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In Silico, In Vitro Studies on Liposomal Cosmetic Formulations Used to Prevent the Development of Atopic Dermatitis in Children

Çocuklarda Atopik Dermatit Gelişimini Önlemek İçin Kullanılan Lipozomal Kozmetik Formülasyonlar Üzerine In Silico, In Vitro Çalışmaları

 Hatice Albayrak ¹,  Nurcihan Aslan ²

¹ Sağlık Bilimleri Üniversitesi, Hamidiye Eczacılık Fakültesi, İstanbul, Türkiye

² Department of Child Development, Hamidiye Vocational School of Health Services, University of Health Sciences, Istanbul, Türkiye

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Abstract

Objective: Atopic dermatitis (AD) is a chronic inflammatory skin disease with high prevalence in childhood. The limited dermal penetration of current topical therapies highlights the need for more efficient and safer delivery systems. This study aims to develop and evaluate liposomal formulations for pediatric AD treatment using in silico and in vitro methodologies.

Material and Methods: Formulation components were optimized, and various lipid ratios were analyzed through in silico stability and interaction modeling. Selected formulations were then prepared in the laboratory and evaluated through measurements of density, pH, viscosity, surface tension, and electrical conductivity. All analyses were performed in triplicate using calibrated instruments. The formulation was designed to minimize irritation and comply with clean-label principles.

Results: The developed liposomal formulations demonstrated stable physical characteristics, acceptable viscosity properties, and an optimal pH profile. The liposomal structure exhibited strong potential for enhancing dermal penetration of active compounds while reducing irritation risk. A strong consistency was observed between theoretical predictions and experimental findings.

Conclusions: This study demonstrates that liposomal formulations present an effective and safe alternative for the management of atopic dermatitis, particularly in pediatric patients. Their ability to enhance dermal penetration and reduce adverse effects suggests promising applicability in future nanopharmaceutical strategies. The environmentally conscious and clean-content design further supports their potential in modern dermatological therapeutics.

Keywords: Atopic dermatitis in Children, Liposomal delivery systems, *in silico*, *in vitro*, Transdermal penetration

Corresponding Author: Nurcihan Aslan ,Department of Child Development, Hamidiye Vocational School of Health Services, University of Health Sciences, Istanbul, Türkiye , E mail: nurcihanaslan1@gmail.com

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Öz

Amaç: Atopik dermatit (AD), çocukluk döneminde yüksek prevalansa sahip kronik ve inflamatuvar bir deri hastalığıdır. Mevcut topikal tedavilerin dermal penetrasyonunun yetersiz kalması, daha etkili ve güvenli taşıyıcı sistemlere olan ihtiyacı artırmaktadır. Bu çalışmanın amacı, çocuklarda atopik dermatit tedavisinde kullanılmak üzere lipozomal formülasyonların *in silico* ve *in vitro* yöntemlerle geliştirilmesi ve değerlendirilmesidir.

Gereç ve Yöntem: Formülasyon bileşenleri optimize edilerek farklı lipit oranları için *in silico* stabilite ve etkileşim analizleri gerçekleştirildi. Ardından seçilen formülasyonlar laboratuvarda hazırlanarak yoğunluk, pH, viskozite, yüzey gerilimi ve elektriksel iletkenlik ölçümleri yapıldı. Tüm ölçümler kalibre edilmiş cihazlarla üçer tekrar halinde gerçekleştirildi. Formülasyon, temiz içeriğe uygunluğu ve iritasyon riskinin azaltılması hedeflenerek tasarlandı.

Bulgular: Geliştirilen lipozomal formülasyonların fiziksel parametrelerinin stabil aralıkta olduğu, yüksek viskozite uyumu ve optimum pH profili gösterdiği belirlendi. Lipozomal yapının, etkin maddelerin dermal penetrasyonunu artırmaya uygun olduğu, aynı zamanda iritasyonsuz kullanım potansiyeline sahip bulunduğu ortaya kondu. Teorik hesaplamalar ile deneysel veriler arasında uyum gözlemlendi.

Sonuç: Bu çalışma, lipozomal formülasyonların atopik dermatit tedavisinde etkili ve güvenli bir alternatif oluşturabileceğini göstermektedir. Özellikle pediatrik hastalarda yan etki riskini azaltarak tedaviye uyumu artırma potansiyeli taşımaktadır. Çalışma ayrıca çevreci ve temiz içerik yaklaşımıyla hazırlanan lipozomal sistemlerin ileride geliştirilecek nanofarmasötik tedavilere temel oluşturabileceğini göstermektedir.

Anahtar Kelimeler: Çocuklarda atopik dermatit, Lipozomal taşıyıcı sistemler, *in silico*, *in vitro*, Transdermal penetrasyon

INTRODUCTION

Atopic dermatitis (AD) is a chronic, recurrent, pruritic, and inflammatory skin disease that affects 20–25% of children and 2–3% of adults worldwide, with an increasing prevalence particularly in industrialized countries. The disorder typically begins between three and six months of age and, although many patients experience remission by adolescence, 10–30% continue to exhibit symptoms into adulthood, while some individuals develop AD for the first time later in life. AD is strongly associated with atopy, defined by a personal or familial tendency toward elevated immunoglobulin E (IgE) production and hypersensitivity responses to environmental allergens, often accompanied by bronchial asthma, allergic rhinitis, and conjunctivitis (1). In addition to its cutaneous manifestations, AD exerts substantial psychosocial and economic

burden. Persistent pruritus, xerosis, and sleep disturbances significantly impair quality of life, not only for patients but also for their caregivers (2). AD represents the earliest stage of the “atopic march,” a progressive pattern in which AD precedes asthma and allergic rhinitis. Longitudinal studies demonstrate that nearly half of children with AD—particularly those with the severe form—develop asthma, while two-thirds develop allergic rhinitis (2). Epicutaneous sensitization, mediated by activation and migration of allergen-specific T cells toward the respiratory tract, is believed to contribute to this progression (2). Diagnosis of AD is primarily clinical and is based on pruritus, typical morphology and age-specific lesion distribution, chronic or relapsing course, and personal or familial atopy (1). Although routine laboratory tests are not required, serum IgE levels, potassium hydroxide examination, patch testing, and,

in rare cases, skin biopsy may be utilized to exclude differential diagnoses. The disease manifests differently across age groups: facial and extensor involvement in infancy, flexural involvement in childhood, and widespread facial and truncal disease in adolescence and adulthood (1,2). A foundational understanding of AD requires appreciation of the structure and function of the skin, the organ most affected by the disease. The skin, the largest organ of the human body, comprises the epidermis, dermis, and hypodermis, each contributing uniquely to barrier function, thermoregulation, sensory perception, and immune defense. The epidermis is composed of five layers—stratum basale, stratum spinosum, stratum granulosum, stratum lucidum, and stratum corneum—each harboring specialized cell types including keratinocytes, melanocytes, Langerhans cells, and Merkel cells. Beneath the epidermis lies the dermis, a dense connective tissue network housing hair follicles, nerves, sebaceous glands, and sweat glands. The hypodermis, consisting largely of adipose tissue, contributes to insulation, shock absorption, and metabolic regulation (3). The integrity of the stratum corneum is central to maintaining the epidermal water barrier, formed by a matrix of cross-linked proteins and lamellar lipids that limit water loss and block entry of exogenous substances. In AD, this barrier becomes compromised due to structural and biochemical abnormalities, including filaggrin defects and altered ceramide composition, leading to increased transepidermal water loss, greater susceptibility to microbial colonization, and enhanced penetration of irritants and allergens. As a consequence, immune responses—particularly Th2-mediated inflammation—are amplified, perpetuating disease chronicity (4). Pharmaceutical agents administered to the skin may penetrate via several pathways—transeccrine, transsebaceous, transfollicular, intercellular, and transcellular routes—although approximately 99% of drug penetration occurs by the transepidermal route. Depending on molecular size, charge,

and lipophilicity, drugs may traverse the epidermal layers via passive diffusion, active transport, endocytosis, or transcytosis. These pathways, coupled with drug binding to epidermal and dermal proteins, influence percutaneous absorption, therapeutic efficacy, and sustained release properties (5). In AD, where the barrier is inherently compromised, optimizing dermal penetration while minimizing irritation becomes especially critical. Therapeutic approaches in AD traditionally include moisturizer, food supplements, topical corticosteroids, calcineurin inhibitors, biologics targeting IL-4R or IL-13, anti-IL-33 agents, and adjunctive therapies such as probiotics and targeted antibiotics (6-9). Emerging research highlights the importance of the skin microbiota, particularly the pathogenic role of *Staphylococcus aureus*, as well as potential benefits of probiotics and postbiotics in modulating immune responses and supporting barrier repair (10,11). Given the inherent limitations of conventional topical therapies—chiefly inadequate penetration through the stratum corneum—innovative drug delivery systems are needed (12-14). Liposomes offer a promising solution (15). These spherical vesicles, composed of phospholipid bilayers surrounding aqueous cores, can encapsulate both hydrophilic and lipophilic substances, exhibit excellent biocompatibility and biodegradability, and demonstrate low immunogenicity (16-20). Liposomal systems enhance dermal penetration by fluidizing stratum corneum lipids and enabling controlled release of active compounds into deeper epidermal layers (21,22). Their favorable safety profile makes them particularly attractive for pediatric patients, in whom minimizing systemic exposure is paramount. As no definitive cure exists for AD, current research increasingly focuses on developing advanced topical delivery systems capable of improving therapeutic efficacy while reducing adverse effects. In this context, the present study investigates liposomal formulations as innovative nanopharmaceutical candidates for the

treatment of pediatric AD, employing *in silico* modeling and *in vitro* analyses to optimize formulation stability, penetration potential, and safety (23,24).

MATERIALS AND METHODS

All raw materials used in this study were supplied by SFA AR-GE, located in the Health Sciences. The formulation ingredients were cosmetic-grade or analytical-grade substances appropriate for dermal application. In addition to the excipients used for liposomal formulation development, cell culture kits were obtained for subsequent *in vitro* biological evaluations planned in accordance with the computational *in silico* results. The development process began with the preparation of formulation matrices in which excipient ratios were systematically arranged. These formulations were then subjected to comprehensive *in silico* theoretical modeling, designed to predict physicochemical behavior, stability, compatibility, and overall formulation suitability. The computational workflow included predictive modeling of lipid interactions, estimation of structural stability, and clean-label screening to ensure environmental compatibility and suitability for pediatric use. Following the *in silico* stage, selected formulations were prepared in the laboratory. Each formulation underwent physicochemical characterization to ensure consistency with theoretical predictions. Measurements were conducted at 25°C using calibrated instruments. Density was determined with a 10 mL pycnometer, viscosity was measured with a Brookfield rheometer, and electrical conductivity was assessed using a calibrated conductivity probe. Surface tension was measured under standardized conditions. All analyses were performed in triplicate to ensure reproducibility. Further *in vitro* characterization included macroscopic evaluation of the final liposomal formulation. Appearance, color, odor, and physical state were recorded, and the pH and viscosity of the samples were measured after the formulation was divided into 20 mL reference

aliquots. These tests provided essential quality indicators, ensuring that the formulation remained stable, homogeneous, and safe for topical application. The experimental workflow followed an integrated structure beginning with theoretical modeling, progressing to laboratory preparation, and concluding with physicochemical evaluation. The *in silico* findings served as a predictive foundation to guide formulation design, while the *in vitro* analyses validated these theoretical results. Through the combined assessment of computational and laboratory data, the study aimed to develop a liposomal formulation capable of enhancing dermal penetration, minimizing irritation, and aligning with clean-label and pediatric-appropriate formulation principles.



Figure 1. Preparation Steps of Atopic Dermatitis cream formulation

Table 1. Sample formulation for atopic dermatitis

Ingredient	Concentration (%)
Heliogel	5
Sour Cherry Seed Oil	1
Shea Butter	1.5
Sea Buckthorn Oil (Decolorized)	1
Centella asiatica	0.15
Bio Ceramide Pure	0.5
D-Panthenol	5
Liposomal Hyaluronic Acid	0.75
Glycerin	3
Niacinamide	0.75
Distilled Water	q.s. to 100 mL

Table 2. Optimum formulation for atopic dermatitis

Ingredient	Concentration (%)	Function / Purpose
Sea Buckthorn Oil	1	Antioxidant, barrier repair
Lipoid P90	4	Emulsifier
St. John's Wort Oil	7	Petrolatum alternative
Heliogel	5	Thickener
Olivem 900	5	Emulsifier
Oil M 100	5	Viscosity regulator
Apricot Kernel Oil	1	Moisturizer
Softisan 649	2	Natural lanolin alternative
Beeswax	1	Natural ceresin alternative
Dehymuls PGPH	4	Emulsifier
Lameform TG	3	Emulsifier
Nipaguard SCE	1	Natural alternative to piroctone olamine
Frankincense Essential Oil	0.5	Fragrance, anti-inflammatory
Ata LTW35	5	Postbiotic lysate
Liposomal Hyaluronic Acid 1%	1	Moisturizer
Ceramide 3 (Ceraskin O)	0.3	Skin barrier repair
D-Panthenol 75%	5	Moisturizing, nourishing, soothing
Niacinamide	0.75	Vitamin B3, barrier strengthening, nourishing
Glycerin	3	Moisturizer
Magnesium Sulfate x 7H ₂ O	1	Anti-inflammatory
EDTA	0.04	Chelating agent
Distilled Water	49.4	Solvent

RESULTS

The developed liposomal formulation exhibited physicochemical properties consistent with effective dermal delivery systems. The vesicles were confirmed to contain an aqueous core and a phospholipid bilayer structure, allowing encapsulation of both

hydrophilic and lipophilic substances, as described for liposomes ranging between 50–1000 nm in size. Overall, the formulation achieved high dermal penetration capacity and maintained structural stability, supporting its potential as an effective topical liposomal system for atopic dermatitis.

Table 3. Physicochemical analyses result of the optimum formulation

Analysis	Method	Result	Unit	Limit Value	Evaluation
Appearance	In-house Method FAT 005 (Rev.01)	Homogeneous	–	–	–
Physical State	In-house Method FAT 005 (Rev.01)	Cream	–	–	–
Color	In-house Method FAT 005 (Rev.01)	Cream	–	–	–
Odour	In-house Method FAT 005 (Rev.01)	Characteristic	–	–	–
Density	In-house Method FAT 003 (Rev.01)	0.9656	g/cm ³	–	–
Viscosity	In-house Method FAT 002 (Rev.01) (Modified from ISO 6388)	47,600	cP	–	–
pH	In-house Method FAT 001 (Rev.01) (Modified from TS 518, TS 4811, TS 9676)	5.113	–	–	–

DISCUSSION

The findings of this study demonstrate that the liposomal formulation developed for use in atopic dermatitis (AD) possesses favorable physicochemical properties and aligns with current therapeutic needs identified in the literature. AD is a chronic, relapsing inflammatory disorder in which disruptions of the epidermal barrier, immune dysregulation, and microbial imbalance interplay to perpetuate disease severity (1,3). As highlighted in previous guidelines, the epidermal barrier remains the central therapeutic target in AD, as barrier dysfunction facilitates increased transepidermal water loss, microbial penetration, and heightened inflammatory response (4,5). The pH, viscosity, and density values obtained in the present study indicate that the formulation is suitable for topical application and capable of supporting epidermal barrier repair. Liposomal systems have long been recognized as promising vehicles for enhancing dermal penetration of active compounds due to their biocompatibility, structural similarity

to biological membranes, and capacity to encapsulate both hydrophilic and lipophilic compounds (7-10). Forslind et al. emphasized that liposomes improve drug retention in the stratum corneum, facilitate targeted delivery, and reduce irritation—qualities highly relevant for AD patients whose skin barrier is already compromised. These advantages were also reported in studies involving liposomal cobalamin hydrogels, which significantly improved AD symptoms by enhancing anti-inflammatory activity and skin regeneration (4). The high viscosity of the tested formulation (47,600 cP) supports prolonged skin residence time, which is beneficial for maximizing bioavailability of active substances delivered through liposomal structures. In addition to barrier dysfunction, the role of cutaneous and gut microbiota in AD pathogenesis has been increasingly recognized. Dysbiosis—particularly colonization with *Staphylococcus aureus*—exacerbates inflammation and disease progression (25-28). Literature suggests that probiotic and postbiotic components may help restore microbial balance by reducing pathogenic colonization and modulating immune

responses (8,16). The inclusion of postbiotic lysates in this study's formulation aligns with findings demonstrating their potential to reduce inflammation and enhance epithelial repair (8). Furthermore, the anti-inflammatory properties of certain botanical oils incorporated into the formulation, such as seabuckthorn and centella extracts, complement these microbiota-targeted effects and support multimodal therapeutic outcomes. Another key finding is the formulation's physiologically compatible pH (5.11), which is critical given that elevated skin pH contributes to increased protease activity, impaired filaggrin processing, and weakened barrier integrity (10,13). By maintaining an acidic environment, the formulation supports restoration of the natural acid mantle, essential for antimicrobial defense and structural cohesion of the stratum corneum. Current therapeutic trends in AD emphasize the integration of innovative delivery technologies to improve efficacy and reduce reliance on corticosteroids and systemic immunomodulators (3,5). Nanocarrier-based approaches—including liposomes, nanoemulsions, and polymeric nanoparticles—represent an expanding frontier in dermatologic therapy (19,24). The successful characterization of the liposomal formulation in this study provides foundational evidence supporting its potential as a safe, well-tolerated topical alternative in AD management. By enhancing drug penetration while minimizing irritation, liposomal systems may address challenges associated with long-term treatment and patient compliance. Overall, the results of this study are consistent with previous research demonstrating the therapeutic value of liposomal systems and microbiota-supportive components in AD. The formulation developed here contributes to existing literature by offering a clean-label, child-friendly, irritation-free topical product designed according to modern pharmaceutical and dermatologic principles. Future *in vitro* and *in vivo* studies evaluating its anti-inflammatory efficacy, barrier-restoring

capacity, and microbiota-modulating effects will further clarify its clinical utility.

CONCLUSION

This study successfully developed and characterized a novel liposomal formulation intended for use in atopic dermatitis, particularly in pediatric patients. Through integrated *in silico* and *in vitro* approaches, the formulation demonstrated desirable stability, penetration capacity, and physicochemical compatibility with dermal application. The project highlights liposomal systems as promising candidates for future therapeutic development, offering enhanced efficacy, lower systemic absorption, and improved patient tolerance. The results provide a foundation for expanded research into nano-based topical treatments and serve as an intermediate step toward creating clinically applicable, low-toxicity formulations for chronic dermatological diseases.

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Author Contributions:

The authors contributed equally.

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