:SDS molar ratio. Notably, a further increase in the SDS amount to a 1:5 ratio resulted in a slight decrease in precipitation efficiency, suggesting that excessive surfactant may lead to resolubilization or destabilization of the formed complex. Interestingly, the precipitation efficiency observed at a 1:3.5 ratio was comparable to that at 1:4, indicating that near-optimal complexation could already be achieved with slightly lower amounts of SDS. These results highlight the importance of fine-tuning the peptide-to-SDS ratio to maximize ion pair formation and ensure high encapsulation performance in subsequent formulation steps.

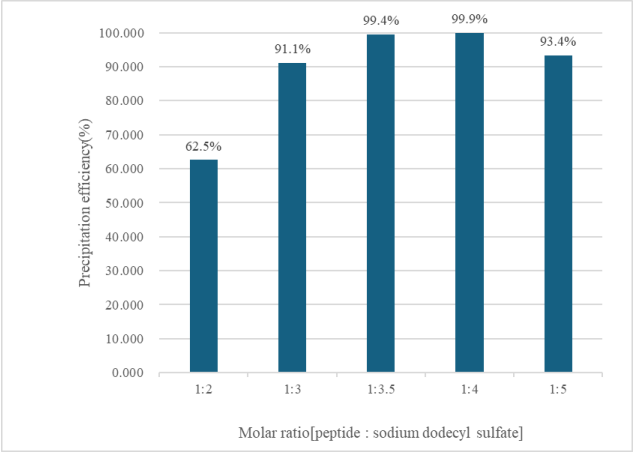

Figure 1: Precipitation efficiency of peptide with sodium dodecyl sulfate (SDS) at different molar ratios. Maximum efficiency (99.9%) was observed at a 1:4 peptide:SDS ratio. A further increase to 1:5 slightly reduced the efficiency.

3.3 The effect of different lipids and surfactants on the total recoveries and encapulation efficicies

(a) Effect of Liquid Lipids on Recovery and Encapsulation Efficiency

The type of liquid lipid significantly influenced both the recovery rate and encapsulation efficiency (EE%) of the peptide-loaded NLCs. Formulations containing castor oil consist-

ently exhibited the highest recovery and encapsulation efficiencies across all surfactant types, with values close to or exceeding 99% EE and 100% recovery. This result suggests that castor oil provides a favorable lipid environment for stabilizing the ion pair-melanostatin complex. In contrast, formulations with isohexadecane (IHD) showed notably lower recovery rates, particularly when combined with P810 and P188, although encapsulation efficiency remained relatively high. Isopropyl myristate (IPM) formulations displayed moderately high EE% values but slightly reduced recovery compared to castor oil. Formulations using caprylic/capric triglyceride (CCT) demonstrated variable results: although high recovery was observed with some surfactants (e.g., P2000 and P188), EE% decreased significantly when combined with Tween 80. Overall, the findings indicate that the chemical compatibility and polarity of the liquid lipid play crucial roles in maintaining the integrity and retention of the peptide ion pair within the lipid matrix.

(b) Effect of Surfactants on Recovery and Encapsulation Efficiency

The choice of surfactant in the aqueous phase also critically affected the recovery and encapsulation outcomes. Plantacare® 2000 (P2000) consistently provided the best performance across all liquid lipid types, maintaining both high recovery and high EE%, suggesting superior stabilization of the emulsion system and effective particle formation. Plantacare® 810 (P810) and Poloxamer 188 (P188) exhibited slightly lower recovery rates, particularly when used with IHD and IPM, but still achieved high EE% values in most cases, indicating that they were able to stabilize the peptide-lipid system during formulation. In contrast, Tween 80 formulations showed a pronounced decrease in EE%, particularly with IHD (75.5%) and CCT (72.0%), despite relatively high recovery percentages. This reduction suggests that Tween 80 may not be optimal for stabilizing the ion pair complex within the lipid matrix, possibly due to its relatively higher hydrophilicity and interaction with the ion-paired peptide, leading to leakage during formulation. These results emphasize the importance of selecting an appropriate surfactant to ensure high encapsulation and stability of sensitive peptide formulations.

Table 2: The total recoveries and encapulation efficiencies of different formulations

	P2000		P810		P188		Tween 80	
	R(%)	EE(%)	R(%)	EE(%)	R(%)	EE(%)	R(%)	EE(%)
Castol oil	100.2	99.9	84.1	99.9	94.9	97.9	109.2	78.6
IHD	93.0	100.0	81.3	92.7	94.9	93.9	110.5	75.5
IPM	88.3	99.8	81.1	97.7	93.6	98.6	102.6	67.9
CCT	102.6	87.1	79.3	98.5	101.7	99.7	107.2	73.0

IHD: Isohexadecane, IPM:Isopropyl Myristate, CCT:Caprylic Capric Triglyceride

3.4 The effect of surfactants and liquids on the partical size and zeta potentional of NLCS

(a) Particle Size and Polydispersity Index (PDI) Analysis

The particle size and polydispersity index (PDI) of the formulations varied significantly depending on the type of liquid lipid and surfactant used. Among all formulations, the smallest particle size (173.8 nm) with a narrow distribution (PDI = 0.175) was observed for the castor oil and Plantacare® 2000 combination (A1-1), indicating efficient nano-

particle formation and good stability. Conversely, formulations containing Plantacare® 810 or Poloxamer 188 generally exhibited larger particle sizes and higher PDI values, suggesting poorer uniformity and potential aggregation tendencies, particularly when castor oil or IPM was used. Notably, the largest particle size (761.6 nm) was recorded for castor oil combined with P188 (A1-3), indicating that this surfactant-lipid combination was less favorable for stable NLC formation.

In terms of liquid lipids, castor oil consistently yielded smaller particle sizes compared to IHD, IPM, and CCT when paired with P2000. IHD- and IPM-based formulations tended to show increased particle sizes and higher PDI values, reflecting less favorable physicochemical compatibility with the ion pair complex. Moreover, Tween 80 resulted in relatively larger particle sizes and variable PDI values, suggesting that it might not efficiently stabilize the nanosystem under the tested conditions.

(c) Zeta Potential Analysis

Zeta potential measurements revealed negative surface charges across all formulations, ranging from -28.1 mV to -43.4 mV. The highest magnitude of zeta potential (-43.4 mV) was observed in the formulation combining IHD and PL2000 , indicating strong electrostatic repulsion between particles, which is favorable for colloidal stability. Generally, formulations with P2000 as the surfactant demonstrated more negative zeta potentials compared to those with P188 or Tween 80, suggesting enhanced particle stabilization in the aqueous medium.

Interestingly, the use of Tween 80 consistently resulted in lower zeta potential values (around -28 to -34 mV), which could contribute to reduced repulsion forces and potentially explain the larger particle sizes and broader size distributions observed in these systems. These findings underscore the critical role of surfactant selection in maintaining both nanoparticle stability and size uniformity in peptide-loaded NLCs.

Table 3: Particle Size , Polydispersity Index (PDI) and zeta potentional of different formulations

Code	lipid oil	surfactant	partical size(nm)	PDI	zeta potentional (mV)
1-1	castol oil	P2000	173.8	0.175	-37.3
1-2	castol oil	P810	234.6	0.426	-38.1
1-3	castol oil	P188	761.6	0.234	-29.4
1-4	castol oil	Tween 80	331.2	0.144	-28.1
2-1	IHD	P2000	281.1	0.284	-43.4
2-2	IHD	P810	299.2	0.481	-37.3
2-3	IHD	P188	336.5	0.399	-29.2
2-4	IHD	Tween 80	532.4	0.078	-30.8
3-1	IPM	P2000	329.9	0.447	-39.8
3-2	IPM	P810	184.3	0.26	-34.8
3-3	IPM	P188	413.9	0.47	-30.1
3-4	IPM	Tween 80	511.4	0.538	-34.0
4-1	CCT	P2000	251.3	0.417	-35.2
4-2	CCT	P810	195.7	0.401	-38.7
4-3	CCT	P188	376.1	0.473	-33.8

4-4	CCT	Tween 80	514.5	0.02	-30.0

1.4 Skin absorption evalution

The skin absorption performance of the peptide-loaded nanostructured lipid carrier (NLC) formulation (composed of CP, CCT, and Plantacare® 2000) was compared with that of a simple peptide solution at the same peptide concentration. As shown in Table 4 , both formulations demonstrated comparable total recovery rates, with 96.0% ± 11.0% for the peptide solution and 95.0% ± 7.0% for the NLC formulation, indicating consistent mass balance in the experimental setup.

However, significant differences were observed in terms of skin absorption behavior. The peptide-loaded NLC exhibited higher skin retention (7.6 ± 1.2 μg) compared to the peptide solution (6.2 ± 0.9 μg), suggesting that the lipid carrier system facilitated better deposition of the peptide within the skin layers. Furthermore, the amount of peptide permeated into the receptor compartment was also higher for the NLC formulation (4.3 ± 1.7 μg) than for the peptide solution (2.6 ± 0.9 μg), indicating enhanced transdermal penetration.

Overall, the skin absorption efficiency of the peptide-loaded NLC (56.7% ± 12.5%) was markedly greater than that of the peptide solution (43.7% ± 7.5%). This enhancement can be attributed to the improved skin interaction and sustained release properties provided by the NLC matrix, as well as the increased lipophilicity imparted by the ion pair complexation with SDS. These findings demonstrate the potential of ion pair-based NLC systems in promoting the dermal and transdermal delivery of hydrophilic peptides.

Table 4 In vitro skin permeation study

	Peptide solution	Peptide loaded NLC
Applied dosage(ug)	20.2±0.4	21.0±0.3
Residue (ug)	10.5±2.5	8.1±1.5
Skin retained (ug)	6.2±0.9	7.6±1.2
recepter(ug)	2.6±0.9	4.3±1.7
Total recovery (%)	96.0±11.0	95.0±7.0
Skin absorption efficiency (%)	43.7±7.5	56.7±12.5

4. Conclusion

An ion pair-based nanostructured lipid carrier (NLC) system was successfully developed to enhance the encapsulation and skin delivery of melanostatin. Castor oil and Plantacare® 2000 were identified as the optimal lipid and surfactant combination. The optimized NLC formulation demonstrated improved skin retention and absorption efficiency compared to a peptide solution, highlighting the potential of this strategy for effective peptide delivery in dermatological applications.

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