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Synthesis and Characterization of Curcumin Loaded Flexible Liposomes Decorated with Hyaluronic Acid and Po Peptide

Alisa Shafira Badrul Hisham[1], Azila Abdul-Aziz[1,*], Guillermo R. Castro[2]

[1] Department of Chemical and Environmental Engineering, Malaysia-Japan International Institute of Technology Universiti Teknologi Malaysia, 54100, Jalan Sultan Yahya Petra, Kuala Lumpur, Malaysia
[2] Nanomedicine Research Unit (Nanomed), Center for Natural and Human Sciences, Federal University of ABC (UFABC), Santo André 09210-580, SP, Brazil

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ABSTRACT

Curcumin is well-known for its antioxidative and anti-inflammatory properties; however, it suffers from poor bioavailability. One strategy to overcome this issue is to encapsulate curcumin in a nanocarrier. In this study, curcumin was loaded into flexible liposomes decorated with hyaluronic acid (HA) and Po peptide (P) to enhance its bioavailability and enable potential active targeting. This research work focused on preparing and characterizing curcumin-loaded flexible liposomes modified with specific peptides derived from Po protein and hyaluronic acid. CD44 and ICAM-1 are cell surface glycoproteins typically expressed during inflammatory events, and HA and Po peptide are ligands that bind to CD44 and ICAM-1, respectively. Flexible liposomes decorated with HA and Po peptide (LHAP) were prepared using the thin-film hydration method. The characterization of LHAP, including size distribution, polydispersity index (PDI), and zeta potential, was performed via dynamic light scattering (DLS), while curcumin encapsulation was analyzed using HPLC. The particle size, PDI, and zeta potential of LHAP were 118.71 nm, 0.195, and -29.3 mV, respectively. The encapsulation efficiency was 90.1%, indicating that most of the curcumin was successfully encapsulated in the flexible liposomes decorated with HA and peptide. FTIR analysis confirmed that HA and Po peptide were successfully conjugated to the flexible liposomes. The physicochemical characteristics of the nanocarrier suggest that it holds potential as an effective delivery system.

1. Introduction

Curcumin, a polyphenol produced from the yellow spice turmeric, is one of several natural compounds accessible for medicinal use. Curcumin has been utilized for a variety of things since prehistoric days, including as a cosmetic, an addictive food spice, and a natural remedy for the treatment of many illnesses, particularly those that are related to inflammation. It is known for its antioxidative and anti-inflammatory properties. In one study by Silvestre *et al.*, [1], it is believed that curcumin may lessen the oxidative stress that psoriatic lesions experience due to its antioxidative

* *Azila Abdul-Aziz.*
E-mail address: r-azila@utm.my

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nature. Topical curcumin also may be used to treat skin disease more effectively and *in vivo*, curcumin has been observed by Mao *et al.,* [2] to suppress the production of pro-inflammatory cytokines (TNF- and IL-6) and lowers levels of the main pro-inflammatory factor IL-17 A.

Even though curcumin has been established as an effective antioxidant and anti-inflammatory agent, its bioavailability and effectiveness are still limited. Curcumin is usually encapsulated in nanocarriers to enhance its water solubility; increase its stability to light, heat, and oxygen; and improve its bioavailability.

Chronic inflammatory skin diseases are long-lasting conditions characterized by persistent inflammation in the skin. Immune-mediated diseases are conditions where the immune system mistakenly attacks the body's tissues, leading to inflammation and tissue damage. Chronic inflammatory skin diseases and immune-mediated diseases often overlap, but they are not synonymous. Detailed reviews on immune mediated disease can be seen in review papers by several authors [3,4].

Cell-surface molecules, such as CD44 and ICAM-1 (Intercellular Adhesion Molecule 1), are involved in immune cell adhesion, migration, and activation during inflammatory responses. Due to their roles in inflammation, they can be recruited as targets for anti-inflammatory therapies. Brown *et al.,* [5] mentioned that hyaluronic acid (HA) is a potential ligand for CD44. Po protein, which is a major glycoprotein that is derived from peripheral nerve myelin has the potential to act as a targeting ligand to ICAM-1. Ueno *et al*., [6], reported that Po-peptide-1 linked to liposomes can target ICAM-1 expressed in certain inflammatory skin diseases. Jaafari *et al*., [7] also suggested that Po-peptide-1 that is connected to liposome can mediate specific binding of liposome with the presence of interferon-γ (IFN-γ).

Lipid-based carriers are well known as promising vehicles for delivering therapeutic agents [8]. Flexible liposomes are deformable liposomes introduced by Cevc *et al.,* [9]. They are similar to conventional liposomes, but a surfactant incorporated in the lipid bilayer provides elasticity [10]. Atlan *et al.,* [11] reported that curcumin has been successfully loaded into flexible liposomes and can be modified for targeted transdermal delivery. In this work, to increase the penetration of curcumin to the inflammatory site of action, curcumin was loaded into hyaluronan-modified flexible liposome, which was decorated with appropriate peptides derived from Po protein.

2. Methodology

2.1 Materials

Curcumin (≥98%) (HPLC) was purchased from Merck, Germany. B-sitosterol, ethanol, chloroform, sodium hyaluronate, Tween 80 and Span 80 were purchased from Sigma Aldrich, Germany. Soybean phospholipid (SPC) and 1,2-distearoyl-sn-glycerophosphaethanolamine (DSPE) were purchased from Sigma Aldrich, Germany. Peptide derived from Po protein was synthesized by Apical Scientific Sdn Bhd. 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and N-Hydroxysuccinimide (NHS) were purchased from Thermo Fisher Scientific, USA. All chemicals were used as received unless otherwise stated.

2.2 Conjugation of HA to DSPE

Conjugation of HA to DSPE was conducted based on the method reported by Franze *et al.,* [12]. 50 mg sodium hyaluronate was dissolved in 10 ml PBS and stirred. 0.05 mM of EDC and NHS was added to the aqueous solution to activate carboxylic acid of sodium hyaluronate and stirred for 2 hours at room temperature. 0.125 mM of DSPE was dissolved in 10 ml of chloroform and mixed at

55°C. DSPE lipid mixture was added dropwise to the HA solution and stirred at 60°C for 6 hours followed by stirring for an additional 18 hours.

2.3 Conjugation of peptides to DSPE

Conjugation of Peptide to DSPE was conducted based on the method reported by Chen *et al.,* [13] with slight modification. 0.08 mmol of EDC was added to 0.5 ml of PBS and stirred. Then PBS was added to make up the solution to 3 ml. 0.5 mmol of NHS was added to 3 ml of PBS. DSPE was dissolved in 5 ml of chloroform under stirring condition for 1 hour prior to mixing dropwise (about 5 times each) with EDC and NHS to activate the lipids. The whole mixture was stirred for 2 hours under 50°C. 0.002 mmol peptide was dissolved in the mixture and stirred overnight at room temperature.

2.4 Conjugation of peptides and HA to DSPC

Flexible liposome was prepared using standard thin-film hydration method, as reported by *Yan et al*., [14], with modifications. Chloroform and ethanol (1:1) were used as solvents. In phase A, 60 mg of β-sitosterol was dissolved in 30 ml of ethanol before mixing with 30 ml of chloroform and 6 mg of curcumin. In phase B, 300 mg of SPC, 0.0476 ml of TWEEN 80, and 0.0567 ml of SPAN 80 were dissolved in chloroform. Phases A and B were then combined and stirred.

Next, DSPE conjugated with HA and DSPE conjugated with peptide were added to the mixture in a 40:6:4 ratio, respectively. The entire mixture was placed in a pear-shaped flask and dried using a rotary evaporator for approximately two hours until complete solvent removal and thin-film formation. The pressure was set to 175 mbar, the cooling chamber to 5°C, vapor temperature to 28°C, heating bath to 60°C, and flask rotation to 90 rpm.

To hydrate the dried thin film, 50 ml of phosphate-buffered saline (PBS, 0.1 mM, pH 6.8) was added. Hydration was conducted at 65°C with rotation at 120 rpm for approximately one hour. The solution was then removed from the flask and left to swell at room temperature for about one hour. Subsequently, the solution was sonicated using an ultrasonic probe sonicator with a net power output of 750 watts, at 30% amplitude, for three cycles of one minute each, with a 30-second rest between cycles. The sample was then stored at 4°C.

2.5 Physicochemical Characterization

2.5.1 Size distribution, polydispersity index and zeta potential

The particle size distribution of the prepared liposomes was measured via dynamic light scattering (DLS) using Litesizer 500 and the zeta potentials were measured simultaneously. The parameters for measurement and calculation were set as follows: 175° backscatter measurement angle, 1.33 material refraction index, 25 °C, and water as a dispersant [15]. Each sample was measured 3 times using a clear disposable cell. The number and duration of the run were optimized for each sample to obtain results meeting measurement quality criteria. The sample was dissolved in deionized water (1:9 dilution) prior to measurement.

2.5.2 Encapsulation efficiency

Freshly prepared curcumin loaded hyaluronan-modified flexible liposomes was separated from non-entrapped extracts using a mini-column centrifuge method as reported by Maghraby *et al.,* [16]. The column was packed with G-50 Sephadex gel (Bio-Rad USA) [17]. The mini column was centrifuged at

1000 x g for 3 minutes to remove excess buffer. 100 µl samples were added to the center of the column followed by centrifugation. The recovered aliquots of encapsulated flexible liposome were disrupted with ethanol at ratio 1:1 [18]. To measure the total amount of encapsulated and non-encapsulated extracts, 1 ml of curcumin loaded hyaluronan-modified flexible liposome was lysed with the same ratio of ethanol and the suspension was vortexed thoroughly and centrifuged at 1000 x g for 3 minutes. The filtrate was prepared, and the curcumin was analysed using HPLC. The EE was calculated according to Eq. (1).

$$Encapsulation\ Efficiency = \frac{W_t - W_f}{W_t} x\ 100 \quad (1)$$

where W_t is the total amount of curcumin in the tested formulation and W_f is the curcumin dispersed outside the nanovesicles.

2.5.3 Morphology Study

The morphology of curcumin loaded flexible liposome decorated with HA and peptide was observed using Transmission Electron Microscopy (TEM). Freshly prepared flexible liposomes were dropped onto a copper grip and dried for 3 minutes at room temperature. The samples were then dyed with uranyl acetate for 1 minute before it was observed under TEM.

2.5.4 Fourier Transform Infrared (FTIR) Spectroscopy

FT-IR spectra were obtained using an FT-IR spectrometer, model Spectrum 1000. Freeze-dried nanoparticles were mixed with a small amount of KBr at a concentration of 1 wt% nanoparticles to obtain the FT-IR spectra. This mixture was then compressed to form a tablet, and the IR spectra were obtained from this tablet in absorbance mode over the spectral range of 400 to 4000 cm^{-1}, with a resolution of 4 cm^{-1} and 64 co-added scans. The method followed was adapted from the procedure by Abdalrahim *et al.,* [19].

3. Results

3.1 Preparation and Physical Characterization of Flexible Liposomes Loaded with Curcumin

Three types of flexible liposomes were prepared: curcumin-loaded liposome decorated with HA and peptide (LHAP), curcumin-loaded liposome decorated with HA (LHA), and curcumin-loaded liposome decorated with peptide (LP). Particle size analysis showed that LHAP had a size of 118.71 ± 1.06 nm (Table 1), with a polydispersity index (PDI) of 0.195 and a zeta potential of -29.3 mV. The optimal values for PDI are below 0.2, while an ideal zeta potential is higher than ±30 mV. The low PDI value (PDI < 0.2) indicates that the designed nanoliposomes have a very narrow size distribution. This is significant, as nanovesicles with diameters of 300 nm or less can penetrate the deeper layers of the skin to some extent, as reported by Verma *et al.,* [20]. The zeta potential indicated fair stability.

The observed particle sizes for LHA and LP were 82.56 nm and 90.19 nm, respectively, with PDI values of around 0.2. The zeta potentials for LHA and LP were -23.4 mV and -30.7 mV, respectively, indicating fair stability. This study utilized soybean phospholipids (SPC) as a natural phospholipid, which is known to influence the physical properties of liposomes, including their permeability. As shown in Table 1, the small particle sizes observed indicate the potential of utilizing the particles for topical applications, as nanovesicles smaller than 300 nm can deliver their contents to deeper skin

layers. Smaller particles are more effective in reaching their intended targets, as supported by the findings from Hoseini *et al.,* [21].

According to Table 1, the encapsulation efficiency for LHAP was relatively high at 90.1%, while LHA and LP had encapsulation efficiencies of 65.8% and 57.2%, respectively. These results indicate that most of the curcumin was successfully encapsulated in the flexible liposomes decorated with HA and peptide.

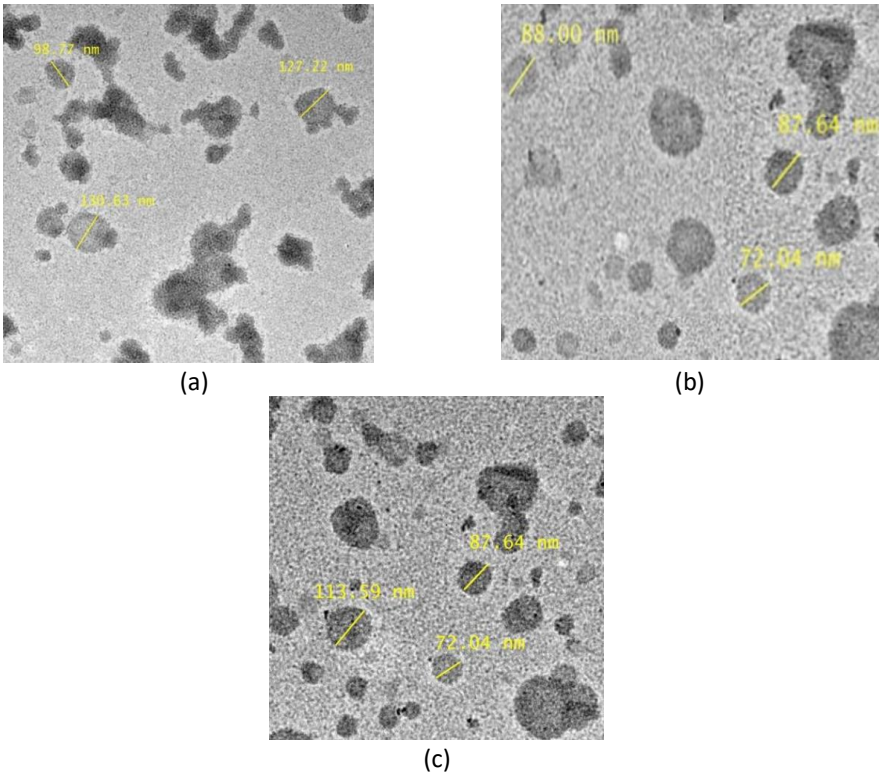
Table 1
Characteristic of modified flexible liposomes (mean ± SD, n = 3)

Liposome Dispersion	Particle Size (nm)	Polydispersity Index	Zeta Potential (mV)	Encapsulation Efficiency (%)
LHAP	118.71 ± 1.05	0.195	-29.3 ± 0.40	90.1
LHA	82.56 ± 0.23	0.233	-23.4 ± 0.08	65.8
LP	90.19 ± 0.75	0.261	-30.7 ± 0.12	57.2

3.1 Liposome Morphology

Transmission Electron Microscopy (TEM) was used to study the morphology of the liposomes. As shown in Figure 1, the shapes of the modified flexible liposomes (LHAP, LHA, and LP) were predominantly spherical. The TEM image of LHAP (Figure 1a) appeared more irregular in shape compared to LHA and LP (Figures 1b and 1c, respectively).

Similarly, a study by Trivedi *et al.,* [22] found that flexible and cationic flexible liposomes exhibit a spherical structure, while anionic flexible liposomes composed of synthetic phospholipids do not. In this analysis, all vesicle types showed a comparable spherical or oval morphology. This shape might be a result of liposome deformation, potentially caused by sample processing.



(a) (b)

(c)

Fig. 1. Morphology of (a) curcumin-loaded liposome decorated with peptide (LP); (b) curcumin-loaded liposome decorated with HA (LHA); and (c) curcumin-loaded liposome decorated with HA and peptide (LHAP)

3.1 Fourier Transform Infrared (FTIR) Spectroscopy

FTIR spectra of DSPE-HA, DSPE-P, and L-HA-P are shown in Figure 2. The spectra of DSPE-HA and DSPE-P display characteristic amide peaks at 1680–1630 cm^{-1}, attributed to the C=O stretching of the carboxylic acid groups in the activated HA and peptide. The N-H stretching peak, typically observed between 3475–3150 cm^{-1}, is absent, likely due to the formation of tertiary amides. Figure 2(d) further confirms amide formation, indicating successful conjugation of HA and peptide to the soybean phospholipids (SPC). The amide peaks at 1680–1630 cm^{-1} result from C=O stretching in the carboxylic acid groups, reflecting the presence of both activated HA and peptides.

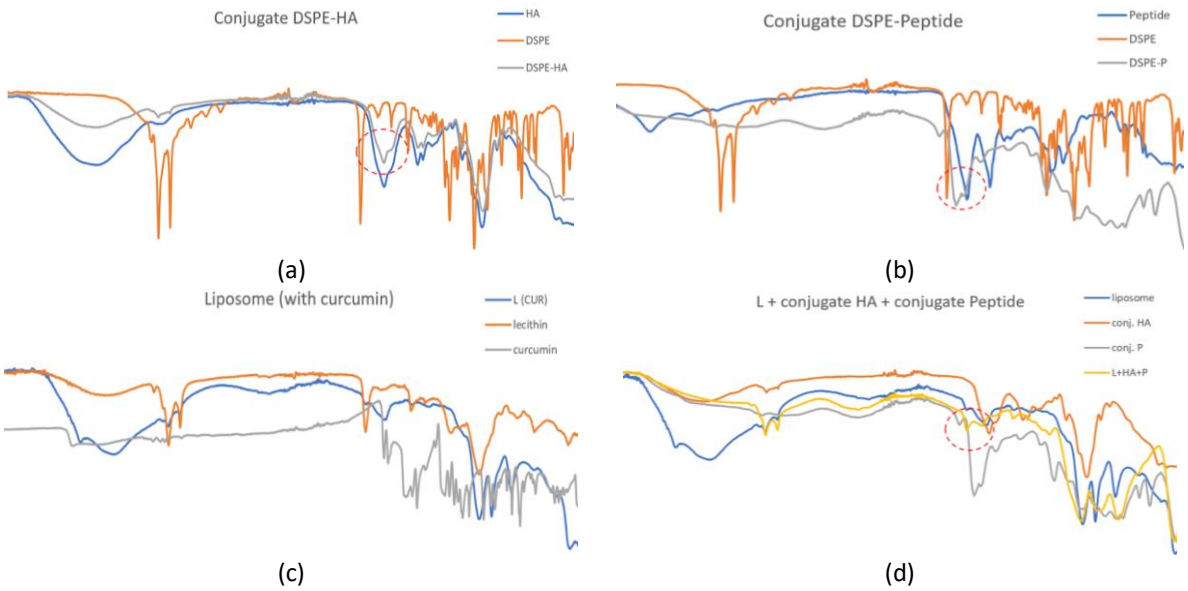


Fig. 2. FTIR Spectra of (a) conjugation of DSPE lipid with HA, (b) conjugation of DSPE lipid with peptide; (c) Liposome of soybean phospholipid without decorated HA and peptide (d) liposomes of soybean phospholipids decorated with HA and peptide

4. Conclusions

Lipid-based nanoparticles have the potential to deliver active ingredients to targeted regions. In this study, curcumin was encapsulated in flexible liposomes decorated with hyaluronic acid (HA) and Po peptide to improve curcumin's bioavailability and facilitate active targeting to inflamed skin cells. Based on the results, a stable formulation of curcumin-loaded, hyaluronan-modified flexible liposomes decorated with peptides derived from Po protein was successfully developed.

The particle size (PDI) and zeta potential values for LHAP were 118.71 ± 1.05 nm and -29.3 mV, respectively, with a high encapsulation efficiency of 90.1%, indicating a stable and uniform distribution of LHAP vesicles. These findings suggest that this formulation could serve as an effective transdermal carrier for curcumin in skin treatments. Additionally, the development of HA modified and Po peptide-targeted flexible liposomes marks a significant advancement in liposomal drug delivery.

The results demonstrate that HA and Po peptide were successfully conjugated to the flexible liposomes, potentially enabling targeted delivery to CD44 and ICAM-1, cell-surface molecules that play key roles in immune cell adhesion, migration, and activation during inflammatory responses. The physicochemical properties of the modified flexible liposomes developed in this study support their potential as an efficient delivery system.

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References

[1] Silvestre, Fernanda, Carolina Santos, Vitória Silva, Alicia Ombredane, Willie Pinheiro, Laise Andrade, Mônica Garcia et al. "Pharmacokinetics of curcumin delivered by nanoparticles and the relationship with antitumor efficacy: a systematic review." *Pharmaceuticals* 16, no. 7 (2023): 943. https://doi.org/10.3390/ph16070943

[2] Mao, Kai-Li, Zi-Liang Fan, Jian-Dong Yuan, Pian-Pian Chen, Jing-Jing Yang, Jie Xu, De-Li ZhuGe et al. "Skin-penetrating polymeric nanoparticles incorporated in silk fibroin hydrogel for topical delivery of curcumin to improve its therapeutic effect on psoriasis mouse model." *Colloids and Surfaces B: Biointerfaces* 160 (2017): 704-714.https://doi.org/10.1016/j.colsurfb.2017.10.029

[3] Fitch, Erin, Erin Harper, Iliyana Skorcheva, Stephen E. Kurtz, and Andrew Blauvelt. "Pathophysiology of psoriasis: recent advances on IL-23 and Th17 cytokines." *Current rheumatology reports* 9, no. 6 (2007): 461-467. https://doi.org/10.1007%2Fs11926-007-0075-1

[4] Lowes, Michelle A., Mayte Suarez-Farinas, and James G. Krueger. "Immunology of psoriasis." *Annual review of immunology* 32, no. 1 (2014): 227-255. https://doi.org/10.1146/annurev-immunol-032713-120225

[5] Brown, Marc B., and Stuart A. Jones. "Hyaluronic acid: a unique topical vehicle for the localized delivery of drugs to the skin." *Journal of the European Academy of Dermatology and Venereology* 19, no. 3 (2005): 308-318. https://doi.org/10.1111/j.1468-3083.2004.01180.x

[6] Ueno, Sho, Min Woo Kim, Gibok Lee, Yong Il Park, Takuro Niidome, and Ruda Lee. "Development of ErbB2-targeting liposomes for enhancing drug delivery to ErbB2-positive breast cancer." *Pharmaceutics* 12, no. 6 (2020): 585.

[7] Jaafari, R., and M. Foldvari. "Targeting of Liposomes to Human Keratinocytes Through Adhesive Peptides from Immunoglobulin Domains in the Presence of IFNM-γ." *Drug delivery* 9, no. 1 (2002): 1-9. https://doi.org/10.1080/107175402753413127

[8] Nordin, Ummu Umaimah Mohd, Noraini Ahmad, Norazlinaliza Salim, and Nor Saadah Mohd Yusof. "Lipid-based nanoparticles for psoriasis treatment: A review on conventional treatments, recent works, and future prospects." *RSC advances* 11, no. 46 (2021): 29080-29101. http://dx.doi.org/10.1039/D1RA06087

[9] Cevc, G., and Gi Blume. "Lipid vesicles penetrate into intact skin owing to the transdermal osmotic gradients and hydration force." *Biochimica et Biophysica Acta (BBA)-Biomembranes* 1104, no. 1 (1992): 226-232. https://doi.org/10.1016/0005-2736(92)90154-E

[10] Marwah, Harneet, Tarun Garg, Amit K. Goyal, and Goutam Rath. "Permeation enhancer strategies in transdermal drug delivery." *Drug delivery* 23, no. 2 (2016): 564-578. https://doi.org/10.3109/10717544.2014.935532

[11] Atlan, Michele, and Josh Neman. "Targeted transdermal delivery of curcumin for breast cancer prevention." *International Journal of Environmental Research and Public Health* 16, no. 24 (2019): 4949. https://pubmed.ncbi.nlm.nih.gov/31817597

[12] Franzé, Silvia, Alessandro Marengo, Barbara Stella, Paola Minghetti, Silvia Arpicco, and Francesco Cilurzo. "Hyaluronan-decorated liposomes as drug delivery systems for cutaneous administration." *International journal of pharmaceutics* 535, no. 1-2 (2018): 333-339. https://doi.org/10.1016/j.ijpharm.2017.11.028

[13] Chen, Cheng-Wei, Da-Wen Lu, Ming-Kung Yeh, Chia-Yang Shiau, and Chiao-Hsi Chiang. "Novel RGD-lipid conjugate-modified liposomes for enhancing siRNA delivery in human retinal pigment epithelial cells." *International journal of nanomedicine* (2011): 2567-2580. http://dx.doi.org/10.2147/IJN.S24447

[14] Chen, Yan, Qingqing Wu, Zhenghai Zhang, Ling Yuan, Xuan Liu, and Lei Zhou. "Preparation of curcumin-loaded liposomes and evaluation of their skin permeation and pharmacodynamics." *Molecules* 17, no. 5 (2012): 5972-5987. https://doi.org/10.3390/molecules17055972

[15] Roslan, Nur Zatul Iradah, Siti Maria Abdul Ghani, Nurul Bashirah Yusof, and Azila Abd Aziz. "Liposome as transdermal carrier for Labisia pumila and Ficus deltoidea water extracts." *Jurnal Teknologi (Sciences & Engineering)* 79, no. 7 (2017). https://doi.org/10.11113/jt.v79.10205

[16] Maghraby, Gamal MM El, Adrian C. Williams, and Brian W. Barry. "Skin delivery of 5-fluorouracil from ultradeformable and standard liposomes in-vitro." *Journal of Pharmacy and Pharmacology* 53, no. 8 (2001): 1069-1077.https://doi.org/10.1211/0022357011776450

[17] Maghraby, Gamal MM El, Adrian C. Williams, and Brian W. Barry. "Skin delivery of 5-fluorouracil from ultradeformable and standard liposomes in-vitro." *Journal of Pharmacy and Pharmacology* 53, no. 8 (2001): 1069-1077. https://doi.org/10.7150%2Fthno.29715

[18] Ghanbarzadeh, Saeed, and Sanam Arami. "Enhanced transdermal delivery of diclofenac sodium via conventional liposomes, ethosomes, and transfersomes." *BioMed research international* 2013, no. 1 (2013): 616810.

[19] Aisha, Abdalrahim FA, Amin Malik Shah Abdul Majid, and Zhari Ismail. "Preparation and characterization of nano liposomes of Orthosiphon stamineus ethanolic extract in soybean phospholipids." *BMC biotechnology* 14 (2014): 1-11. https://doi.org/10.1186/1472-6750-14-23

[20] Verma, Dr D., S. Verma, G. Blume, and A. Fahr. "Particle size of liposomes influences dermal delivery of substances into skin." *International journal of pharmaceutics* 258, no. 1-2 (2003): 141-151. https://doi.org/10.1016/s0378-5173(03)00183-2

[21] Hoseini, Benyamin, Mahmoud Reza Jaafari, Amin Golabpour, Amir Abbas Momtazi-Borojeni, Maryam Karimi, and Saeid Eslami. "Application of ensemble machine learning approach to assess the factors affecting size and polydispersity index of liposomal nanoparticles." *Scientific reports* 13, no. 1 (2023): 18012. https://doi.org/10.1038/s41598-023-43689-4

[22] Trivedi, Rashmi, Milind Umekar, Nandkishore Kotagale, Smita Bonde, and Jayshree Taksande. "Design, evaluation and in vivo pharmacokinetic study of a cationic flexible liposomes for enhanced transdermal delivery of pramipexole." *Journal of drug delivery science and technology* 61 (2021): 102313. https://doi.org/10.1016/j.jddst.2020.102313