

Development and characterization of ivermectin loaded liposomes prepared by lipid film hydration and ethanol injection method

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Introduction

Ivermectin (IVM) is an FDA-approved drug used for treating rare tropical diseases as an antiparasitic agent (Formiga et al., 2021). Despite its long history of use and excellent safety profile, its potential as an antiviral treatment is limited due to poor water solubility. To address this challenge, the researchers aimed to develop IVM-loaded liposomes that can improve solubility, provide controlled release, and reduce toxicity.

Liposomes are lipid-based vesicles that have advantages as a solubilization matrix for poorly soluble agents. They can act as reservoirs for controlled drug release. In this study, liposomes were prepared using soy phosphatidylcholine (SPC) as the lipid component with molar ratios of 1.85:1:0.15 and 7:2:1 for SPC:cholesterol (CHOL):dicetyl phosphate (DCP) (Arisoy, Kocas, Comoglu, Guderer, & Banerjee, 2022; Bassissi, Lespine, & Alvinerie, 2006).

In the lipid film hydration method, the calculated amounts of lipid and IVM were dissolved in a chloroform/methanol mixture and dried in a rotating evaporator. Ultrapure water was added to achieve a final lipid concentration of 30 µmol/ml. The liposome dispersion was then sonicated to adjust the particle size and extruded through 0.05 µm polycarbonate membrane filters using a mini-extruder (Arisoy et al., 2022; Bassissi et al., 2006).

For the ethanol injection method, IVM, soybean SPC, CHOL, and DCP were dissolved in ethanol. The resulting

ethanolic solution was injected into ultrapure water mixed with a magnetic stirrer at a controlled rate, resulting in a final lipid concentration of 30 mM (Croci et al., 2016).

The specific lipid content and preparation methods for each formulation can be found in Table 1 of the original study.

In vitro characterization of ivermectin loaded liposomes

Particle size and zeta potential measurement: Samples were prepared by taking 50 µL of the liposome dispersions and diluting them with 25 mL of ultrapure water. Particle size (nm), PDI and zeta potential (mV) were measured with Malvern NANOZS(n=3).

Encapsulation efficiency: Liposome dispersion was centrifuged at 15.000 rpm for 30 minutes at 4 °C to determine the loading efficiency. By this method, the amount of unloaded IVM was found from the supernatant obtained by the precipitation of liposomes, and then the loading efficiency was calculated indirectly.

Encapsulation efficiency (EE) % = (Total amount of ivermectin in the liposomes - amount of drug in the supernatant) / (Total amount of ivermectin in liposomes) x 100

In vitro drug dissolution test: The in vitro dissolution test was performed by using dialysis bag method. The formulation was added to the dialysis bag and was placed in 50 ml pH 7.4 0.2% SDS phosphate buffer medium in a 37 °C 50 rpm shaking water bath (Lu et al., 2017)). After the determined time intervals, the samples were taken from

the release medium and analyzed by HPLC method and the released amounts were calculated (n=3).

Table 1. Formulations' lipid content and methods

Formulation	Method	Lipid Molar Ratio
F1	EI	1.85
F2	EI	7
F3	FH-E	1.85
F4	FH-E	7
F5	FH-S	1.85
F6	FH-S	7

*EI-Ethanol injection; FH-E Film hydration-ekstruder; FH-S Film hydration sonikation

Results and discussion

In vitro characterization results of the prepared liposomes were given in Table 2. Studies have shown that particle size increases with increasement in the lipid concentration.

Table 2. In vitro characterization results of formulations

Formulation	Particle Size (nm)	Zeta Potential (mV)	%EE*
F1	164 ±40.99	-48.4 ±2.31	98.10±1.21
F2	271.3±3.88	-49.9±2.31	86.98±0.43
F3	389.9±12.46	-43.9±0.2	96.94±0.65
F4	446.9±23.62	-51.1±0.872	95.96±0.52
F5	151.8±1.986	-44.4±0.651	77.29±0.23
F6	165.5±5.108	-52.1±0.872	77.08±0.50

*EE: Encapsulation efficiency

The highest particle size and EE was seen in the formulations produced with the FH-E method, and the lowest particle size and EE was obtained in the formulations produced with the FH-S method. It is probable that this is due to the fact that the sonication technique has the ability to apply a greater amount of energy. The zeta potential values of all formulations were excellent.

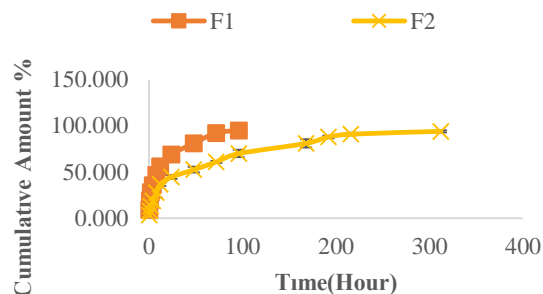


Fig. 1. Release profiles of IVM loaded liposomes (mean ± SD, n=3)

The observed decrease in the lipid content seems to result in a diminished dissolution rate, as IVM is highly lipid-bound.

Conclusion

IVM-loaded liposomes were successfully prepared by using the film hydration extrusion method. The particle size of optimum formulation(F1) was 164 ± 40.99 nm; zeta potential was -48.4 ± 2.31 mV and $98.10 \pm 1.21\%$ encapsulation efficiency. The IVM release from the F1 formulation reached 90% at 72 hours.

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