IFSCC 2025 full paper (IFSCC2025-1029)

"Enhanced Solubility and Stability of Resveratrol Using Deep Eutectic Solvent and Liposome Encapsulation"

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1. Introduction

Resveratrol is a naturally occurring antioxidant belonging to the polyphenol family, and is found in high concentrations in red grapes, berries, and peanuts [1,2]. It has been reported to offer various health benefits, including anti-wrinkle, skin-whitening, and soothing effects, primarily due to its potent antioxidant and skin-protective properties [3]. However, its poor water solubility and instability in the presence of light, oxygen, and oxidative enzymes limit its applicability in cosmetic formulations. In this study, deep eutectic solvents (DES) and liposomal encapsulation were employed to enhance the solubility and stability of resveratrol. DES are formed by mixing two or more components in a specific molar ratio, resulting in a eutectic mixture with a melting point significantly lower than that of each individual component [4]. This allows DES to remain liquid at room temperature, exhibiting properties similar to those of ionic liquids (ILs). Unlike ILs, however, DES offer several advantages, including lower toxicity, reduced environmental impact, and improved biodegradability [5]. Moreover, DES are easy to synthesize, exhibit good solubility in chemical systems, and remain in a liquid state even at low temperatures, making them attractive alternatives to conventional organic solvents and ILs in various industrial applications. Liposomes are spherical vesicles composed of a phospholipid bilayer, structurally analogous to cellular membranes, which endows them with high biocompatibility. Owing to their amphiphilic nature, liposomes are capable of encapsulating both hydrophilic and hydrophobic substances, thereby enabling the stable storage and efficient delivery of a wide range of active compounds. Consequently, liposomes have been extensively studied in drug delivery systems and have also found significant applications in the cosmetic field [6,7].

2. Materials and Methods

A deep eutectic solvent (DES) was obtained by adding betaine and glycerin in a 1:2 molar ratio and stirring at 80°C. The resveratrol-DES (ReDES) compound was prepared by dissolving resveratrol in DES. ReDES liposomes were prepared using a microfluidizer with ReDES, phospholipids, cholesterol, and purified water in the ratios listed in Table 1. The four samples of ReDES liposomes obtained in the experiment were characterized, and the content of resveratrol entrapped in the ReDES liposomes was analyzed using ultraviolet-visible spectroscopy (UV-Vis) [Orion Aquamate 8000, Thermo scientific]. The chemical bonding of Re-DES

liposomes was analyzed using Fourier transform infrared spectroscopy (FT-IR) [NICOLET Summit, Thermo scientific]. The particle size was measured using dynamic light scattering (DLS) [Photal ELS-Z, Otsuka Electronics Co.]. The morphology of ReDES liposomes was analyzed using cryo-electron microscopy (cryo-TEM)[Tecnai G2 F20, FEI].

Table 1. ReDES Composition

		A	B	C	D
A)	Cholesterol	0.50	0.50	0.50	0.50
	Hydrogenated Lecithin	0.50	1.00	3.00	5.00
	Ethanol	10.00	10.00	10.00	10.00
B)	DES	40.00	50.00	60.00	70.00
	Water	To100	To100	To100	To100
	Resveratrol	5.00	5.00	5.00	5.00

3. Results

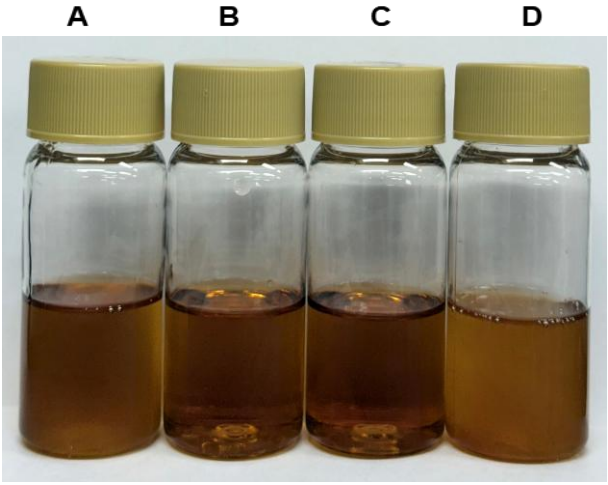

Figure 1. Photograph of liposome morphology

As Figure 1 shows, the morphological appearance of four liposome formulations prepared according to the ratios described in Table 1. ReDES Liposomes A and D appeared as brown, opaque liquids, whereas ReDES Liposomes B and C were observed as brown, transparent liquids. The absence of phase separation or precipitation in all four liposome formulations indicates successful encapsulation of resveratrol dissolved in DES.

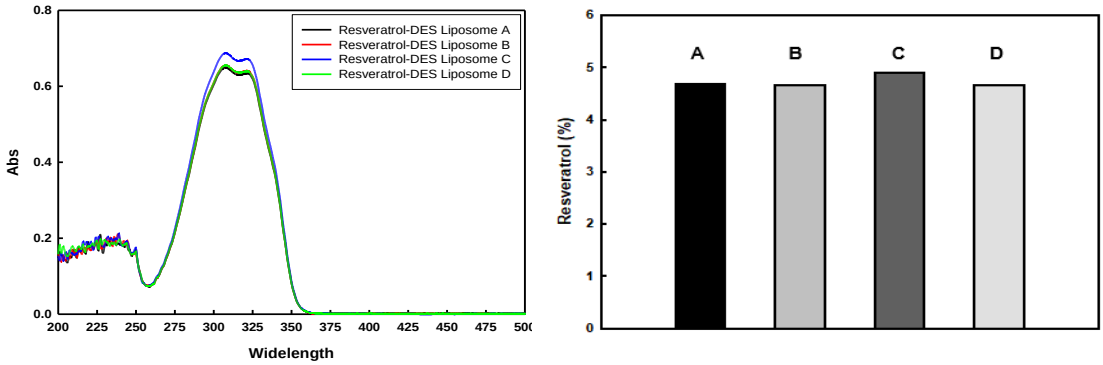

Figure 2. UV spectra of ReDES Liposomes

Figure 2 illustrates the resveratrol content measured in ReDES liposomes. An analysis of the resveratrol content in ReDES liposomes was conducted using a UV spectrophotometer. The results demonstrated that ReDES Liposome A contained 4.69% resveratrol, Liposome B 4.66%, Liposome C 4.89%, and Liposome D 4.66%. Collectively, these findings support the ability of ReDES liposomes to stably encapsulate resveratrol.

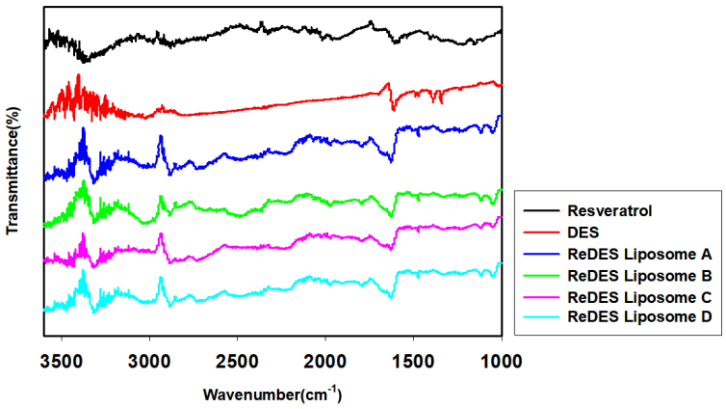

Figure 3. FT-IR spectra of ReDES Liposomes

Figure 3 presents the FT-IR spectra of resveratrol, DES, and ReDES liposomes (A–D). FT-IR analysis confirmed the chemical interactions between resveratrol, DES and the components within the ReDES liposomes (A–D). Both resveratrol and DES exhibited O-H stretching vibration peaks, and similar peaks were observed in the ReDES liposome samples. However, some peaks showed shifts and changes in width, suggesting the presence of hydrogen bonding or intermolecular interactions between resveratrol and DES within the liposomal structure. In addition, resveratrol-specific peaks corresponding to C=C and C=O stretching vibrations were retained in the ReDES liposomes, although some spectral shifts were observed. These results indicate that resveratrol was successfully encapsulated in the liposomes by interaction with DES rather than by simple physical mixing.

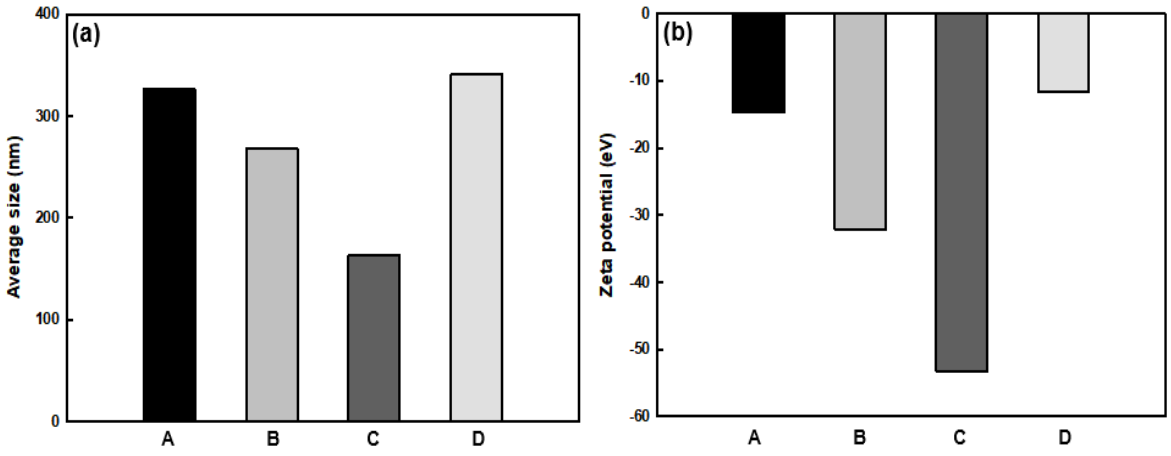

Figure 4. (a) Particle size of ReDES liposomes (b) Zeta potential of ReDES liposomes

Table 2. Particle Size and Zeta Potential Data of ReDES Liposomes

Sample Name	Particle Size(nm)	Zeta-Potential(mV)
Resveratrol-DES Liposome A	326.00	-14.70
Resveratrol-DES Liposome B	268.00	-32.00
Resveratrol-DES Liposome C	164.00	-53.20
Resveratrol-DES Liposome D	341.00	-11.60

The particle size and zeta potential of ReDES liposomes were analyzed using dynamic light scattering (DLS). The results showed that ReDES Liposome A had an average particle size of 326 nm and a zeta potential of -14.7 mV; Liposome B had 268 nm and -32.1 mV; Liposome C had 164 nm and -53.2 mV; and Liposome D had 341 nm and -11.6 mV. Among these, ReDES Liposome C exhibited the smallest particle size and a more negative zeta potential, indicating relatively high particle stability.

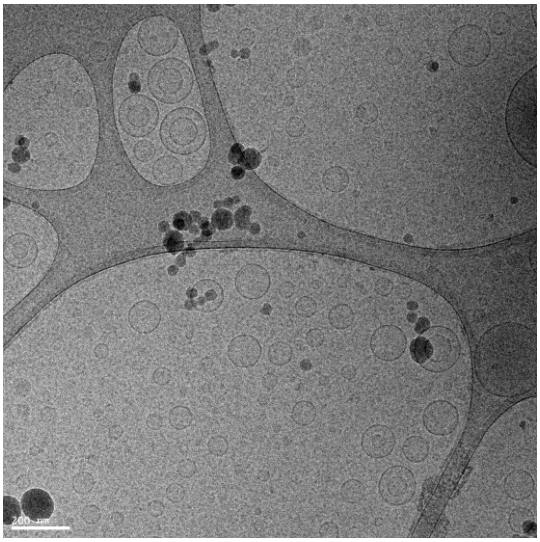

Figure 5. Cryo-TEM images of ReDES Liposome C

Based on the preceding comparative analysis, ReDES Liposome C demonstrated a more stable liposomal structure and effectively encapsulated resveratrol compared to ReDES Liposome (A, B and D). Consequently, Cryo-TEM analysis was performed to investigate the structural characteristics of ReDES Liposome C in detail. Figure 5 presents an image of ReDES Liposome C, showing that the particles exhibit a relatively uniform size distribution, with distinct spherical morphology and phospholipid bilayers. These results suggest that ReDES Liposome C possesses high structural stability.

4. Discussion

In this study, an encapsulation system utilizing deep eutectic solvent (DES) and liposomes was developed to stably entrap resveratrol, a poorly water-soluble bioactive compound. The spherical morphology and bilayer structure of the ReDES liposomes were clearly observed through TEM analysis, while DLS measurements and zeta potential analysis confirmed the formation of stable, nanoparticle-sized liposomes. These findings suggest that the presence

of DES positively influenced the structural stability and physicochemical properties of the liposomes. Furthermore, FT-IR analysis revealed the presence of specific chemical bonds or intermolecular interactions between DES and resveratrol within the liposomes, indicating that resveratrol was successfully encapsulated. Although the characteristic peaks of resveratrol were retained in the liposomal formulation, slight peak shifts were observed, supporting the successful and stable entrapment of resveratrol during the DES-based encapsulation process.Collectively, these results demonstrate that the combination of DES and liposomes can function as an effective delivery system for hydrophobic drugs. This formulation holds significant potential for future applications in drug delivery and the development of functional cosmetics.

5. Conclusion

ReDES liposomes have been shown to effectively encapsulate resveratrol dissolved in a deep eutectic solvent (DES), thereby preserving its physiological activity. The use of DES offers several advantages, including low toxicity, high biodegradability, and environmental friendliness. These properties enable ReDES liposomes to deliver active substances in a safer and more sustainable manner than conventional delivery systems. Furthermore, DES has been shown to enhance both the solubility and stability of resveratrol, and this technology may be applicable to other poorly soluble substances, suggesting the potential for developing liposomes with enhanced solubility. These ReDES liposomes hold promise for applications in pharmaceuticals, food, and cosmetics, and are poised to attract attention as an eco-friendly and innovative method for the safe and effective delivery of bioactive compounds.

6. References.

[1] Valletta, A.; Iozia, L.M.; Leonelli, F. Impact of Environmental Factors on Stilbene Biosynthesis. Plants 2021, 10, 90.
[2] Burns, J.; Yokota, T.; Ashihara, H.; Lean, M.E.; Crozier, A. Plant foods and herbal sources of resveratrol. J. Agric. Food Chem. 2002, 50, 3337–3340.
[3] Va ˇnková, E.; Paldrychová, M.; Kašparová, P.; Lokoˇcová, K.; Kodeš, Z.; Maťátková, O.; Kolouchová, I.; Masák, J. Natural antioxidant pterostilbene as an effective antibiofilm agent, particularly for gram-positive cocci. World J. Microbiol. Biotechnol. 2020, 36, 101.
[4] Zhang, Q., De Oliveira Vigier, K., Royer, S., & Jérôme, F. (2012). Deep eutectic solvents: Syntheses, properties and applications. *Chemical Society Reviews*, 41(21), 7108–7146.
[5] Kudłak, B.; Owczarek, K.; Namiesnik, J. Selected issues related to the toxicity of ionic liquids and deep eutectic solvents—A review. Environ. Sci. Pollut. Res. 2015, 22, 11975–11992.
[6] Mazur, F.; Bally, M.; Städler, B.; Chandrawati, R. Liposomes and lipid bilayers in biosensors. Adv. Colloid Interface Sci. 2017, 249, 88–99
[7] Nastruzzi C, Esposito E, Menegatti E, Walde P. Use ano stability of liposomes in dermatological preparations. J Appl Cosmetol. 1993;11:77–91.