Liposomal Delivery of Caffeine and Cafestol for Enhanced Skin Absorption

Nubul Albayati [1,2], Bozena Michniak –Kohn [1,2]

[1] Ernest Mario School of Pharmacy, Rutgers-The State University of New Jersey, 160 Frelinghuysen Road, Piscataway, NJ 08854.

[2] Center for Dermal Research, Rutgers-The State University of New Jersey, 145 Bevier Road, Piscataway, NJ 08854.

CENTER FOR DERMAL RESEARCH

RUTGERS UNIVERSITY

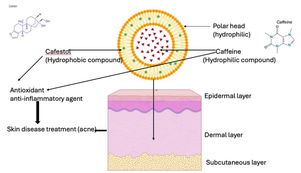
Background

Green coffee beans are well-known for their diverse health-promoting properties, including antioxidant, anti-inflammatory, anti-cancer, anti-diabetic, and antihypertensive effects. These beneficial attributes are largely due to the presence of key compounds like caffeine (an alkaloid) and cafestol (a diterpene).

Both caffeine (CF) and cafestol (CA) have attracted considerable research attention owing to their significant pharmacological activities, particularly their potent antioxidant, antimicrobial, and anti-inflammatory effects. These properties suggest a strong therapeutic potential for treating various skin conditions, such as acne.

Despite their promising benefits, the effective topical delivery of these compounds faces substantial challenges. Caffeine's high-water solubility and low log P value limit its ability to penetrate the stratum corneum, the outermost layer of the skin, and reach deeper affected tissues. CA, while beneficial, is highly hydrophobic and chemically unstable, making it prone to oxidative degradation when exposed to air, light, or acidic conditions, which consequently leads to poor bioavailability in pharmaceutical formulations.

To address these delivery hurdles and enhance the transdermal penetration of both hydrophilic caffeine and lipophilic cafestol, the aim of this study was to develop a novel liposomal formulation and enhanced skin delivery.. Liposomes are proposed as an effective system to transport both compounds across the skin, simultaneously protecting the encapsulated substances from environmental degradation, controlling their release, and ultimately ensuring excellent biocompatibility and safety profiles.



Formulations Preparation and Characterization

Four combinations of caffeine and cafestol in liposomes, two with caffeine in liposomes, and four with cafestol in liposomes were formulated with varying lipid types, concentrations, and controls. Lipids (Lipoid 75 S and Phospholipon 90G -Ph90) with cholesterol at a ratio of 80:20 were used as the lipid components for liposome preparation. Ethanol was used as the organic component, and phosphate-buffered saline (PBS) or HPLC water served as the aqueous phase.

Table 2. Formulation compositions investigated in this study.

Formulation	CA (%W/W)	CF (%W/W)	Lipoid S75 (% W/W)	Ph 90 (%W/W)	*Ethanol (ml)	Cho. (%W/W)	**Water
F1	-	1	0.5	-	6	0.062	q.s
F2	-	1	-	0.5	6	0.062	q.s
F3	0.07	1	0.5	-	6	0.062	q.s
F4	0.07	1	-	0.5	6	0.062	q.s
F5	0.07	-	0.5	-	6	0.062	q.s
F6	0.07	-	-	0.5	6	0.062	q.s
F7	0.133	-	0.5	-	6	0.062	q.s
F8	0.133	-	-	0.5	6	0.062	q.s
F9	0.133	1	0.5	-	6	0.062	q.s
F10	0.133	1	-	0.5	6	0.062	q.s
Blank	-	-	0.5	-	6	0.062	q.s

CA: cafestol, CF: caffeine, Ph 90: Phospholipon® 90 G, Cho.: cholesterol.
* Used to dissolved CA & evaporated completely does not account while calculating final weight.
** q.s to produce 10 g

Table 3. Characterization parameters of different Liposomes: EE, PDI, and Visual Assessment (Mean ± S.D., n=3).

Formula Code	Vesicle size, nm	%EE of CF	%EE of CA	PDI	Zeta potential (mv)	Visual Observation
F1	193.8 ± 2.7	91.1 ± 0.8	-	0.31 ± 0.04	-46.7 ± 2.82	Clear
F3	71.4 ± 0.24	91 ± 0.70	99.8 ± 0.22	0.32 ± 0.005	-44.2 ± 0.12	Clear
F5	230.6 ± 0.7	-	99.8 ± 0.35	0.32 ± 0.01	-49.7 ± 0.21	Clear
F7	257.8 ± 1.4	-	99.7 ± 0.07	0.32 ± 0.20	-50.9 ± 0.68	Clear
F9	76.84 ± 0.5	90.8 ± 0.35	99.9 ± 0.21	0.32 ± 0.006	-46.9 ± 0.65	Clear

EE of CF: entrapment efficiency of caffeine, EE of CA: entrapment efficiency of CA, PDI: polydispersity index.

Table 4. Stability of liposomes at 4°C for 6 Months N=3. Zeta potential (mv) ± S.D. of F1(1% CF-L), F3 (1% CF + 0.07% CA-L); F5 (0.07% CA-L); F9 (1% CF +0.13% CA-L); and F7 (0.13% CA-L).

	Zeta potential (mv) up to 6 months		
Formula code	Freshly prepared (0 day)	30 Days	180 days
Blank	-54.2 ± 1.23	-48.2 ± 0.3	-28.7 ± 0.2
F1	-46.7 ± 2.82	-43.9 ± 3.61	-19.2 ± 0.6
F3	-44.2 ± 0.12	- 43.3 ± 0.24	-18.9 ± 1.7
F5	-49.7 ± 0.21	-48.6 ± 0.34	-19.9 ± 0.3
F7	-50.9 ± 0.68	- 54 ± 2.95	-24.3 ± 3
F9	-46.9 ± 0.65	- 40.5 ± 2.1	-27±1.6

Methods and Materials

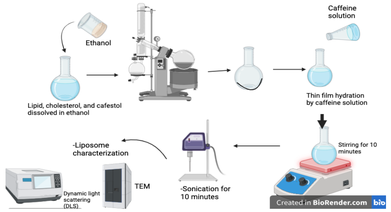
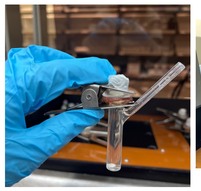


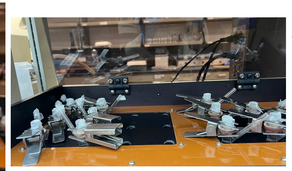
Table 1. Experimental conditions for HPLC quantification of the active compounds.

Active compounds	CF	CA
Wavelength	280 (nm)	230 (nm)
Retention time	4.9 (minute)	13.7 (minute)
Run time	17 (min)	
Flow rate	0.8 (mL/minute)	
Injection Volume	10 microliters (µL)	
Mobile Phase	45:55 (HPLC water: Acetonitrile) ratio by volume	
Column Temperature	25 °C	
HPLC Column	Partisil 10 ODS-1 4.6mm X 250 mm	

In Vitro Permeation study



Setup of Franz Diffusion Cell and Dosing with different liposomal Formulations



Maintaining the Franz diffusion cell setup at 32 °C during the permeation study

Detection of Cafestol in Epidermis and Dermis Layers Using HPLC Analysis

Results

Figure 1. Peak area for HPLC assay plotted against concentration of caffeine.

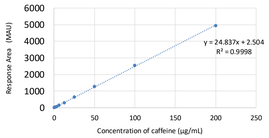


Figure 2. Peak area for HPLC assay plotted against concentration of cafestol.

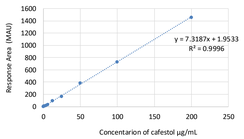
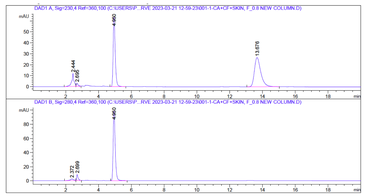


Figure 3. Caffeine and cafestol chromatogram peaks at retention times of 4.9 min, 13.7 min respectively.



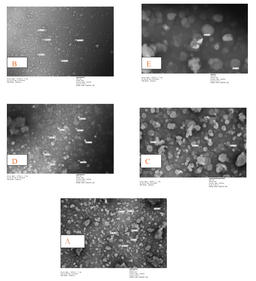
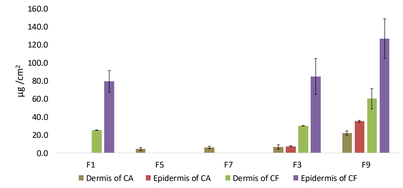


Figure 4. Morphology of different liposomal formulations using TEM. (A) F1(1% CF-L); (B) F3 (1% CF + 0.07% CA-L); (C) F5 (0.07% CA-L); (D) F9 (1%CF +0.13% CA-L); and (E) F7 (0.13% CA-L).



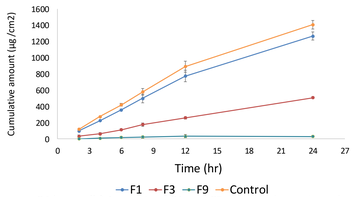


Figure 5. (a) Ex vivo permeation profiles of caffeine-loaded liposome formulations over 24 hours; **(b)** Amounts of CA and CF deposited in various skin layers following a 24-hour permeation study using the tested formulations (N = 5). Data are presented as means ± SD.

Cytotoxicity Study

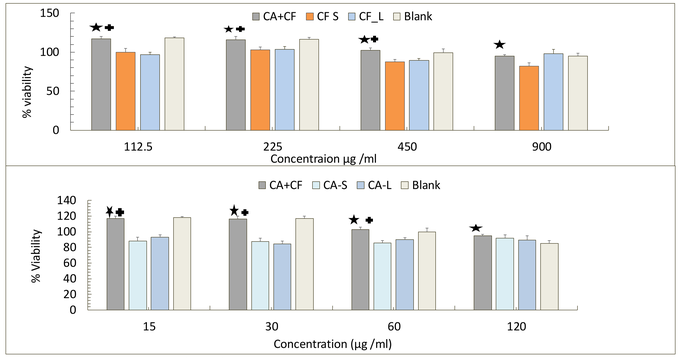


Figure 6: Viability of Keratinocyte cell line (HaCaT) as measured by AlamarBlue assay. A- after 24 hr incubation with CF solution, or CF loaded in liposome, or CF +CA loaded in liposome.
B- after 24 hr incubation with hydroethanolic CA solution, or CA loaded in liposome, or CF +CA loaded in liposome. Data represent average values ± SD (n >3). Statistically significant differences: (p <0.005), ★(p < 0.0005 ★vs. control solution at the same concentration.

Conclusion and future work

- The morphology of liposomes was characterized by Transmission Electron Microscopy (TEM) and Dynamic Light Scattering (DLS) both confirmed that F9 show smaller vesicle size compared to other formulation. The individual compound vesicles, F1, F7 had larger particle sizes of approximately 193.8 nm, and 257.8 nm, respectively compared to the liposome formulation loaded with both CF and CA which was about 76.8 nm.
- The combination of 1% caffeine with 0.13% CA-L significantly enhanced cafestol penetration into the dermis compared to other formulations. Additionally, F9 markedly enhanced caffeine penetration into the dermis, reaching the highest observed level among all tested groups. Caffeine penetration followed the trend: 1% CF solution < 1% CF-L < 1% CF + 0.07% CA-L < 1% CF + 0.13% CA-L with respective values of 14 ± 0.3, 25 ± 0.4, 30 ± 0.14, and 60 ± 11.2 µg. The synergistic combination of these compounds allows them to penetrate deeper into the skin layers, owing to the consistent size of the nanosized vesicles in our liposomal blend, which improves the solubility profile of both compounds.
- Additionally, in vitro cytotoxicity tests reveal that encapsulating caffeine and cafestol in liposomes notably increases cell viability in HaCaT monolayer cells compared to when each compound is used separately.
- These results highlight the potential of caffeine and cafestol liposomal formulations as a safe and effective option for topical skin treatments, with further research needed to explore their clinical applications.

Acknowledgements

Funding provided by the Center for Dermal Research (CDR) at Rutgers - The State University of New Jersey., Piscataway, NJ.

References

1. Cornelis, M. Nutrients. 2019:11(2).
2. Barbosa, M.,Scholz, M., Kitzberger, C., Benassi, M. Food Chemistry. 2019:275-280.
3. Lee, K., Jeong, H. Toxicology Letters. 2007:80-87.
4. Vieira, A., Gaspar,E., Santos, P. Radiation Physics and Chemistry. 2020:174.
5. Guercia, E., Forzato, C., Navarini, L., Berti, F.Food chemistry.2016:502-508.