

Article

Liposomal Formulations Containing Amino Acid Menthol Ester Naproxenate: Physicochemical Characterization and Transdermal Delivery Potential

Aleksandra Bil ska ¹, Karolina Bil ska ¹, Anna Nowak ², Grzegorz Story ³, Łukasz Struk ⁴ and Paula Ossowicz-Rupniewska ^{1,*}

¹ Department of Organic Chemical Technology and Polymer Materials, Faculty of Chemical Technology and Engineering, West Pomeranian University of Technology in Szczecin, Piastów Ave. 42, 71-065 Szczecin, Poland

² Department of Cosmetic and Pharmaceutical Chemistry, Pomeranian Medical University in Szczecin, Powstańców Wielkopolskich Ave. 72, 70-111 Szczecin, Poland

³ Department of Chemical and Process Engineering, Faculty of Chemical Technology and Engineering, West Pomeranian University of Technology in Szczecin, Piastów Ave. 42, 71-065 Szczecin, Poland

⁴ Department of Organic Chemistry and Physical Chemistry, Faculty of Chemical Technology and Engineering, West Pomeranian University of Technology in Szczecin, Piastów Ave. 42, 71-065 Szczecin, Poland

* Correspondence: possowicz@zut.edu.pl

Featured Application

The synthesized amino acid-based naproxen derivative incorporated into liposomal carriers may be applied in the development of transdermal pharmaceutical formulations for the treatment of pain and inflammatory disorders, providing improved skin penetration and potentially reducing adverse effects associated with oral administration of NSAIDs.

Abstract

Non-steroidal anti-inflammatory drugs (NSAIDs), including naproxen, are widely used for the treatment of pain and inflammation; however, their therapeutic application is limited by poor aqueous solubility and low bioavailability. This study aimed to synthesize and characterize a novel amino acid-based naproxen derivative, L-phenylalanine menthol ester naproxenate ([PheOMent][NAP]), develop liposomal formulations containing the obtained compound, and evaluate their physicochemical properties and transdermal delivery potential. The derivative was synthesized via a three-step procedure and characterized using NMR, FT-IR, TG, DSC, and XRD analyses. Compared with naproxen, [PheOMent][NAP] exhibited lower lipophilicity ($\log P = 1.36$ vs. 1.70). Liposomal formulations containing the modified derivative showed high encapsulation efficiency (89.6–90.7%), higher than that observed for naproxen-loaded liposomes (51.7–54.7%). The prepared systems exhibited bimodal particle size distributions, comprising both submicrometre and micrometre vesicle populations depending on the preparation method, as well as negative zeta potential values (-18.17 to -23.72 mV) and pH values ranging from 6.18 to 7.07, demonstrating physicochemical characteristics suitable for topical formulations. In vitro permeation studies using porcine skin demonstrated markedly enhanced transdermal delivery of [PheOMent][NAP]. After 24 h, cumulative permeation exceeded that of naproxen formulations by more than 1.5-fold (416.7 vs. 281.6 $\mu\text{g cm}^{-2}$). These findings indicate that liposomal formulations containing amino acid-modified naproxen derivatives represent a promising strategy for improving transdermal NSAID delivery.

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Keywords: naproxen derivatives; amino acid esters; liposomal drug delivery; transdermal delivery systems; skin permeation; encapsulation efficiency; physicochemical characterization; modified NSAIDs

1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used in the treatment of pain and inflammatory disorders due to their high therapeutic efficacy, broad availability, and relatively low cost [1–3]. Among them, naproxen is commonly applied in the management of musculoskeletal diseases, rheumatoid arthritis, postoperative pain, and other inflammatory conditions [4,5]. Its pharmacological activity is associated with inhibition of cyclooxygenase enzymes (COX-1 and COX-2), resulting in reduced prostaglandin synthesis and alleviation of inflammation and pain [5,6]. Despite its effectiveness, naproxen therapy is often associated with gastrointestinal adverse effects, particularly during long-term administration, as well as poor aqueous solubility and limited bioavailability, which may reduce its therapeutic performance [2,3,5,7–11].

To overcome these limitations, increasing attention has been directed toward structural modification of NSAIDs aimed at improving their physicochemical and pharmacokinetic properties [12–15]. One promising approach involves conjugation of NSAIDs with amino acid derivatives, which may improve drug solubility, lipophilicity, stability, and membrane permeability while maintaining therapeutic activity [13–21]. In particular, amino acid ester derivatives containing naturally occurring terpenes, such as menthol, have attracted considerable interest because menthol is a well-established skin penetration enhancer capable of reversibly modifying the lipid organization of the stratum corneum [22–28]. In the present study, menthol was selected due to its penetration-enhancing properties and compatibility with lipid-based delivery systems, whereas L-phenylalanine was chosen because its hydrophobic aromatic side chain may promote favorable interactions with biological membranes and liposomal bilayers [15,22–30].

Lipid-based drug delivery systems, particularly liposomes, have emerged as promising carriers for poorly soluble drugs due to their biocompatibility, biodegradability, and ability to encapsulate lipophilic compounds [28,31–35]. Liposomal systems can improve drug stability, enable controlled release, and facilitate transdermal delivery by enhancing penetration through the skin barrier [31–41]. Therefore, combining structurally modified naproxen derivatives with liposomal carriers may constitute a promising strategy for improving transdermal NSAID delivery and enhancing therapeutic efficacy.

We hypothesized that the structural modification of naproxen through the formation of an amino acid menthol ester naproxenate ([PheOMent][NAP]) combined with encapsulation into liposomal carriers would improve its physicochemical properties, enhance encapsulation efficiency, and increase transdermal permeation compared with unmodified naproxen while maintaining formulation characteristics suitable for dermal application.

The novelty of this work lies in combining rational molecular modification of naproxen with liposomal encapsulation to simultaneously improve drug loading and transdermal delivery. To the best of our knowledge, this is the first study describing liposomal formulations containing L-phenylalanine menthol ester naproxenate.

Therefore, the aim of this study was to synthesize and characterize a novel amino acid-based naproxen derivative, L-phenylalanine menthol ester naproxenate ([PheOMent][NAP]), and to investigate its suitability for transdermal delivery. Furthermore, liposomal formulations containing the obtained compound were developed and characterized in terms of their physicochemical properties. Finally, the influence of

structural modification and liposomal encapsulation on the in vitro skin permeation behavior of naproxen was evaluated.

2. Materials and Methods

2.1. Chemicals and Reagents

All chemicals and solvents used in this study were of analytical grade and used without further purification unless otherwise stated. N-Boc-L-leucine (97%) and N-Boc-L-phenylalanine (99.9%) were purchased from AmBeed (Buffalo Grove, IL, USA). L-Phenylalanine (99%) and n-octanol (99%) were obtained from Alfa Aesar (Ward Hill, MA, USA). L-Menthol (>99%), aqueous ammonia solution (>25%), and acetonitrile (gradient grade, $\geq 99.9\%$) were supplied by Sigma-Aldrich (St. Louis, MO, USA). Dichloromethane (99.9%) and isopropanol (99.9%) were purchased from Eurochem BGD (Tarnów, Poland). 4-Dimethylaminopyridine (DMAP, >99%) was obtained from Glentham Life Sciences (Corkham, UK), while N,N'-dicyclohexylcarbodiimide (DCC, 99%) was purchased from Acros Organics (Geel, Belgium). Naproxen ((S)-(+)-2-(6-methoxynaphthalen-2-yl)propanoic acid, >98%) was obtained from AmBeed (Buffalo Grove, IL, USA). Soy lecithin containing more than 94% phosphatidylcholine and less than 2% triglycerides was purchased from POL-AURA (Morąg, Poland). Cholesterol ((3 β)-cholest-5-en-3-ol, 98%) was obtained from Chemat (Konin, Poland). Chloroform (99.9%) and methanol (99.9%) were supplied by STANLAB (Lublin, Poland). Dimethyl sulfoxide (DMSO, 99.7%), sodium chloride (>99.9%), diethyl ether (99.5%), and absolute ethanol (99.8%) were purchased from Chem-pur (Piekary Śląskie, Poland) and POCH (Gliwice, Poland), respectively. PBS tablets (10 mM phosphate buffer and 3 mM KCl, pH 7.4 at 25 °C) were obtained from Merck (Darmstadt, Germany). Deuterated chloroform (CDCl_3 , 99.8% + 0.03% TMS) and DMSO- d_6 (99.8% + 0.03% TMS) were supplied by Deutero GmbH (Kastellaun, Germany).

2.2. Synthesis Procedure of L-Phenylalanine Menthol Ester Naproxenate

L-phenylalanine menthol ester naproxenate ([PheOMent][NAP]) was synthesized using a three-step procedure (Figure 1) involving: (i) esterification of N-Boc-L-phenylalanine with L-menthol, followed by deprotection to obtain L-phenylalanine menthol ester hydrochloride; (ii) neutralization of the hydrochloride salt to the corresponding free base; and (iii) proton transfer reaction between the free ester and naproxen.

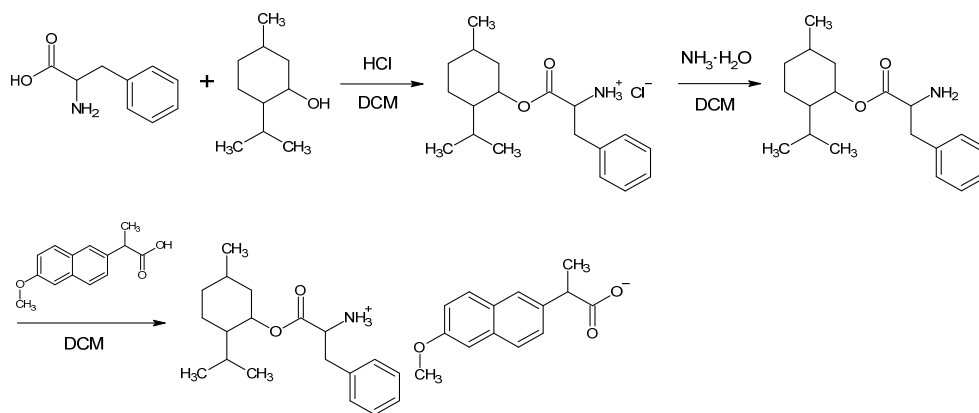


Figure 1. Synthetic pathway for the preparation of L-phenylalanine menthol ester naproxenate ([PheOMent][NAP]) via esterification, deprotection, neutralization, and subsequent salt formation with naproxen.

In the first step, N-Boc-L-phenylalanine (approximately 3.0 mmol) was esterified with L-menthol (approximately 3.3 mmol) in the presence of N,N'-

dicyclohexylcarbodiimide (DCC; 3.6 mmol) as a coupling agent and 4-dimethylaminopyridine (DMAP; 0.8 mmol) as a catalyst, using dichloromethane as the reaction medium (20 mL) under cooling, followed by stirring at room temperature for 24 h. After completion of the coupling reaction, the protected ester was isolated by filtration, extraction and solvent removal, and subsequently subjected to acid-mediated deprotection to remove the Boc protecting group, yielding L-phenylalanine menthol ester hydrochloride ([PheOMent][HCl]). When necessary, the product was additionally purified by washing with diethyl ether before vacuum drying.

In the second step, [PheOMent][HCl] (approximately 1–2 mmol) was neutralized with an aqueous ammonia solution using a substrate-to-base molar ratio of 1:6 to convert the hydrochloride salt into the corresponding free amine, L-phenylalanine menthol ester ([PheOMent]). The resulting free amine was extracted with dichloromethane, and the organic phase was concentrated under reduced pressure to obtain L-phenylalanine menthol ester ([PheOMent]).

In the third step, [PheOMent] (approximately 2 mmol) was reacted with an equimolar amount of naproxen ((S)-(+)-2-(6-methoxynaphthalen-2-yl)propanoic acid) in dichloromethane at room temperature. After 30 min of stirring, the solvent was removed under reduced pressure. The acid–base proton transfer between the amino group of the L-phenylalanine menthol ester and the carboxylic group of naproxen led to the formation of the organic salt–L-phenylalanine menthol ester naproxenate ([PheOMent][NAP]). The final product was purified and dried under reduced pressure to constant mass.

2.3. Nuclear Magnetic Resonance (NMR) Spectroscopy

The chemical structures of the synthesized compounds were confirmed using proton (^1H NMR) and carbon (^{13}C NMR) nuclear magnetic resonance spectroscopy. Approximately 30 mg of each compound was dissolved in 0.7 mL of the appropriate deuterated solvent (CDCl_3 or DMSO-d_6), transferred into 5 mm NMR tubes, and analyzed without further purification. Spectra were recorded using a Bruker Avance 400 MHz spectrometer (Bruker, Mannheim, Germany) at room temperature. Deuterated chloroform (CDCl_3) and dimethyl sulfoxide (DMSO-d_6) containing tetramethylsilane (TMS) as an internal standard were used as solvents. Chemical shifts (δ) were expressed in parts per million (ppm), while coupling constants (J) were reported in Hz.

2.4. Fourier Transform Infrared (FT-IR) Spectroscopy

FT-IR spectra were recorded using a Thermo Scientific Nicolet iS5 FT-IR spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) equipped with an ATR accessory. Spectra were collected in the range of $4000\text{--}400\text{ cm}^{-1}$ with a resolution of 4 cm^{-1} and 32 scans per sample. The obtained spectra were analyzed to identify characteristic absorption bands corresponding to amino, ester, carboxyl, and aromatic functional groups. Samples were analyzed directly using the ATR crystal without additional preparation. Background spectra were collected before each measurement and automatically subtracted from the sample spectra.

2.5. Solubility Determination

The solubility of the synthesized compounds was evaluated according to the procedure described by Vogel [42]. Approximately 10 mg of each compound was accurately weighed into a glass vial, and the selected solvent was added stepwise in 100 μL portions at room temperature. After each addition, the sample was mixed and visually inspected. Solvent addition was continued until a clear solution was obtained or until the total added volume exceeded 300 μL .

Five solvents with different polarities were used: deionized water, absolute ethanol, dimethyl sulfoxide (DMSO), chloroform, and diethyl ether. The solubility of each compound was assessed based on the minimum volume of solvent required to achieve complete dissolution at room temperature. The compounds were classified according to the following criteria: (i) readily soluble, requiring less than 100 μL of solvent; (ii) slightly soluble, requiring 100–300 μL of solvent; and (iii) practically insoluble, requiring more than 300 μL of solvent to form a clear solution.

All measurements were performed in triplicate, and the results were expressed as the average solubility classification for each tested solvent.

2.6. Optical Rotation Measurement Procedure

Optical rotation measurements were carried out using a Rudolph Research Analytical Autopol polarimeter (Hackettstown, NJ, USA) at room temperature. Samples were dissolved in appropriate solvents, and measurements were performed at a wavelength of 589 nm using a 1 dm optical cell.

2.7. Thermogravimetric Analysis (TG) Procedure

The thermal stability of the synthesized compounds was evaluated by thermogravimetric analysis (TG) using a Proteus Thermal Analysis TG 209 F1 Libra instrument (NETZSCH, Selb, Germany). Samples weighing approximately 5 mg were placed in corundum crucibles (without any additional pretreatment) and heated from 25 to 1000 $^{\circ}\text{C}$ under a controlled atmosphere. The measurements were performed using a nitrogen flow rate of 10 mL min^{-1} and an air flow rate of 25 mL min^{-1} . Changes in sample mass were continuously recorded as a function of temperature, enabling the determination of thermal stability and decomposition behavior of the analyzed compounds.

2.8. Differential Scanning Calorimetry (DSC) Procedure

The phase transition behavior of the synthesized compounds was investigated using a TA Instruments Q-100 differential scanning calorimeter (TA Instruments, New Castle, DE, USA). Measurements were performed over a temperature range from 25 $^{\circ}\text{C}$ to the decomposition temperature previously determined by thermogravimetric analysis (TG). Samples weighing approximately 10 mg were sealed in aluminum pans and analyzed under a nitrogen atmosphere. The obtained thermograms were used to determine phase transition temperatures and evaluate the thermal properties of the investigated compounds.

2.9. X-Ray Diffraction (XRD) Procedure

The crystalline structure of the synthesized compounds was investigated by powder X-ray diffraction (XRD) using an Aeris X-rays ON diffractometer (Malvern Panalytical, Malvern, UK). Diffractograms were recorded over a 2θ range of 7–90 $^{\circ}$ employing $\text{CuK}\alpha$ radiation with a wavelength of $\lambda = 1.54056 \text{ \AA}$. The obtained diffraction patterns were used to evaluate the crystallinity and structural organization of the analyzed compounds. Powdered samples were gently homogenized and placed on the sample holder without preferred orientation before analysis.

2.10. Determination of Partition Coefficient (Log P)

The lipophilicity of naproxen and its derivative was determined using the shake-flask method based on the n-octanol/water partition coefficient (log P). Equal volumes of mutually saturated n-octanol and water phases were mixed with the tested compounds (10 mg) and shaken until equilibrium was reached. After phase separation, compound concentrations were determined by HPLC, and log P values were calculated.

2.11. Preparation Procedure of Liposomal Formulations

Liposomal formulations were prepared using the thin-film hydration method (Bangham method), as described by Torchilin and Weissig [43]. Briefly, soy lecithin and cholesterol were mixed at a molar ratio of 1:1 and dissolved either in chloroform or in a chloroform–methanol mixture (2:1, *v/v*). The resulting lipid solution was transferred to a 50 mL round-bottom flask.

For formulations containing active substances, naproxen or L-phenylalanine menthol ester naproxenate ([PheOMent][NAP]) was added to the lipid mixture prior to dissolution in the organic solvent. Naproxen and L-phenylalanine menthol ester naproxenate were added in equimolar amounts during lipid film preparation (0.434 mmol), corresponding to approximately 100 mg of naproxen and 232 mg of [PheOMent][NAP], respectively.

Organic solvents were removed using a rotary evaporator (Heidolph, Schwabach, Germany) under reduced pressure at approximately 60 °C until a thin lipid film was formed on the inner wall of the flask. The obtained film was subsequently hydrated with 10 mL of phosphate-buffered saline (PBS, pH 7.4), resulting in spontaneous self-assembly of phospholipid vesicles.

The resulting suspensions were subjected to ultrasonic homogenization to reduce vesicle size and improve formulation homogeneity. Subsequently, the dispersions were centrifuged to remove non-encapsulated material and obtain stable liposomal formulations.

Two preparation variants differing in the solvent system used during lipid film formation were investigated: (i) chloroform–methanol (2:1, *v/v*), designated as formulation type 1, and (ii) chloroform alone, designated as formulation type 2. Blank liposomes and liposomes containing naproxen or [PheOMent][NAP] were prepared using both approaches.

2.12. Surface Tension Measurement Procedure

The surface tension of the prepared liposomal formulations was determined using a DCAT 15 tensiometer (DataPhysics Instruments, Filderstadt, Germany) equipped with a platinum–iridium Wilhelmy plate (PT 11). Measurements were performed according to the Wilhelmy plate method for surface force analysis. Surface tension was calculated based on the wetting force acting on the plate during its contact with the liquid surface. Data acquisition and analysis were carried out using DCATS software (version 4.1.86.164) supplied by the instrument manufacturer.

2.13. Particle Size Analysis Procedure

The particle size distribution of the liposomal formulations was determined by laser diffraction using a Mastersizer 3000 analyzer (Malvern Instruments, Malvern, UK) equipped with a Hydro EV wet dispersion unit. Measurements were performed according to Mie theory. Demineralized water at 20 °C, with a refractive index of 1.33, was used as the dispersion medium. The samples were dispersed under continuous stirring at 1200 rpm using a propeller stirrer.

The refractive index of each formulation was determined prior to analysis. Samples were gradually added to the dispersion medium until an obscuration value between 2 and 5% was achieved. Measurements were carried out using a red laser (632.8 nm) for 20 s, followed by a blue laser (470 nm) for an additional 20 s. Each analysis consisted of 77–81 measurement repetitions. The obtained data were used to evaluate the particle size distribution and homogeneity of the liposomal formulations.

2.14. Zeta Potential Measurement Procedure

The zeta potential of the prepared liposomal formulations was determined using a Litesizer DLS 700 analyzer (Anton Paar, Graz, Austria) based on electrophoretic light scattering (ELS). Measurements were performed using Kalliope software (version 2.40) supplied by the instrument manufacturer and Omega cuvettes dedicated to zeta potential analysis. Samples were analyzed in phosphate-buffered saline (PBS, pH 7.4) at 20 °C. The obtained zeta potential values were used to assess the surface charge and colloidal stability of the liposomal systems. Data acquisition and analysis were performed using Kalliope™ software (version 1.8, Anton Paar, Graz, Austria).

2.15. pH Determination Procedure

The pH of the prepared liposomal formulations was determined using an HI 221 microprocessor pH meter (Hanna Instruments, Smithfield, RI, USA) equipped with a Hanna HI 1131B combined glass electrode. Measurements were performed at room temperature following instrument calibration with standard buffer solutions of pH 4.0, 7.0, and 9.0 (Chempur, Piekary Śląskie, Poland). The electrode was rinsed with distilled water between successive measurements to prevent sample cross-contamination. pH values were recorded after stabilization of the instrument reading. The obtained results were used to assess the suitability of the formulations for dermal application.

2.16. Encapsulation Efficiency Determination

The encapsulation efficiency (EE) of the active substances in the liposomal formulations was determined based on the amount of non-encapsulated compound remaining in the aqueous phase after separation of the liposomal fraction from the dispersion medium. The concentration of free naproxen or L-phenylalanine menthol ester naproxenate in the aqueous phase was quantified by high-performance liquid chromatography (HPLC). The chromatographic conditions and analytical procedure are described in Section 2.19 (HPLC Analysis).

Encapsulation efficiency was calculated as the percentage of the initial amount of active substance successfully incorporated into the liposomal carrier according to Equation (1):

$$EE(\%) = \frac{m_{total} - m_{free}}{m_{total}} \times 100 \quad (1)$$

where m_{total} is the total amount of active substance used during formulation preparation, and m_{free} is the amount of non-encapsulated active substance determined in the aqueous phase.

The obtained EE values were used to evaluate the ability of the liposomal systems to incorporate and retain the investigated active compounds.

2.17. Microscopic Observations Procedure

Microscopic observations of the liposomal formulations were performed using a Genetic Pro optical microscope (Delta Optical, Nowe Osiny, Poland). Analyses were carried out in transmitted light using objectives with magnifications of 4×, 10×, 40×, and 100×, allowing evaluation of the morphology and dispersion characteristics of the prepared liposomal systems.

Samples were prepared by placing a small amount of each formulation onto a micro-metric glass slide equipped with a 1/100 mm scale. The obtained micrographs were used for qualitative assessment of vesicle morphology, size distribution, and the presence of possible aggregates within the formulations.

2.18. In Vitro Skin Permeation Study Procedure

In vitro skin permeation studies were performed using Franz diffusion cells (Phoenix DB-6, Teledyne Hanson Research, Chatsworth, CA, USA). The receptor chambers were filled with phosphate-buffered saline (PBS, pH 7.4) maintained at 37.0 ± 0.2 °C and continuously stirred at 350 rpm. Liposomal formulations dispersed in PBS were applied to the donor chambers. The experiments were conducted for 24 h, and the donor chambers were sealed to prevent solvent evaporation.

Samples (0.3 mL) were withdrawn from the receptor phase at predetermined time intervals (0.5, 1, 2, 3, 5, 8, and 24 h). After each sampling, the receptor chamber volume was replenished with an equal volume of fresh PBS to maintain sink conditions. The concentration of permeated active substance was determined by high-performance liquid chromatography (HPLC).

The cumulative amount of permeated active substance (A , $\mu\text{g cm}^{-2}$) was plotted as a function of time, and permeation parameters were calculated according to the following equation:

$$A = J_{ss}(t - LT)$$

where A is the cumulative amount of permeated active substance ($\mu\text{g cm}^{-2}$), J_{ss} is the steady-state flux ($\mu\text{g cm}^{-2} \text{ h}^{-1}$), t is the permeation time (h), and LT is the lag time (h).

The steady-state flux (J_{ss}) was determined from the slope of the linear portion of the cumulative permeation curve, while the lag time (LT) was obtained from the intercept of the extrapolated line with the time axis.

The permeability coefficient (K_p) was calculated according to Equation (2):

$$K_p = \frac{J_{ss}}{C} \quad (2)$$

where C is the concentration of the active substance in the donor phase.

The diffusion coefficient (D) was calculated using Equation (3):

$$D = \frac{h^2}{6LT} \quad (3)$$

where h is the thickness of the skin membrane (mm).

The skin partition coefficient (K_m) was determined according to Equation (4):

$$K_m = \frac{K_p \cdot h}{D} \quad (4)$$

The calculated permeation parameters were used to compare the transdermal delivery performance of liposomal formulations containing naproxen and L-phenylalanine menthol ester naproxenate.

2.19. High-Performance Liquid Chromatography (HPLC) Analysis

Quantitative analyses of naproxen and L-phenylalanine menthol ester naproxenate were performed using a Nexera-i LC-2040C 3D high-performance liquid chromatography system (Shimadzu, Kyoto, Japan) equipped with a diode-array detector (DAD). Chromatographic separation was achieved on a Kinetex F5 column (2.6 μm particle size, 100 Å pore size; Phenomenex, Torrance, CA, USA).

The mobile phase consisted of water and acetonitrile (50:50, v/v) delivered at a flow rate of 1.0 mL min^{-1} . Samples were maintained in the autosampler at 25 °C throughout the analysis. Detection was performed at a wavelength of 230 nm, and chromatograms recorded at this wavelength were used for quantitative evaluation.

For each sample, at least three injections were performed, and the obtained results were averaged. The concentrations of naproxen and L-phenylalanine menthol ester naproxenate were determined based on peak areas using the external calibration curve method. Calibration curves were prepared for each analyte and used for the quantitative

determination of active substances in solubility, encapsulation efficiency, and permeation studies.

2.20. Statistical Analysis

Unless otherwise stated, all experiments were performed in triplicate ($n = 3$), and the results are presented as mean $\pm$ standard deviation (SD).

3. Results and Discussions

3.1. Synthesis of L-Phenylalanine Menthol Ester Naproxenate

L-phenylalanine menthol ester naproxenate ([PheOMent][NAP]) was successfully obtained through a three-step synthetic route involving esterification, deprotection, neutralization, and subsequent salt formation with naproxen. The synthetic pathway enabled the preparation of the target compound in satisfactory overall yield and purity. The isolated yields obtained during individual synthetic steps are summarized in Table 1.

Table 1. Isolated yields obtained during the synthesis of menthol ester amino acid derivatives and the target naproxenate salt.

Compound	Yield (%)
[LeuOMent][HCl]	1–30
[PheOMent][HCl]	39–63
[PheOMent]	96–98
[PheOMent][NAP]	75–83

The variability observed in the isolated yields of [PheOMent][HCl] was mainly attributed to differences in purification efficiency and product isolation following the esterification and deprotection steps.

The first synthetic step, involving esterification followed by Boc deprotection, represented the most challenging stage of the synthesis. Although satisfactory reaction conversion was achieved, the isolated yield varied between individual syntheses because additional purification was required to remove reaction by-products. Consequently, the variability reflected purification losses rather than poor reproducibility of the synthetic procedure. In contrast, the neutralization of the hydrochloride salt and the subsequent proton-transfer reaction with naproxen proceeded reproducibly and consistently afforded high isolated yields (96–98% and 75–83%, respectively).

The synthesis of the phenylalanine derivative proceeded with satisfactory reproducibility, whereas the corresponding leucine derivative exhibited considerably lower and less reproducible yields. Consequently, the leucine derivative was excluded from further investigation, and only [PheOMent][NAP] was selected for physicochemical characterization and formulation studies.

3.2. Structural Characterization of the Synthesized Compounds by NMR and FT-IR Spectroscopy

The successful formation of [PheOMent][NAP] was confirmed by NMR and FT-IR analyses, which evidenced the presence of both the phenylalanine menthol ester cation and naproxenate anion as well as the disappearance of signals characteristic of the protonated hydrochloride precursor. Representative ^1H and ^{13}C NMR spectra of L-phenylalanine menthol ester hydrochloride, L-phenylalanine menthol ester, and L-phenylalanine menthol ester naproxenate are presented in Figures S1–S6, while the corresponding FT-IR spectra are shown in Figures S7 and S8 of the Supplementary Materials.

The ^1H NMR spectra of [PheOMent][HCl] and [PheOMent][NAP] exhibited characteristic singlet signals assigned to the protonated amino group (NH_3^+) observed at approximately 9 and 5 ppm, respectively, confirming the successful formation of both ionic compounds. The observed shift in the NH_3^+ resonance reflects the different electronic environments provided by the chloride and naproxenate counterions. Furthermore, spectra confirmed the presence of the menthol and L-phenylalanine moieties in all synthesized compounds, while additional resonances corresponding to the naproxenate fragment were identified in the spectrum of [PheOMent][NAP]. The ^{13}C NMR spectra were consistent with the proposed structures and confirmed the expected number of carbon atoms. In particular, signals originating from both the phenylalanine menthol ester cation and the naproxenate anion were observed for [PheOMent][NAP], including a characteristic resonance of the carboxyl carbon atom at approximately 174 ppm.

The FT-IR spectra further supported the successful formation of [PheOMent][NAP]. A broad absorption band at 3322 cm^{-1} was assigned to the N–H stretching vibrations of the protonated amino group (NH_3^+), whereas the bands at 2944, 2926, and 2852 cm^{-1} corresponded to the aliphatic C–H stretching vibrations of the menthol fragment. The strong absorption band at 1742 cm^{-1} was attributed to the ester carbonyl (C=O) stretching vibration, confirming the presence of the menthol ester moiety. The bands observed at 1628 and 1376 cm^{-1} were assigned to the asymmetric ($\nu_{\text{as}}(\text{COO}^-)$) and symmetric ($\nu_{\text{s}}(\text{COO}^-)$) stretching vibrations of the carboxylate group, respectively. The separation between these two bands ($\Delta\nu \approx 252\text{ cm}^{-1}$) confirmed the ionic character of the carboxylate functionality and the successful formation of the naproxenate salt. Furthermore, the absorption bands at 1603, 1570, 1523, and 1505 cm^{-1} originated from aromatic C=C skeletal vibrations of the phenylalanine and naproxen aromatic rings. The bands in the $1261\text{--}1037\text{ cm}^{-1}$ region were attributed to C–O stretching vibrations of the ester group and methoxy functionality, while the absorptions below 900 cm^{-1} corresponded to aromatic C–H out-of-plane bending vibrations, characteristic of substituted aromatic rings. Overall, the FT-IR results were fully consistent with the proposed structure of the synthesized naproxenate salt.

3.3. Solubility Studies

The solubility of [PheOMent][HCl], naproxen, and [PheOMent][NAP] was evaluated in selected polar and non-polar solvents. The obtained results are summarized in Table 2. Differences in solubility behavior were observed among the investigated compounds, reflecting changes in their physicochemical properties resulting from structural modification.

Table 2. Solubility of the investigated compounds in selected solvents.

Solvent	[PheOMent][HCl]	NAP	[PheOMent][NAP]
Diethyl ether	–	–	–
Chloroform	–	+/–	+
DMSO	+	+	–
Ethanol	+	+/–	+
Water	–	–	–

Symbols indicate the minimum solvent volume required for complete dissolution: (+) readily soluble ($<100\text{ }\mu\text{L}$ of solvent); (+/–) slightly soluble ($100\text{--}300\text{ }\mu\text{L}$); (–) practically insoluble ($>300\text{ }\mu\text{L}$).

The hydrochloride salt exhibited solubility only in polar solvents, namely ethanol and DMSO, indicating its highly polar character. In contrast, [PheOMent][NAP] retained solubility in ethanol and additionally showed good solubility in chloroform, suggesting an increase in lipophilic character following naproxenate formation. Naproxen was readily soluble in DMSO and exhibited limited solubility in ethanol and chloroform. Overall,

the results indicate that conversion of the hydrochloride salt into the corresponding naproxenate altered the polarity profile of the compound, yielding a derivative with intermediate polarity between [PheOMent][HCl] and naproxen.

Solubility in Water and Phosphate-Buffered Saline (PBS)

The solubility of naproxen and L-phenylalanine menthol ester naproxenate ([PheOMent][NAP]) was determined in deionized water and phosphate-buffered saline (PBS). The obtained results are summarized in Table 3. To facilitate comparison, the solubility values were additionally expressed as the equivalent concentration of naproxen present in the investigated compounds.

Table 3. Solubility of naproxen and [PheOMent][NAP] in water and PBS. Values are expressed as mean $\pm$ SD ($n = 3$).

Compound	Solubility in Water		Solubility in PBS	
	[g/dm ³]	[g NAP/dm ³]	[g/dm ³]	[g NAP/dm ³]
NAP	0.066 $\pm$ 0.004	0.066 $\pm$ 0.004	1.378 $\pm$ 0.133	1.378 $\pm$ 0.133
[PheOMent][NAP]	0.239 $\pm$ 0.028	0.103 $\pm$ 0.012	0.514 $\pm$ 0.022	0.222 $\pm$ 0.009

The solubility of [PheOMent][NAP] in water was approximately 3.6-fold higher than that of naproxen (0.239 $\pm$ 0.028 vs. 0.066 $\pm$ 0.004 g/dm³). When expressed as naproxen content, the solubility remained approximately 1.6-fold higher than that of the parent drug. In contrast, the derivative exhibited substantially lower solubility in PBS, corresponding to approximately 0.222 $\pm$ 0.009 g NAP/dm³ compared with 1.378 $\pm$ 0.133 g NAP/dm³ for naproxen.

These results demonstrate that the conversion of naproxen into the amino acid-based organic salt altered its solubility profile depending on the dissolution medium. The enhanced aqueous solubility of [PheOMent][NAP] is consistent with its lower logP value and may be associated with the ionic character of the compound. Furthermore, the reduced crystallinity observed in DSC and XRD analyses may contribute to the improved dissolution behavior in water. The lower solubility observed in PBS suggests that the ionization state of the compound and interactions with buffer components strongly influence its behavior in physiological media.

3.4. Optical Rotation Measurements

The optical activity of the synthesized compounds and selected starting materials was evaluated by polarimetric measurements. The obtained values of optical rotation, specific rotation, and molar rotation are summarized in Table 4.

Table 4. Optical rotation, specific rotation, and molar rotation values of the investigated compounds. Values are expressed as mean.

Compound	Solvent	Concentration [%]	Optical Rotation α_D^{25} [°Arc]	Specific Rotation $[\alpha]_D^{25}$	Molar Rotation $[M]_D^{25}$
L-Phe	Water	1.005	−0.166	−33.234	−43.593
Boc-L-Phe	Chloroform	1.005	+0.116	+22.886	+60.719
Menthol	Chloroform	1.006	−0.216	−42.942	−67.105
[PheOMent][HCl]	DMSO	1.004	−0.067	−13.347	−45.470
NAP	Chloroform	1.003	+0.339	+67.597	+155.649
[PheOMent][NAP]	Chloroform	1.007	−0.022	−4.171	−22.293

The measured specific rotation of L-phenylalanine in water was -33.23° , which is in good agreement with literature data reported for this amino acid [44]. All investigated compounds exhibited optical activity, confirming the preservation of chirality throughout the synthetic procedure. While Boc-L-phenylalanine and naproxen displayed positive specific rotation values, menthol, [PheOMent][HCl], and [PheOMent][NAP] rotated plane-polarized light in the opposite direction.

The hydrochloride salt [PheOMent][HCl] was insoluble in chloroform; therefore, DMSO was used as the solvent for polarimetric measurements. Compared with [PheOMent][HCl], the final naproxenate salt exhibited a lower magnitude of specific rotation. This behavior can be attributed to the incorporation of the optically active naproxenate anion, whose contribution to the overall chiroptical properties differs from that of the optically inactive chloride counterion. Consequently, the observed optical rotation of [PheOMent][NAP] reflects the combined influence of both the chiral phenylalanine menthol ester cation and the chiral naproxenate anion. These results confirm that replacement of the chloride ion with naproxenate alters the chiroptical properties of the system while preserving its optical activity.

3.5. Thermogravimetric Analysis (TG)

The thermal properties of the investigated compounds were evaluated by thermogravimetric analysis (TG) and differential thermal analysis (c-DTA). The characteristic thermal parameters obtained from the measurements are summarized in Table 5.

Table 5. Thermal properties of the investigated compounds determined by TG and c-DTA analyses. Values are expressed as mean.

Compound	Melting Temperature		Maximum Mass Loss Rate Temperature [°C]	5% Mass Loss Temperature [°C]	50% Mass Loss Temperature [°C]	Number of Decomposition Stages
	Onset [°C]	Endset [°C]				
L-Phe	-	-	212.1	199.4	220.5	3
Boc-L-Phe	82.8	94.8	200.1	145.3	201.6	3
Menthol	43.8	48.5	144.3	91.6	131.5	1
[PheOMent][HCl]	139.4	154.8	249.3	203.8	255.0	2
NAP	155.8	164.9	284.6	221.0	270.6	1
[PheOMent][NAP]	106.6	116.2	314.3	184.3	293.1	2

The TG analysis demonstrated that [PheOMent][NAP] remained thermally stable up to approximately 180 °C, as indicated by its 5% mass loss temperature (T5%) of 184.3 °C. Although this value was lower than that observed for naproxen (221.0 °C), the obtained derivative still exhibited sufficient thermal stability for potential pharmaceutical applications. The maximum decomposition rate for [PheOMent][NAP] occurred at 314.3 °C, which was higher than that of naproxen (284.6 °C), indicating a different degradation pathway after salt formation. Furthermore, c-DTA analysis revealed a melting range of 106.6–116.2 °C for [PheOMent][NAP]. In contrast, determination of the melting point of L-phenylalanine was not possible due to the overlap of melting and decomposition processes. A comparison of the TG curves of naproxen and [PheOMent][NAP] is presented in Figure 2.

Naproxen exhibited higher initial thermal stability, with a 5% mass loss occurring at 221.0 °C, whereas for [PheOMent][NAP] the corresponding temperature was 184.3 °C. Despite the lower onset of decomposition, the thermal degradation of the naproxenate salt proceeded over a broader temperature range and involved two decomposition stages, while naproxen decomposed predominantly in a single step. These results indicate that incorporation of the phenylalanine menthol ester cation altered the thermal properties of

naproxen, leading to a different degradation profile while maintaining sufficient thermal stability for further pharmaceutical development.

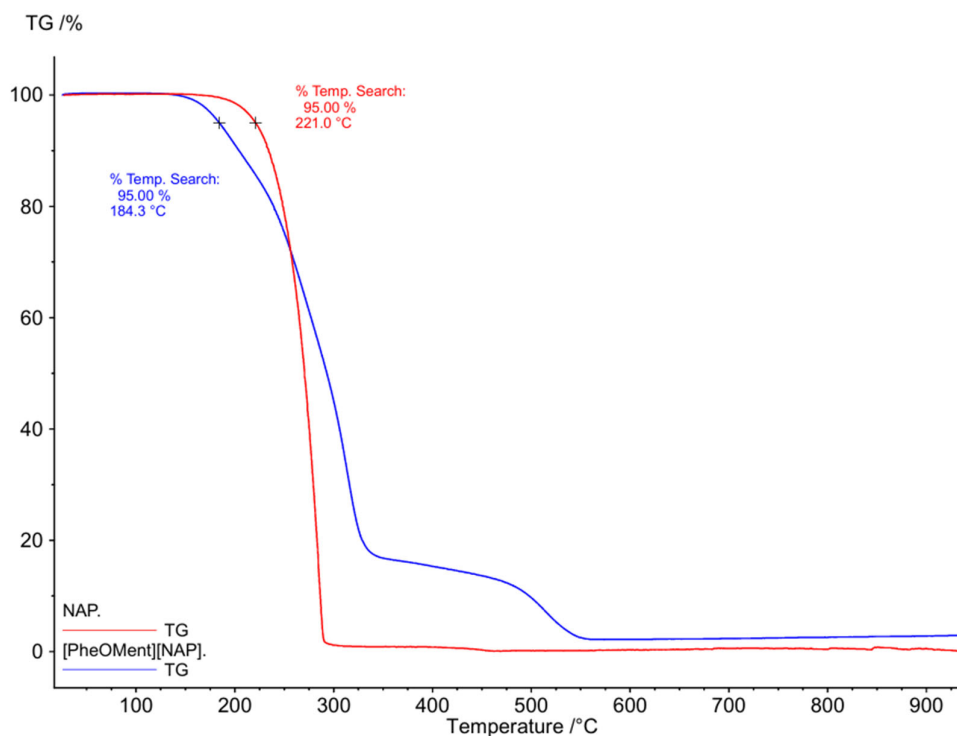


Figure 2. A comparison of the TG curves for naproxen and L-phenylalanine menthol ester naproxenate, with the temperatures corresponding to a 5% loss in mass marked on the graph.

Thermogravimetric analysis provides important information regarding the thermal stability of the synthesized compound during manufacturing and handling. Knowledge of the onset of thermal degradation is essential for selecting appropriate processing and drying conditions and for ensuring that no degradation occurs during formulation development. Therefore, TG analysis complements the DSC results and contributes to the overall physicochemical characterization of the developed naproxen derivative.

Although the developed formulations are intended for topical application under physiological conditions, evaluation of thermal stability is an important part of preformulation studies. Knowledge of the decomposition profile supports the selection of appropriate processing, drying, sterilization, and storage conditions while confirming that the compounds remain stable under conventional pharmaceutical manufacturing conditions.

3.6. Differential Scanning Calorimetry (DSC)

The DSC thermograms of L-phenylalanine menthol ester hydrochloride (orange curve) and L-phenylalanine menthol ester naproxenate (navy blue curve), recorded during two consecutive heating/cooling cycles, are presented in Figure 3. The direct comparison of both thermograms enables evaluation of the influence of the counterion on the thermal properties, phase transition behavior, and crystallization ability of the obtained ionic compounds.

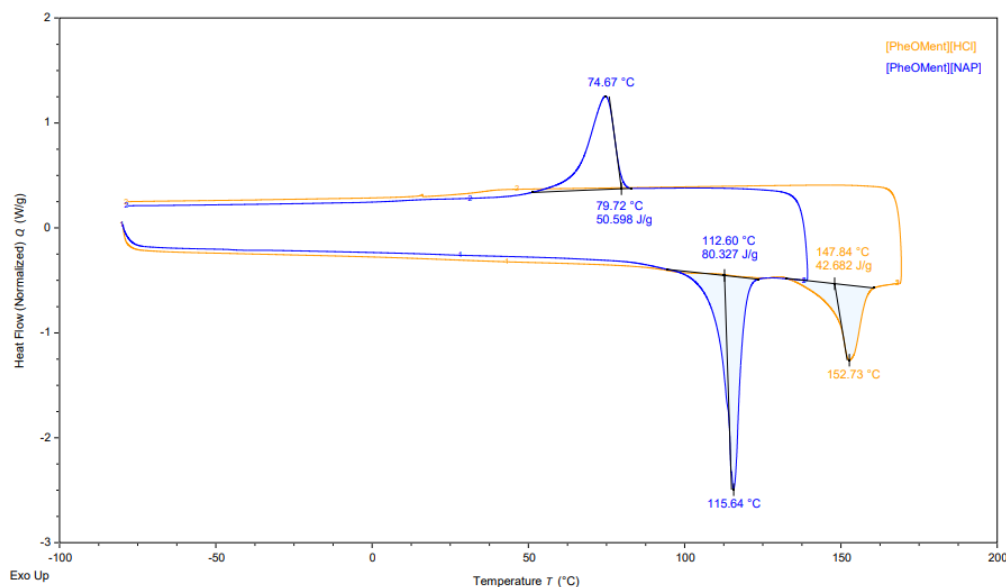


Figure 3. DSC thermograms of L-phenylalanine menthol hydrochloride and L-phenylalanine menthol ester naproxenate recorded during two consecutive heating/cooling cycles.

For L-phenylalanine menthol hydrochloride, the heating cycle revealed a distinct endothermic melting peak with an onset at 147.84 °C and maximum at 152.73 °C, indicating the presence of a crystalline phase in the initial material. During the subsequent cooling stage, no noticeable exothermic crystallization event was observed, suggesting that after melting the compound lacks sufficient structural mobility to reorganize into an ordered crystalline lattice under the applied cooling conditions. This behavior is indicative of a tendency to form an amorphous phase upon solidification.

In contrast, L-phenylalanine menthol ester naproxenate exhibited markedly different thermal behavior. The heating curve showed a sharp endothermic melting peak with an onset at 112.60 °C and a maximum at 115.64 °C, a well-defined crystalline structure in the initial sample and a substantially higher degree of crystalline order compared to the hydrochloride analogue. Unlike the hydrochloride analogue, the cooling cycle showed a pronounced exothermic crystallization peak with a maximum at 74.67 °C, demonstrating a strong ability of the compound to undergo structural reorganization and reform an ordered crystal lattice after melting.

Comparison of both thermograms clearly demonstrates that the nature of the counterion affects the thermal behavior and phase transition characteristics of the investigated ionic systems. L-phenylalanine menthol hydrochloride, despite melting at a higher temperature, shows no tendency to recrystallize upon cooling, suggesting that the presence of the small chloride anion promotes amorphous solidification. In contrast, L-phenylalanine menthol ester naproxenate, bearing a bulky organic naproxenate anion, exhibits a substantially higher melting enthalpy and pronounced recrystallization ability, indicating stronger and more directional intermolecular interactions within the crystal lattice. These results confirm that the incorporation of a large organic anion fundamentally alters the intermolecular interaction network, leading to enhanced crystalline order and reversible phase transition behavior compared with the hydrochloride analogue.

The thermal behaviour observed by DSC is also relevant from a pharmaceutical formulation perspective. Thermal analysis provides valuable information during preformulation studies by identifying phase transitions, changes in crystallinity, and possible interactions between the active compound and formulation excipients. Such information is important for selecting appropriate manufacturing conditions, predicting formulation

performance, and minimizing the risk of undesirable physical changes during processing. Although the developed liposomal formulations are intended for topical administration under physiological conditions, thermal characterization remains an important component of the comprehensive physicochemical evaluation of newly synthesized pharmaceutical compounds.

DSC analysis provides valuable information on the solid-state properties of the synthesized compound, including phase transitions and crystallinity, which may influence formulation stability, drug encapsulation, and long-term storage. These data therefore contribute to the rational design and optimization of lipid-based transdermal delivery systems.

3.7. X-Ray Diffraction (XRD) Analysis

The crystalline properties of naproxen and its derivatives were evaluated by X-ray diffraction (XRD). The obtained diffractograms are presented in Figure 4.

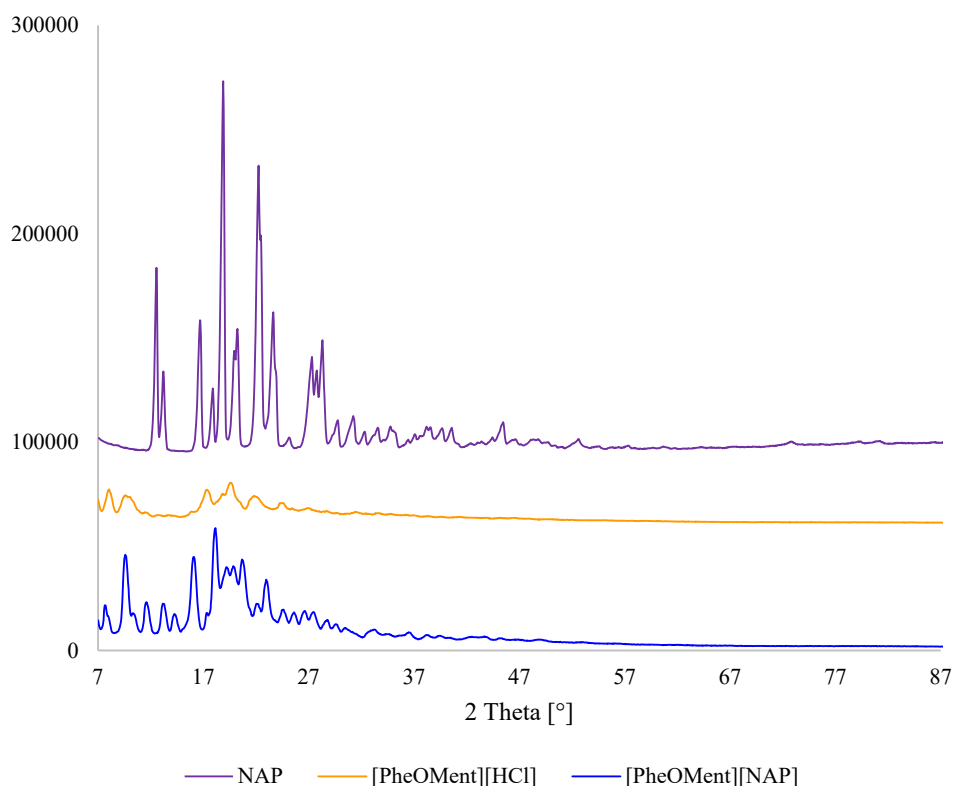


Figure 4. X-ray diffraction patterns of naproxen, L-phenylalanine menthol ester hydrochloride ([PheOMent][HCl]), and L-phenylalanine menthol ester naproxenate ([PheOMent][NAP]).

Naproxen exhibited numerous sharp and intense diffraction peaks throughout the 2θ range, confirming its highly crystalline nature. In contrast, the diffraction patterns of [PheOMent][HCl] displayed only weak and broad reflections, indicating a substantial reduction in long-range molecular order compared with the parent drug. The diffraction pattern of [PheOMent][NAP] retained several characteristic reflections; however, their positions and relative intensities differed markedly from those observed for naproxen, confirming the formation of a new solid phase rather than a simple physical mixture of the starting components.

The reduced intensity and broadening of the diffraction peaks observed for [PheOMent][HCl] and [PheOMent][NAP] indicate a lower degree of crystallinity, which

can be attributed to disruption of the original crystal packing following esterification and subsequent salt formation. Such structural changes are commonly associated with altered intermolecular interactions and modified molecular packing within the crystal lattice.

These observations are consistent with the TG and DSC results, which also demonstrated changes in the thermal behavior of the synthesized derivatives relative to naproxen. A reduced degree of crystallinity may facilitate drug incorporation into lipid-based carriers and may contribute to improved formulation performance by promoting more favorable solid-state properties during pharmaceutical development.

3.8. Determination of Partition Coefficient (Log P)

The lipophilicity of naproxen and its derivative, L-phenylalanine menthol ester naproxenate ([PheOMent][NAP]), was evaluated using the n-octanol/water partition coefficient. The obtained results are summarized in Table 6.

Table 6. Lipophilicity parameters of naproxen and [PheOMent][NAP]. Values are expressed as mean.

Compound	Log P
NAP	1.709
[PheOMent][NAP]	1.360

The experimentally determined log P value for naproxen was 1.709, whereas [PheOMent][NAP] exhibited a lower value of 1.360. Although the literature log P value reported for naproxen is approximately 3.18 [10], differences between literature and experimental data may arise from variations in analytical methodology, measurement conditions, and the influence of ionization in the biphasic system. Nevertheless, the obtained results clearly demonstrate that conversion of naproxen into the corresponding amino acid-based organic salt reduced its lipophilicity. This decrease may be associated with the ionic nature of [PheOMent][NAP] and could contribute to improved interactions with aqueous environments, potentially affecting its solubility and biopharmaceutical performance.

The observed decrease in the log P value of [PheOMent][NAP] compared with naproxen indicates a more balanced hydrophilic–lipophilic character. Such modification is advantageous for transdermal delivery, as compounds with moderate lipophilicity generally exhibit improved partitioning into both the liposomal bilayer and the skin barrier. Similar observations have been reported for amino acid-based ionic derivatives of NSAIDs, where reduced lipophilicity contributed to enhanced skin permeation.

3.9. Preparation of Liposomal Formulations

Liposomes were prepared using the thin-film hydration method according to the procedure described in Section 2.11 of the Materials and Methods. Three types of formulations were developed: drug-free liposomes, liposomes loaded with naproxen, and liposomes loaded with L-phenylalanine menthol ester naproxenate. For each formulation type, two variants were prepared, differing in the solvent system employed: a methanol–chloroform mixture and chloroform alone.

3.9.1. Preparation of Drug-Free Liposomes

Upon dissolution of the lipid components in both solvent variants, a clear, homogeneous solution with a pale-yellow color was obtained. Evaporation of the organic solvents resulted in the formation of a thin, yellowish lipid film deposited on the inner walls and bottom of the round-bottom flask. Subsequent addition of phosphate-buffered saline (PBS) followed by ultrasonication induced turbidity of the system, indicative of liposome

formation. The resulting suspension exhibited a milky appearance, and sedimentation of the dispersed phase was observed at the bottom of the flask after a period of time.

3.9.2. Preparation of Drug-Loaded Liposomes

Incorporation of the active substance into the lipid components required prior ultrasonication for approximately 5 min, due to the limited solubility of both naproxen and its salt derivative in the organic solvent systems. Following this step, the solvents were evaporated, yielding a white lipid film. Addition of PBS buffer induced gradual turbidity of the system, which was accelerated by ultrasonication. The duration of the ultrasonication step differed substantially between formulations: approximately 1 h for naproxen-loaded liposomes and 8 h for those containing L-phenylalanine menthol ester naproxenate.

In the case of naproxen, a homogeneous milky suspension was obtained. However, liposomes loaded with L-phenylalanine menthol ester naproxenate failed to form a uniform dispersion; the resulting suspension contained agglomerates of the salt compound varying in size. The difficulties encountered in dispersing L-phenylalanine menthol ester naproxenate may be attributed to its increased hydrophilicity and limited compatibility with both the lipid and aqueous phases of the liposomal system. In comparison, naproxen demonstrated superior dispersibility within the liposomal matrix. In both drug-loaded formulations, sedimentation of the dispersed phase was observed upon standing.

3.10. Surface Tension Measurements

Surface tension of the investigated systems was determined using the tensiometric method with a platinum Wilhelmy plate, according to the procedure described in Section 2.12 of the Materials and Methods. The results are summarized in Table 7.

The reduced surface tension values observed for the lipid mixtures (L1 and L2) relative to the individual components confirm the ability of phospholipids to undergo self-assembly and form liposomal structures. The incorporation of an active substance did not induce changes in surface tension—the values remained comparable to those of the drug-free lipid mixture, indicating successful integration of the active compound within the lipid bilayer structure.

Table 7. Surface tension of the investigated liposomal formulations. Values are expressed as mean.

Designation	Surface Tension [mN/m]
PBS	72.42
Soy lecithin1	26.14
Cholesterol1	44.79
L1	23.35
L2	23.29
LNAP1	23.35
LNAP2	23.22
L[PheOMent][NAP]1	22.71
L[PheOMent][NAP]2	22.88

(1) Solvent: CHCl₃/MeOH (2:1, *v/v*) (2) Solvent: CHCl₃.

The phospholipid bilayer of soy lecithin gives rise to liposomes characterized by a pronounced reduction in surface tension relative to PBS buffer—the surface tension of the lipid mixtures (L1: 23.35 mN/m, L2: 23.29 mN/m) was approximately three times lower than that of pure PBS (72.42 mN/m), confirming the high surface activity of soy lecithin and its capacity for spontaneous self-organization into liposomal structures. Notably, soy lecithin alone reduced surface tension to 26.14 mN/m, whereas cholesterol, as a single component, exhibited considerably weaker surface activity (44.79 mN/m), approximately

1.7-fold higher than that of lecithin. Nevertheless, the presence of cholesterol in the combined lipid system contributed to a further slight decrease in surface tension and stabilization of the interfacial layer—from 26.14 mN/m for lecithin alone to 23.35 mN/m for the L1 mixture—consistent with the classical model of liposomal membranes, in which cholesterol is known to condense and rigidify the lipid bilayer. The addition of naproxen or its derivative to the soy lecithin–cholesterol system did not produce changes in surface tension, with values remaining within the narrow range of 22.71–23.35 mN/m across all drug-loaded formulations, suggesting that these compounds are predominantly localized within the hydrophobic core of the lipid bilayer without disrupting phospholipid adsorption at the phase boundary.

3.11. Particle Size Analysis

A total of six liposomal formulations were characterized: drug-free liposomes, naproxen-loaded liposomes, and liposomes containing L-phenylalanine menthol ester naproxenate, each prepared by two variants of the thin-film hydration method differing in the organic solvent system employed. An overview of all investigated systems is presented in Figure 5.

The results revealed an influence of both the solvent system used during preparation and the type of active substance on the particle size characteristics of the liposomal formulations. All obtained systems exhibited a polydisperse character, with at least two distinct maxima in the size distribution profiles corresponding to particle populations of different dimensions.

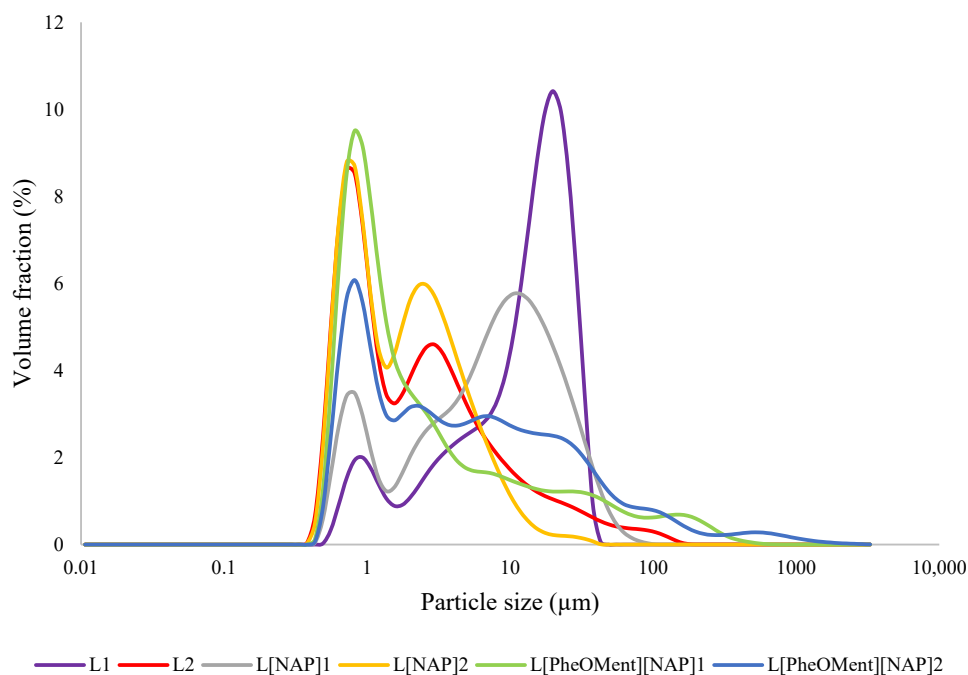


Figure 5. Particle size distribution of all investigated liposomal formulations.

In formulations L1 and LNAP1, prepared using the chloroform–methanol mixture ($\text{CHCl}_3/\text{MeOH}$, 2:1, *v/v*), a marked shift in the size distribution toward larger particles was observed (Figure 6). The dominant fraction comprised particles in the size range of approximately 1.548–42.792 μm , with the volumetric distribution maximum at a particle diameter of 19.984 μm . This indicates an increased tendency toward particle aggregation

when a binary solvent mixture is employed during formulation preparation, regardless of the presence of naproxen.

These micrometric dimensions are consistent with values reported for multilamellar vesicles (MLV) obtained by thin-layer hydration without a subsequent size-reduction step. Maestrelli et al. reported empty MLV liposomes of $1.58 \pm 0.08 \mu\text{m}$, increasing to $3.16\text{--}3.71 \mu\text{m}$ upon loading with a ketoprofen–cyclodextrin complex [45], confirming that vesicles obtained by simple film hydration—without extrusion, sonication, or homogenization—inherently fall within the low-micrometre range, in agreement with the dominant population observed here for L1 and LNAP1.

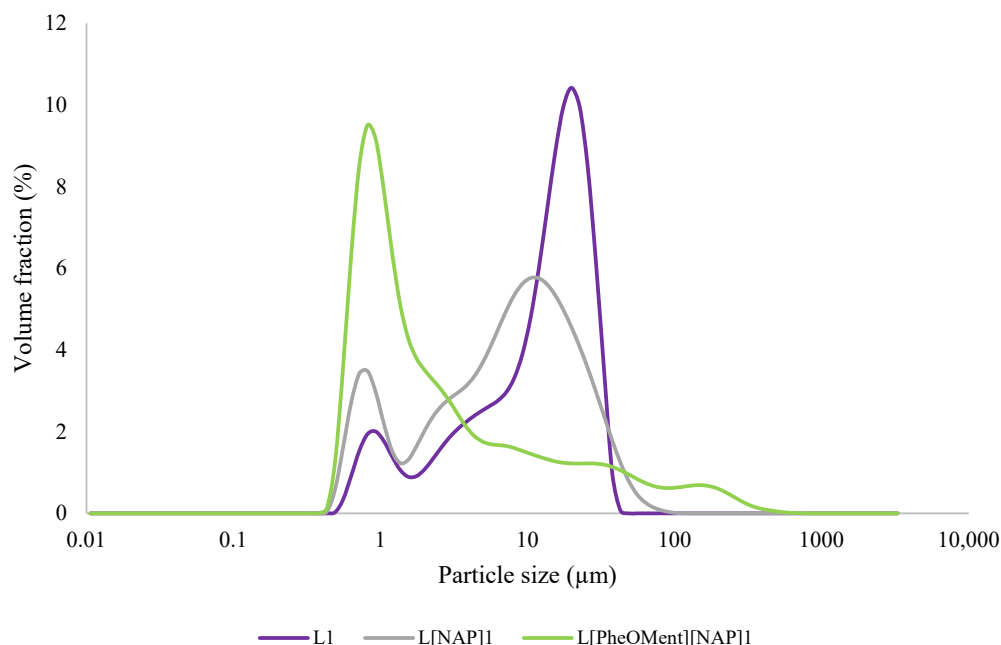


Figure 6. Particle size distribution of formulations prepared using the chloroform–methanol solvent mixture ($\text{CHCl}_3/\text{MeOH}$).

In contrast, formulations L2 and LNAP2, prepared using chloroform as the organic solvent during the thin-film preparation step, exhibited considerably narrower and more comparable size distributions (Figure 7). All particle size measurements were performed after hydration of the lipid film in distilled water. In both systems, a fine particle fraction was identified in the range of approximately $0.380\text{--}1.548 \mu\text{m}$, with the greatest volumetric contribution from particles of approximately $0.720 \mu\text{m}$ in diameter. A second, larger particle fraction was also present, spanning the range of $1.548\text{--}134.999 \mu\text{m}$; for formulation LNAP2, the distribution maximum of this fraction was located at a diameter of $2.271 \mu\text{m}$. These results demonstrate that the use of chloroform alone as the preparation solvent favors the formation of smaller particles compared to the binary solvent system.

This sub-micrometre fraction, although still above the nanometric range typical of ethanol-injection or homogenization-based methods, is markedly closer to sizes reported for other liposomal NSAID systems. Ibuprofen liposomes (IBU-LP) prepared by ethanol injection, without any extrusion or sonication step, showed a mean diameter of $228.00 \pm 8.60 \text{ nm}$ with a narrow PDI of 0.15 ± 0.02 [46], indicating that the preparation technique itself, and not merely the absence of a mechanical size-reduction step, can be decisive for achieving nanometric vesicles.

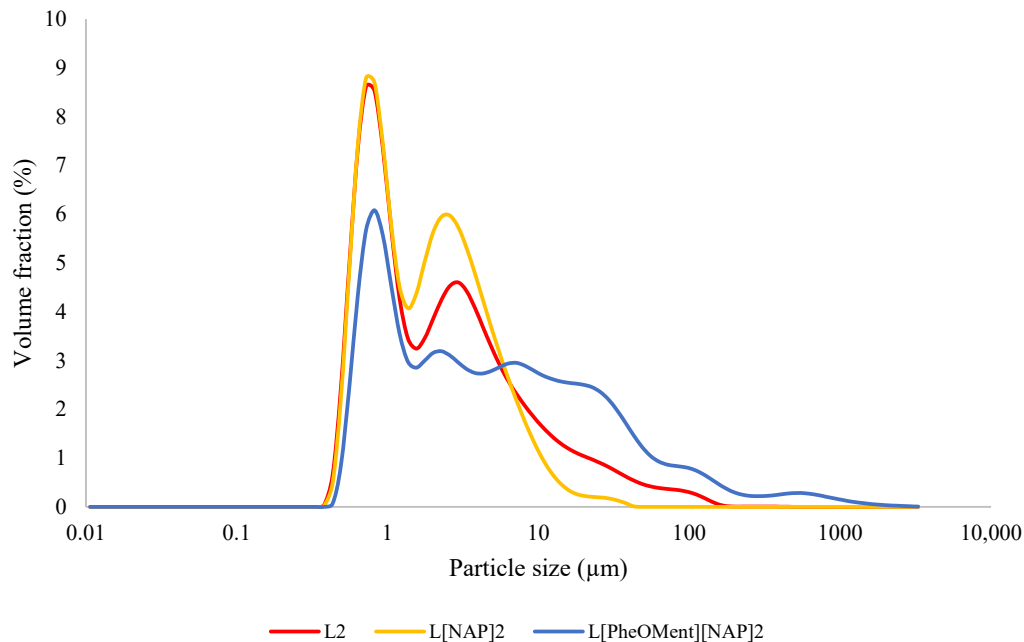


Figure 7. Particle size distribution of formulations prepared using chloroform as the sole solvent.

Comparison of liposomal systems prepared with the chloroform–methanol mixture versus chloroform alone indicates that the choice of solvent system exerts a greater influence on particle size distribution than the incorporation of the unmodified active substance. The addition of L-phenylalanine menthol ester naproxenate did not produce changes in particle size when chloroform was used as the sole solvent; however, in the binary solvent variant, it led to the formation of a fraction of very small liposomes, predominantly in the range of approximately 0.432–4.885 μm . In formulations prepared with chloroform alone, no significant differences in particle size distribution were observed regardless of the active substance incorporated, with particle sizes falling predominantly in the range of approximately 0.432–29.177 μm and the volumetric distribution maximum occurring at a particle diameter of approximately 0.818 μm .

A similar pattern of drug-related size heterogeneity was described for diclofenac-loaded nanovesicles prepared by double solvent displacement: most formulations showed homogeneous vesicles of 101–133 nm, but the formulation with sodium diclofenac at the highest phospholipid concentration displayed a bimodal distribution, with a second population around 4 μm attributed to uneven partitioning of the hydrophilic drug fraction [47]. This offers a useful parallel to the bimodal distributions observed here for L2, LNAP2, and L[PheOMent][NAP]2, suggesting that such heterogeneity may recur whenever the encapsulated drug retains partial hydrophilic character, regardless of the specific NSAID or preparation method used.

Particle size is a critical quality attribute of liposomal formulations, affecting colloidal stability, release kinetics, and the ability of vesicles to permeate the stratum corneum. It has been reported that the average width of intercellular skin pathways is about 20–40 nm, and that flexible liposomes remain effective as skin penetrants only up to a liposome-to-pore diameter ratio of about 6, while smaller lipid nanocapsules (51 nm) support markedly higher dermal permeability than larger ones (289 nm) [48]. In this context, the micrometric fraction dominating L1/LNAP1 would be expected to favour surface deposition and a reservoir effect, whereas the sub-micrometre fraction identified in L2/LNAP2 and

L[PheOMent][NAP]2 is more consistent with genuine transdermal transport—in line with the higher cumulative permeation observed here for the chloroform-based formulations.

3.12. Zeta Potential Measurements

Zeta potential is a key parameter characterizing the colloidal stability of liposomal systems, as it reflects the surface charge of particles and the magnitude of electrostatic repulsive forces acting between them. The zeta potential values determined for all investigated formulations are summarized in Table 8.

Table 8. Zeta potential values of the investigated liposomal formulations. Values are expressed as mean.

Designation	Zeta Potential [mV]
L1	−18.29
L2	−20.78
LNAP1	−22.14
LNAP2	−18.17
L[PheOMent][NAP]1	−23.72
L[PheOMent][NAP]2	−20.20

(1) Solvent: CHCl₃/MeOH (2:1, *v/v*) (2) Solvent: CHCl₃.

All measured zeta potential values fell within the range of −18.17 to −23.72 mV, indicating a negative surface charge and moderate colloidal stability of the investigated formulations. The most negative value was recorded for L[PheOMent][NAP]1 (−23.72 mV), while the least negative value was obtained for LNAP2 (−18.17 mV). It should be noted, however, that all values remain within a relatively narrow range, and the differences between individual formulations are modest, suggesting a broadly comparable electrokinetic character across the series.

These values sit at the less negative end of the range typically reported for liposomal NSAID systems. Meloxicam-loaded liposomes prepared from phosphatidylcholine, with or without cholesterol and various anionic surfactants, showed zeta potentials of −23 to −43 mV, attributed to the combined negative charge of the zwitterionic phospholipid above its isoelectric point, the anionic surfactants, and the ionized drug at physiological pH [49]. Diclofenac-loaded nanoliposomes prepared by double solvent displacement showed even more negative values, from -34 ± 2 to -62 ± 3.2 mV [47], and flurbiprofen-loaded ethanolic liposomes reached -51.6 ± 0.2 mV at their most negative [50]. By contrast, ibuprofen liposomes prepared by ethanol injection showed a near-neutral charge of only -7.15 ± 1.55 mV [46]. This spread indicates that the magnitude of surface charge in liposomal NSAID systems depends strongly on the preparation technique and lipid composition rather than being an intrinsic property of the drug class, situating the present formulations within an intermediate, moderately stable range.

The incorporation of active substances resulted in a shift in the zeta potential toward more negative values relative to the drug-free lipid systems (L1: −18.29 mV, L2: −20.78 mV), indicative of interactions between the encapsulated compounds and the liposomal lipid membrane. This effect may be attributed to the presence of ionizable functional groups within the molecular structures of the active substances, as well as their spatial localization within or at the interface of the lipid bilayer, which can modulate the overall surface charge density of the liposomal particles.

The negative zeta potential values observed across all formulations are characteristic of stable or moderately stable liposomal dispersions and confirm the effectiveness of the thin-film hydration method employed for liposome preparation. Furthermore, the

absence of significant differences between formulations prepared with different organic solvent systems suggests that the choice of solvent does not substantially affect the final electrokinetic properties of the resulting liposomes, and that the surface charge characteristics are primarily governed by the lipid composition and the nature of the incorporated active substance.

All formulations exhibited negative zeta potential values, indicating satisfactory colloidal stability resulting from electrostatic repulsion between vesicles. The absence of substantial differences between formulations suggests that incorporation of [PheOMent][NAP] did not adversely affect liposome stability.

3.13. pH Determination

The pH of liposomal formulation is a critical physicochemical parameter that influences both the stability of the lipid bilayer and the ionization state of encapsulated active substances. Maintaining an appropriate pH is particularly important for pharmaceutical and dermatological applications, where deviations from physiological or skin-compatible values may affect membrane integrity, drug release profiles, and tolerability upon administration. Furthermore, pH can influence the surface charge of liposomal particles and, consequently, their colloidal stability. In the present study, the pH values of all investigated liposomal formulations were determined and are summarized in Table 9.

Table 9. pH values of the investigated liposomal formulations. Values are expressed as mean.

Designation	pH
L1	6.78
L2	7.07
LNAP1	6.21
LNAP2	6.18
L[PheOMent][NAP]1	6.64
L[PheOMent][NAP]2	6.49

(1) Solvent: CHCl₃/MeOH (2:1, *v/v*) (2) Solvent: CHCl₃.

All investigated formulations exhibited pH values within the range of 6.18 to 7.07, corresponding to a neutral or slightly acidic reaction. No significant differences in pH were observed between formulations prepared using the chloroform–methanol mixture and those prepared with chloroform alone, suggesting that the choice of organic solvent employed during preparation does not exert a substantial influence on the final pH of the liposomal formulations.

The incorporation of naproxen resulted in a slight decrease in pH relative to the drug-free baseline formulations (L1: 6.78, L2: 7.07), with values dropping to 6.21 and 6.18 for LNAP1 and LNAP2, respectively. This reduction is consistent with the acidic character of naproxen ($pK_a \approx 4.2$) and its capacity to modestly acidify the aqueous environment of the liposomal dispersion. In contrast, the incorporation of L-phenylalanine menthol ester naproxenate led to a partial recovery of pH toward higher values (6.64 and 6.49 for L[PheOMent][NAP]1 and L[PheOMent][NAP]2, respectively) compared to the naproxen-loaded formulations. This effect may be attributed to the ionic nature of the salt compound and the partial neutralization of the acidic properties of the naproxen moiety upon salt formation.

The pH values recorded across all formulations fall within the range broadly considered acceptable for lipid-based formulations intended for pharmaceutical and dermatological applications, supporting the suitability of the developed systems for further biological evaluation.

3.14. Encapsulation Efficiency

Encapsulation efficiency (EE) is a fundamental parameter in the characterization of liposomal drug delivery systems, as it directly determines the amount of active pharmaceutical ingredient (API) retained within the liposomal structure and thus available for controlled release and subsequent transdermal permeation. High EE values are indicative of favorable compatibility between the active substance and the lipid bilayer, as well as the effectiveness of the preparation method employed. In the present study, the degree of entrapment of the active substances within the liposomal structure was evaluated, and the results are presented in Table 10.

Table 10. Encapsulation efficiency of active substances in the investigated liposomal formulations. Data are presented as mean $\pm$ SD ($n = 3$).

Designation	Unencapsulated API [mg/L]	Encapsulated API [mg/L]	Encapsulation Efficiency [%]
LNAP1	4827.6 $\pm$ 8.5	5172.5 $\pm$ 8.5	51.7 $\pm$ 0.1
LNAP2	4530.8 $\pm$ 6.0	5469.2 $\pm$ 6.0	54.7 $\pm$ 0.1
L[PheOMent][NAP]1	2156.2 $\pm$ 11.0	21,038.7 $\pm$ 11.0	90.7 $\pm$ 0.1
L[PheOMent][NAP]2	2410.8 $\pm$ 5.7	20,784.2 $\pm$ 5.7	89.6 $\pm$ 0.1

The results demonstrate that encapsulation efficiency is primarily governed by the nature of the incorporated API rather than by the organic solvent system used during liposome preparation. Naproxen-loaded formulations exhibited moderate encapsulation efficiency, with values ranging from 51.7% to 54.7% for LNAP1 and LNAP2, respectively.

In contrast, the use of L-phenylalanine menthol ester naproxenate as the active substance led to a substantial increase in encapsulation efficiency, reaching values of approximately 90.7% and 89.6% for L[PheOMent][NAP]1 and L[PheOMent][NAP]2, respectively—nearly a 1.7-fold improvement over the naproxen-loaded counterparts. This markedly higher EE suggests stronger interactions between the modified drug and the phospholipid bilayer: the presence of both the amino acid residue and the menthol moiety likely increases affinity toward the lipid membrane, facilitating more efficient incorporation into the liposomal structure.

The minor differences in encapsulation efficiency between formulations prepared with the chloroform–methanol mixture and those prepared with chloroform alone confirm that the choice of organic solvent does not significantly affect the degree of API entrapment, and that lipid bilayer–drug compatibility is the dominant factor determining encapsulation performance in the investigated systems.

The encapsulation efficiency obtained for unmodified naproxen (51.7–54.7%) is markedly lower than the entrapment efficiencies reported for other liposomal NSAID systems, such as ibuprofen liposomes (86.68 $\pm$ 1.43%) [46], diclofenac nanoliposomes (58 $\pm$ 3% to 87 $\pm$ 5%) [47], and ketoprofen conventional and deformable liposomes (71.87% to 78.10%) [51]. It is, however, comparable to encapsulation efficiencies reported for ketoprofen–hydroxypropyl- β -cyclodextrin complexes in multilamellar liposomes (42.4–75.1%, depending on complex concentration), where entrapment efficiency was likewise strongly dependent on drug solubilization and its affinity for the aqueous liposomal phase rather than for the lipid bilayer itself [45]. These differences may largely be explained by the relatively low lipophilicity and high aqueous ionization of unmodified naproxen (log $P = 1.70$, $pK_a \approx 4.2$), which limits its retention within the hydrophobic bilayer core and favours partitioning into the aqueous phase during liposome formation. In contrast, the markedly higher EE obtained for L[PheOMent][NAP] (89.6–90.7%) is comparable to, and even exceeds, values reported for flurbiprofen ethanolic liposomes (93.51 $\pm$ 2.1%) [50]. This supports the conclusion that encapsulation efficiency in liposomal NSAID systems is

governed primarily by the lipophilicity, ionization state, and molecular architecture of the active substance rather than by the liposome composition itself, in agreement with the marked EE improvement observed here upon amino acid–menthol ester modification of naproxen, which reduced its log P from 1.70 to 1.36 while increasing its overall compatibility with the phospholipid bilayer.

3.15. Microscopic Observations

Optical microscopy was employed as a complementary technique to assess the morphology and structural integrity of the prepared liposomal formulations. Microscopic examination provides direct visual confirmation of vesicle formation and allows qualitative evaluation of particle shape, size heterogeneity, and the potential presence of aggregates or irregular structures. The microscopic image of formulation L2, acquired at 100× magnification, is presented in Figure 8.

The microscopic image reveals the presence of spherical lipid vesicles, confirming the successful formation of liposomal structures. The observed vesicles exhibit a relatively uniform shape; however, variation in particle diameter is apparent, which is consistent with the polydisperse character of the system established by laser diffraction measurements. Notably, no large aggregates or irregularly shaped structures were detected, which may be indicative of satisfactory physical stability of the investigated formulation.

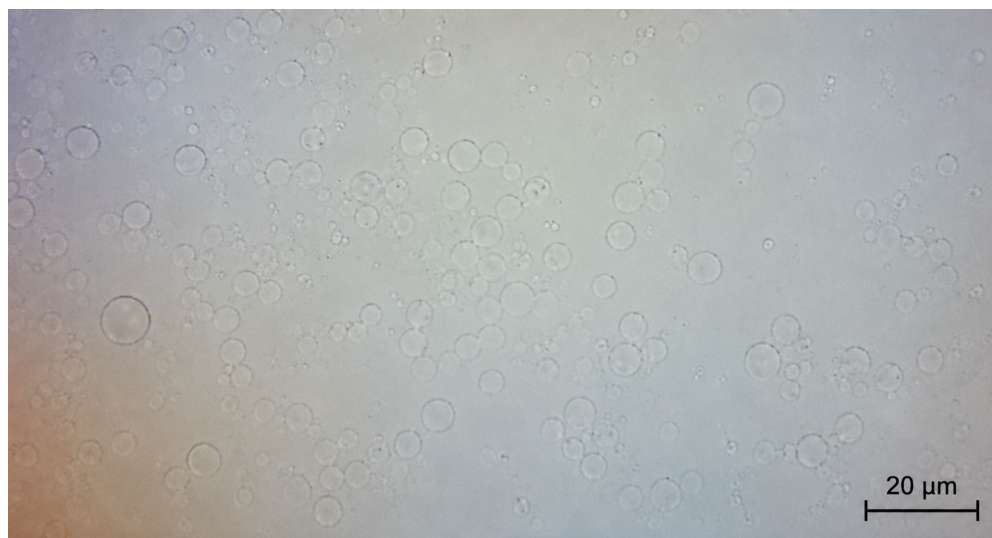


Figure 8. Optical microscopy image of liposomal formulation L2 (magnification 100×).

The microscopic observations confirmed the effective formation of liposomal vesicles and provided qualitative morphological characterization complementary to the quantitative particle size and zeta potential data discussed in the preceding sections.

3.16. In Vitro Skin Permeation Studies

The permeation behavior of liposomal formulations containing unmodified naproxen (LNAP1) and L-phenylalanine menthol ester naproxenate (L[PheOMent][NAP]1) was evaluated over a 24 h period. The cumulative mass of the active substance permeating through the skin as a function of time is presented in Figure 9.

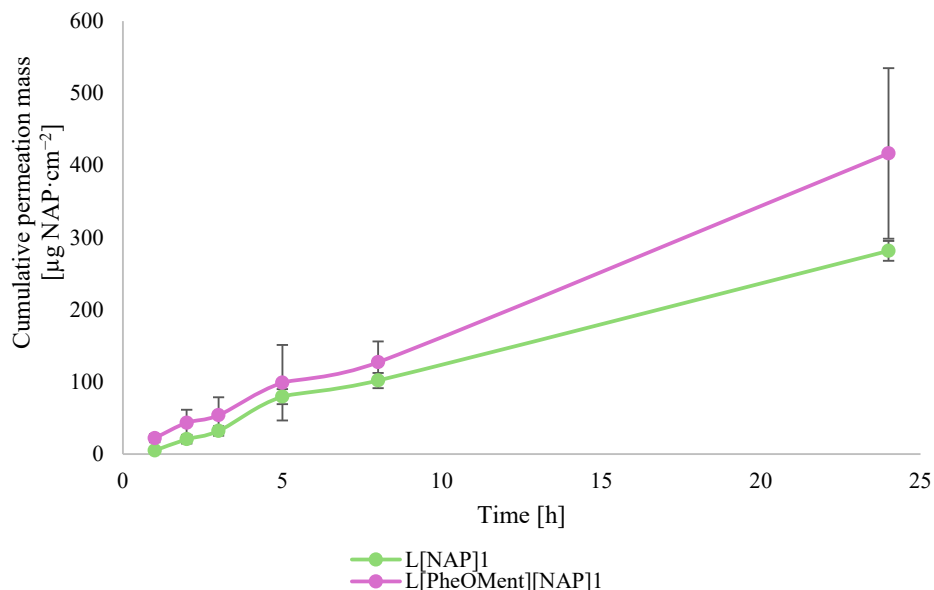


Figure 9. Skin permeation profiles expressed as cumulative amount of active compound released from liposomal formulations containing naproxen and L-phenylalanine menthol ester naproxenate during a 24 h study.

As shown in Figure 9, clear differences in permeation profiles were observed between the investigated formulations. The L[PheOMent][NAP]1 formulation exhibited a steeper increase in cumulative permeated mass over time, indicating faster and more efficient transport of the active compound across the skin barrier compared with the formulation containing unmodified naproxen (LNAP1). These differences suggest that structural modification of naproxen influences the transdermal transport process.

The total amount of active compound permeating through the skin after 24 h is summarized in Table 11. To enable direct comparison, the obtained values were additionally recalculated as the equivalent amount of naproxen. After 24 h, the amount of compound permeating from L[PheOMent][NAP]1 reached $965.88 \pm 118.20 \mu\text{g}\cdot\text{cm}^{-2}$, corresponding to $416.71 \pm 51.00 \mu\text{g NAP}\cdot\text{cm}^{-2}$, whereas for LNAP1 the permeated amount was significantly lower ($281.60 \pm 13.73 \mu\text{g}\cdot\text{cm}^{-2}$). These results clearly demonstrate that the modified compound exhibits superior skin permeation efficiency compared with the non-modified drug.

Table 11. Total amount of permeated active compound after 24 h for the investigated liposomal formulations. Data are presented as mean $\pm$ SD ($n = 3$).

Amount of the Compound	L[PheOMent][NAP]1	LNAP1
$[\mu\text{g}\cdot\text{cm}^{-2}]$	965.877 ± 118.202	281.595 ± 13.730
$[\mu\text{g NAP}\cdot\text{cm}^{-2}]$	416.711 ± 50.996	281.595 ± 13.730

These cumulative permeation values are of a comparable order of magnitude to those reported for other liposomal NSAID systems evaluated over 24 h using analogous Franz diffusion methodology. For example, conventional diclofenac sodium liposomes incorporated into Carbopol gel permeated $786.0 \pm 86.3 \mu\text{g}\cdot\text{cm}^{-2}$ after 24 h, with a flux of $29.13 \pm 5.24 \mu\text{g}\cdot\text{cm}^{-2}\cdot\text{h}^{-1}$, while non-gelled liposomal dispersions of the same drug reached $421.2 \pm 35.6 \mu\text{g}\cdot\text{cm}^{-2}$ (flux $16.32 \pm 2.35 \mu\text{g}\cdot\text{cm}^{-2}\cdot\text{h}^{-1}$)—values considerably lower than the corresponding ethosomal and transfersomal formulations of the same drug (952.6 and $1026.6 \mu\text{g}\cdot\text{cm}^{-2}$, respectively), reflecting the comparatively rigid bilayer structure of conventional

liposomes relative to elastic vesicular carriers [52]. The permeation obtained here for both LNAP1 and L[PheOMent][NAP]1 therefore falls within the range typically reported for conventional (non-deformable) liposomal NSAID carriers.

The permeation parameters calculated from the permeation profiles are presented in Table 12. When expressed as naproxen equivalent, the steady-state flux of L[PheOMent][NAP]1 was $21.61 \pm 4.94 \mu\text{g NAP}\cdot\text{cm}^{-2}\cdot\text{h}^{-1}$, compared with $20.30 \pm 1.89 \mu\text{g NAP}\cdot\text{cm}^{-2}\cdot\text{h}^{-1}$ for LNAP1. Although the naproxen-equivalent flux values were comparable, L[PheOMent][NAP]1 exhibited a higher permeability coefficient ($5.02 \pm 1.15 \times 10^{-3} \text{ cm}\cdot\text{h}^{-1}$) than LNAP1 ($2.03 \pm 0.19 \times 10^{-3} \text{ cm}\cdot\text{h}^{-1}$), reflecting the lower naproxen-equivalent concentration in the donor phase and the improved intrinsic permeability of the modified compound.

Table 12. Calculated transdermal permeation parameters for the investigated liposomal formulations. Data are presented as mean $\pm$ SD ($n = 3$).

Designation	J_{ss} [$\mu\text{g NAP}/\text{cm}^2\cdot\text{h}$]	$K_p\cdot 10^3$ [cm/h]	L_T [h]	$D\cdot 10^4$ [cm^2/h]	K_m	$Q\%_{24h}$ [%]
LNAP1	20.303 ± 1.888	2.030 ± 0.189	1.181 ± 0.255	3.651 ± 0.856	0.284 ± 0.037	2.816 ± 0.168
L[PheOMent][NAP]1	21.612 ± 4.943	5.020 ± 1.148	0.131 ± 0.058	27.595 ± 9.890	0.093 ± 0.013	9.960 ± 1.527

A directly comparable enhancement pattern has been reported for other amino acid ester ionic derivatives of NSAIDs evaluated by an almost identical set of permeation parameters using porcine skin and Franz diffusion cells. L-proline alkyl ester naproxenates showed a 2.68- to 3.94-fold higher permeability coefficient than unmodified naproxen, together with a 2.80- to 3.83-fold higher steady-state flux, with the isopropyl ester derivative increasing overall naproxen permeation almost four-fold [13]. Similarly, amino acid isopropyl ester ibuprofenates showed a 1.48- to 6.84-fold higher permeability coefficient and a 3.16- to 21.91-fold higher flux than unmodified ibuprofen [18]. The 2.5-fold increase in K_p obtained here for L[PheOMent][NAP]1 relative to LNAP1 is therefore consistent in direction and magnitude with these previously reported amino acid ester NSAID derivatives, even though in the present case the modification was combined with liposomal encapsulation rather than tested as a free compound in solution.

A significant difference was also observed for the lag time (L_T), which was markedly shorter for L[PheOMent][NAP]1 ($0.13 \pm 0.06 \text{ h}$) than for LNAP1 ($1.18 \pm 0.26 \text{ h}$), indicating faster attainment of steady-state permeation. Furthermore, the diffusion coefficient (D) was considerably higher for the modified compound ($27.60 \times 10^{-4} \pm 9.89 \times 10^{-4} \text{ cm}^2\cdot\text{h}^{-1}$) compared with unmodified naproxen ($3.65 \times 10^{-4} \pm 0.86 \times 10^{-4} \text{ cm}^2\cdot\text{h}^{-1}$), suggesting facilitated diffusion through the stratum corneum.

The partition coefficient (K_m) was lower for L[PheOMent][NAP]1 (0.093 ± 0.013) compared with LNAP1 (0.284 ± 0.037), indicating more favorable interactions between the modified compound and the skin barrier, which may contribute to improved permeation efficiency. In addition, the percentage of the applied dose permeating after 24 h ($Q\%_{24h}$) was significantly higher for L[PheOMent][NAP]1 ($9.96 \pm 1.53\%$) than for LNAP1 ($2.82 \pm 0.17\%$), confirming a greater fraction of the active compound delivered through the skin over the experimental period.

The permeation rate profiles presented in Figure 10 further confirm these observations. The L[PheOMent][NAP]1 formulation exhibited a higher and more stable permeation rate throughout the experiment, indicating efficient release of the active compound from the liposomal carrier and effective transport across the skin barrier. In contrast, LNAP1 showed a lower permeation rate and reached steady-state conditions later, which may result from the less favorable physicochemical properties of unmodified naproxen.

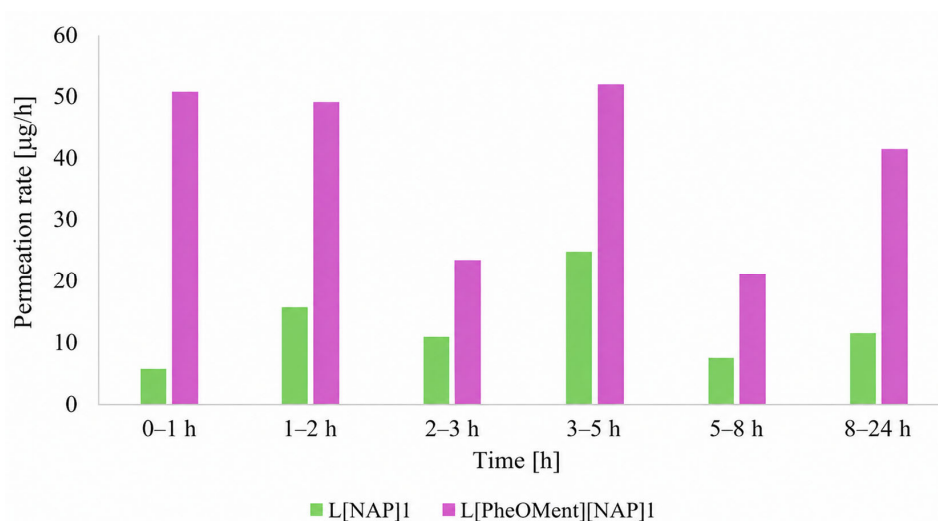


Figure 10. Permeation rate profiles of active compounds from investigated liposomal formulations during 24 h of skin permeation.

The observed differences in permeation behavior can be attributed to the synergistic effect of structural modification of the active compound combined with the liposomal delivery system. The introduction of the L-phenylalanine menthol ester naproxenate structure likely improved interactions with the lipid domains of the stratum corneum, while the liposomal carrier facilitated compound deposition within the skin layers and enabled controlled release.

This interpretation is consistent with earlier reports on amino acid ester ionic derivatives of naproxen and ibuprofen, in which the observed permeation enhancement was attributed to the temporary neutralization of the drug's charge by ion-pair formation, allowing the ion pair to more readily traverse the lipophilic stratum corneum before dissociating in the lower epidermal layers [13,18]. However, those studies evaluated the amino acid ester salts as free compounds in saturated aqueous solution, without the use of nanocarrier systems. In contrast, the present work combines structural modification with liposomal encapsulation. The more than threefold increase in cumulative permeation observed for L[PheOMent][NAP] suggests that these two strategies act synergistically rather than redundantly: the phenylalanine–menthol modification improves affinity for stratum corneum lipids, whereas the liposomal vesicles promote controlled release and compound deposition within the skin. These findings indicate that chemical modification of NSAIDs should be considered not only as a strategy for tailoring physicochemical properties, but also as an approach to optimize compatibility with lipid-based nanocarriers, thereby improving transdermal drug delivery.

Overall, the obtained results demonstrate that the proposed formulation strategy enhances transdermal delivery performance and may represent a promising approach for the development of advanced transdermal drug delivery systems. However, the present study extends these findings by integrating molecular modification with liposomal encapsulation. To the best of our knowledge, this is the first report describing liposomal formulations containing L-phenylalanine menthol ester naproxenate and demonstrating that the combination of both approaches synergistically improves encapsulation efficiency and skin permeation. These findings highlight the potential of integrating rational chemical modification with lipid-based nanocarriers for the development of advanced transdermal drug delivery systems.

4. Conclusions

In this study, a novel amino acid-based naproxen derivative, L-phenylalanine menthol ester naproxenate ([PheOMent][NAP]), was successfully synthesized, incorporated into liposomal formulations, and evaluated as a potential transdermal delivery system.

Compared with unmodified naproxen, the developed derivative exhibited reduced lipophilicity, markedly higher encapsulation efficiency, and enhanced in vitro skin permeation while maintaining physicochemical properties suitable for dermal application. These findings indicate that the combination of molecular modification and liposomal encapsulation effectively improves the transdermal delivery potential of naproxen.

The present study demonstrates that combining rational molecular modification of NSAIDs with lipid-based nanocarriers represents a promising strategy for the development of next-generation transdermal drug delivery systems. This is in line with earlier reports showing that amino acid ester ionic derivatives of naproxen and ibuprofen alone can markedly enhance skin permeation compared with the parent drugs; the present work indicates that this effect can be further extended when the modified compound is additionally incorporated into a liposomal carrier. Moreover, this approach may be extended to other poorly permeable anti-inflammatory drugs requiring improved dermal bioavailability.

Although the obtained results are promising, the study was limited to in vitro characterization and skin permeation experiments. Future work should include long-term stability studies, biological safety evaluation, and in vivo investigations to further assess the therapeutic potential of the developed formulations.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/app16157813/s1>, Figure S1: ^1H NMR spectrum of L-Phenylalanine Menthol Ester Hydrochloride (CDCl_3); Figure S2: ^{13}C NMR spectrum of L-Phenylalanine Menthol Ester Hydrochloride (CDCl_3); Figure S3: ^1H NMR spectrum of L-Phenylalanine Menthol Ester (DMSO); Figure S4: ^{13}C NMR spectrum of L-Phenylalanine Menthol Ester (DMSO); Figure S5: ^1H NMR spectrum of L-Phenylalanine Menthol Ester Naproxenate (CDCl_3); Figure S6: ^{13}C NMR spectrum of L-Phenylalanine Menthol Ester Naproxenate (CDCl_3); Figure S7: FT-IR spectrum of L-Phenylalanine Menthol Ester Hydrochloride; Figure S8: FT-IR spectrum of L-Phenylalanine Menthol Ester Naproxenate; Figure S9: TG, c-DTA, DTG curves of L-Phenylalanine Menthol Ester Hydrochloride; Figure S10: TG, c-DTA, DTG curves of L-Phenylalanine Menthol Ester Naproxenate; Figure S11: Individual cumulative permeation profiles of naproxen through porcine skin obtained from three independent permeation experiments (grey, yellow, and green). The red line represents the mean permeation profile ($n = 3$) used for the calculation of permeation parameters presented in the manuscript; Figure S12: Individual cumulative permeation profiles of L-phenylalanine menthol ester naproxenate through porcine skin obtained from three independent permeation experiments. The red line represents the mean permeation profile ($n = 3$) used for the calculation of permeation parameters presented in the manuscript; Table S1: Mean particle sizes ($\text{Dx}10$, $\text{Dx}50$, $\text{Dx}90$) and Span values of the investigated liposomal formulations (mean $\pm$ SD).

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Abbreviations

The following abbreviations are used in this manuscript:

NSAIDs	Non-Steroidal Anti-Inflammatory Drugs
[PheOMent][NAP]	L-Phenylalanine Menthol Ester Naproxenate
NMR	Nuclear Magnetic Resonance
FT-IR	Fourier Transform Infrared Spectroscopy
TG	Thermogravimetric Analysis
DSC	Differential Scanning Calorimetry
XRD	X-ray Diffraction
log P	Octanol/Water Partition Coefficient
COX-1	Cyclooxygenase-1
COX-2	Cyclooxygenase-2
DMAP	4-Dimethylaminopyridine
DCC	N,N'-Dicyclohexylcarbodiimide
DMSO	Dimethyl Sulfoxide
PBS	Phosphate-Buffered Saline
mM	Millimolar
KCl	Potassium Chloride
CDCl ₃	Deuterated Chloroform
TMS	Tetramethylsilane
DMSO-d ₆	Deuterated Dimethyl Sulfoxide
ATR	Attenuated Total Reflectance
μL	Microliter
HPLC	High-Performance Liquid Chromatography
v/v	Volume per Volume
DLS	Dynamic Light Scattering
ELS	Electrophoretic Light Scattering
EE	Encapsulation Efficiency
DAD	Diode-Array Detector
FLD	Fluorescence Detector
L-Phe	L-Phenylalanine
Boc	tert-Butoxycarbonyl
Boc-L-Phe	N-Boc-L-Phenylalanine
NAP	Naproxen
c-DTA	Calculated Differential Thermal Analysis
T5%	Temperature Corresponding to 5% Mass Loss
CHCl ₃	Chloroform
L1	Liposomal Formulation Type 1
L2	Liposomal Formulation Type 2
LNAP1	Naproxen-Loaded Liposomes Type 1
LNAP2	Naproxen-Loaded Liposomes Type 2
L[PheOMent][NAP]1	Liposomes loaded with L-phenylalanine menthol ester naproxenate Type 1
L[PheOMent][NAP]2	Liposomes loaded with L-phenylalanine menthol ester naproxenate Type 2
J _{ss}	Steady-State Flux
L _T	Lag Time
K _p	Permeability Coefficient
D	Diffusion Coefficient
K _m	Skin Partition Coefficient

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