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Effect of formulation parameters on the characteristics of binary ethosomes loaded with Fluconazole

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Abstract

Background: The emergence of fungal resistance to most conventional topical antifungal drugs remains a serious problem. New topical drug delivery systems, such as ethosomes, particularly binary ones, have proven to be effective due to their ability to efficiently target the deeper layers of the skin, due to the presence of a high amount of ethanol in their structure. They deliver an optimal drug dose with reduced toxicity. These are soft and flexible vesicles, composed of phospholipids, a binary alcohol phase and water. The combination of ethanol with other alcohols is believed to give them smaller vesicle sizes, higher skin permeability and better entrapment efficiency. This study aimed to formulate and characterize binary ethosomes loaded with Fluconazole to investigate the effect of formulation parameters on their characteristics.

Methods: A preliminary trial consisted on the preparation of blank classical ethosomes without active substance by varying the sonication time to select the optimal time, allowing for the subsequent the preparation of binary ethosomes loaded with Fluconazole, where ethanol and soybean lecithin concentrations were varied. The ethosomes were characterized by determining the particle size, zeta potential and the percentage of entrapment efficiency of Fluconazole.

Results: The particle size of the blank classical ethosomes ranged from 412,2±1,7nm to 5816±9,6nm, while the binary ethosomes ranged from 742,5±11,1nm to 2288±10nm. The zeta potential was between -8.15±0.1mV and -41.1±0.2mV for classical ethosomes and -9.91±0.1mV and -48.4±0.35mV for binary ethosomes. Finally, the entrapment efficiency percentage of the binary ethosomes ranged from 48,89±3.5% to 89,89±0.1%.

Conclusion: Based on the obtained results, the formulation parameters had a significant impact on the characteristics of the formulated ethosomes.

Keywords: Nanomedicine; Ethosomes; Fluconazole; Nanocarriers; Zeta potential

1. Introduction

The emergence of fungal resistance to most conventional topical antifungal drugs remains a serious problem [1, 2]. Conventional topical delivery systems have some drawbacks that limit the delivery of antifungal drugs through the skin layers due to the stratum corneum barrier [3-6]. To address this issue, an increase in the prescribed drug dose is

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necessary to achieve the desired therapeutic effect; however, the potential for local and systemic side effects cannot be ruled out [5-7]. Consequently, the development of new topical drug delivery systems, such as vesicular nanocarriers, has proven to be effective carriers for antifungal drugs due to their ability to efficiently target the deeper layers of the skin and deliver an optimal drug dose [4, 6, 8-12]. Ethosomes were first introduced by Touitou and al. in 2002 [13, 14] ; They are soft and flexible vesicles mainly composed of phospholipids, ethanol and water [4-6, 12, 14, 15]. The presence of a large amount of ethanol in their structure differentiates them from other vesicular systems and gives them the ability to modify the dense alignment of lipid bilayers in the stratum corneum, thus ensuring deeper drug penetration [4-6, 12, 14, 16, 17]. Ethanol also imparts a net negative charge to the surface of the vesicles, providing them with better stability due to electrostatic repulsion [14, 18]. Compared to other systems, ethosomes are less toxic and cause less skin irritation, making them more suitable for transdermal administration [4-6, 12, 14, 19]. Ethosomes are easy to prepare, stable and safe to use; therefore, various ethosomal formulations are currently available on the market [5, 6, 14, 20-23].

Binary ethosomes (BE), particularly, constitute an innovative vesicular nanocarrier system primarily composed of phospholipids, a binary alcoholic phase that includes ethanol, short-chain alcohols, and water [24-26]. In addition, propylene glycol is an agent that is also part of the ethosome wall composition due to its skin penetration enhancer effect [17, 20, 26]. Moreover, the combination of ethanol with other alcohols is intended to provide binary ethosomes with a smaller vesicle size, higher skin permeability and better entrapment efficiency [17, 20, 26].

In light of the above, this study aimed to formulate and characterize binary ethosomes loaded with Fluconazole in order to investigate the effect of formulation parameters on their characteristics.

2. Material and methods

Initially, during a preliminary trial, conventional blank ethosomes (without active substance) were prepared while varying the sonication duration. The aim was to select the optimal sonication time, which would later be used for the preparation of binary ethosomes loaded with Fluconazole.

2.1. Method of preparation of blank conventional ethosomes

Nine conventional ethosomal formulations were prepared using the cold method [7, 27]. A precise amount of soybean lecithin (AROMA-ZONE, France) [2–5% (w/v)] is first dissolved in varying amounts of absolute ethanol (VWR Chemicals, France) [25–50% (v/v)] under continuous stirring using a magnetic stirrer (VELP Scientifica, Italy) at a speed of 700 revolutions per minute (rpm). The mixture is then heated to 30°C in a water bath (VELP Scientifica, Italy) for 10 minutes. A precise volume of distilled water (QSP 100%) is also heated to the same temperature and then added dropwise while stirring for 15 minutes to prepare the colloidal ethosomal dispersion. Stirring continued for 5 minutes at room temperature. Each prepared formula was subjected to three different sonication times: 15 minutes, 22.5 minutes and 30 minutes, in order to reduce the particle size [7, 28, 29] using an ultrasonic bath (Haver USC 2000, Germany). The composition of the classical blank ethosomal formulations is reported in Table 1:

Table 1 Composition of the classical blank ethosomal formulations

Formula	Soya lecithin (%)	Ethanol (%)	Water (%)
F1	2	20	Up to 100
F2	2	35	
F3	2	50	
F4	3.5	20	
F5	3.5	35	
F6	3.5	50	
F7	5	20	
F8	5	35	
F9	5	50	

2.2. Method of preparation of binary ethosomes

Nine binary ethosomal formulations based on Fluconazole were prepared using the cold method [27]. In this case, a precise amount of soybean lecithin (AROMA-ZONE, France) [2–5% (w/v)] is dissolved in a mixture of absolute ethanol (VWR Chemicals, France) and isopropyl alcohol (IPA) (Sigma-Aldrich, Germany) [20–50% (v/v)], to which 0.5% Fluconazole (kindly provided by MERINAL Laboratories, Algeria) and 1% cholesterol (Alfa Aesar, USA) are added under continuous stirring using a magnetic stirrer (VELP Scientifica, Italy) at a speed of 700 rpm. The mixture is then heated to 30°C in a water bath (VELP Scientifica, Italy) for 5 minutes before adding 20% of propylene glycol (Sigma-Aldrich, USA), and stirring continues for 5 minutes. A precise volume of preheated distilled water at the same temperature (QSP 100%) is added drop by drop under magnetic stirring for 15 minutes to prepare the colloidal ethosome dispersion. The stirring of the prepared ethosomal dispersion continued for 5 minutes at room temperature. Each prepared formula was subjected to sonication in an ultrasonic bath (Haver USC 2000, Germany) for 22.5 minutes [28]. The composition of formulations is reported in Table 2:

Table 2 Composition of binary ethosomal formulations

Formula	LS %	Alcohol mixture %		Fluconazole e%	Cholesterol %	PG %	Water %
		Ethanol	IPA				
F 1	2	15	5	0.5	1	20	Up to 100
F 2	2	12.5	7.5				
F 3	2	10	10				
F 4	2	30	5				
F 5	2	27.5	7.5				
F 6	2	25	10				
F 7	5	30	5				
F 8	5	27.5	7.5				
F 9	5	25	10				

LS: Soya lecithin; IPA: Isopropyl Alcohol; PG: Propylene glycol.

2.3. Characterization

2.3.1. Particle Size and Zeta potential

The particle size was measured using dynamic light scattering (DLS) at 25°C with the particle size measurement system (Zetasizer, Malvern Instruments, UK) [30, 31].

Electrokinetic phenomena, such as electrophoresis, reflect the surface charge and are represented by the zeta potential which is considered as a good indicator of the stability of colloidal suspensions. Experimentally, it is possible to measure the particle mobility under the influence of an electric field (called electrophoretic mobility) in the solution and deduce the zeta potential from it [30, 32].

2.3.2. Entrapment efficiency (% EE)

The maximum absorption wavelength of Fluconazole (λmax = 250nm) was determined by performing a scan between 200 and 400 nm of a pure Fluconazole solution. A volume of 5 ml of each binary ethosomal formulation is diluted in distilled water, then centrifuged at 15,000 rpm for 45 minutes at a temperature of 4°C using a refrigerated centrifuge (Sigma 2-16 K, Germany) [15]. The concentration of Fluconazole in the supernatant was analyzed using a UV-visible spectrophotometer (Optizen, South Korea) at 250 nm after diluting the supernatant. The entrapment efficiency (EE%) of the binary ethosomes was then calculated using the following equation [33] :

$$EE\% = \frac{\text{Total Fluconazole - Non encapsulated Fluconazole}}{\text{Total Fluconazole}} \times 100$$

3. Results and discussion

The results of the characterization of blank and binary ethosomes are presented in Tables 3 and 4, respectively. They revealed an average vesicle size ranging from a minimum and a maximum values of 412.2 ± 1.7 nm and 5816 ± 9.6 nm for the blank ethosomes, and from 742.5 ± 11.1 nm to 2288 ± 10 nm for the binary ethosomes.

Table 3 Particle sizes and zeta potential values of blank classical ethosomes according to sonication time

Sonification time	**15 min**		**22.5 min**		**30 min**	
Formula	**Particle sizes (nm)**	**Zeta potential (mV)**	**Particle sizes (nm)**	**Zeta potential (mV)**	**Particle sizes (nm)**	**Zeta potential (mV)**
F1	1083±8.2	-25±0.5	963.6±3.2	-35.7±0.1	735±4.5	-22.5±0.3
F2	656.5±4.5	-21±0.3	577.3±2.1	-19.7±0.5	414.2±1.7	-9.35±0.1
F3	1747.5±6.1	-11.5±0.3	3893±5	-15±0.2	5816±9.6	-15.5±0.1
F4	1669±9.8	-41.1±0.2	1492.5±6.8	-13.4±0.1	889.2±3.3	-12.4±0.6
F5	1320±3.2	-12.9±0.1	1260±4.7	-14.2±0.3	1059±1.2	-15.1±0.3
F6	3472±15.8	-11.8±0.6	2592.5±8.9	-19.5±0.4	1878±2.3	-8.15±0.1
F7	2023±7.6	-11±0.5	1678±6.8	-9.9±0.2	2419±5.4	-11.5±0.2
F8	1305±3.3	-10.8±0.1	983.8±2.1	-39.6±0.1	789±2.8	-9.12±0.3
F9	4070±8.8	-10.3±0.2	1105±2.4	-12.1±0.3	978.5	-10±0.7

nm : nanometer ; mV : millivolts

Table 4 Characteristics of Fluconazole-loaded binary ethosomes

Formula	**Particle sizes (nm)**	**Zeta potential (mV)**	**Entrapment efficiency (%)**
F 1	742.5±11.1	-15±0.3	87.75±0.1
F 2	1007.6±15.4	-11.5±0.09	89.89±0.1
F 3	1111.5±19.5	-9.91±0.1	87.34±0.1
F 4	1171±13	-18.6±0.1	88.62±0.05
F 5	2052.5±20	-48.4±0.35	74.87±2.2
F 6	2288±10	-23.1±0.5	74.24±3.1
F 7	1113.6±14.3	-10.21±0.5	82.12±1.3
F 8	1219.5±10.1	-14.3±0.3	75.57±2.4
F 9	1273±17.8	-22.5±0.2	48.89±3.5

nm : **nanometer ; mV : millivolts**

3.1. Effect of sonication time on particle size

The effect of sonication time on the particle size of blank conventional ethosomes was observed by varying the sonication duration (15, 22.5 and 30 minutes), as shown in **Figure 1**:

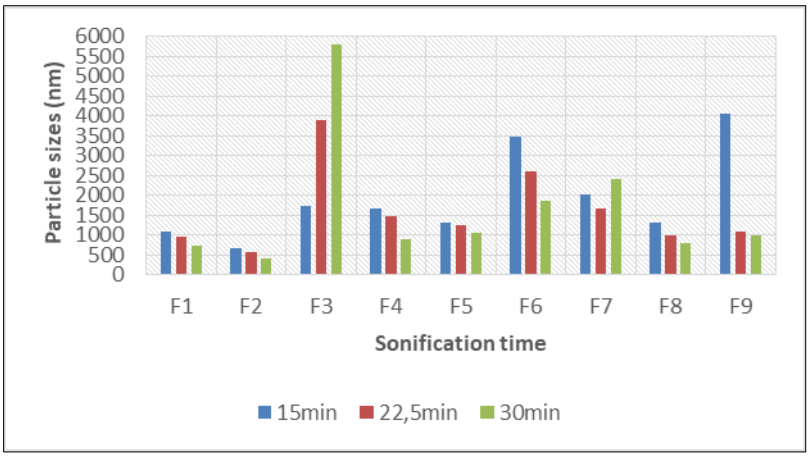

Figure 1 Effect of sonication time on the particle size of blank classical ethosomes

It was observed that the sonication time had a negative impact on the vesicle size, an increasing of the sonication time from 15 minutes to 22.5 minutes resulted in a reduction in particle size for the majority of the formulations. This can be attributed to the fact that sonication breaks down large droplets into nanodroplets, resulting in ethosomes with smaller particle sizes [29, 34]. However, an increase in particle size was noted after 30 minutes of sonication, up to 5816 nm for formula F3, this can be due to an excessive sonication [35].

According to the obtained results, the optimal sonication time chosen for the preparation of Fluconazole-loaded binary ethosomes was 22.5 minutes. Therefore, the three formulations F1, F2, and F8, which were the most stable and had the smallest particle sizes, were selected to formulate the binary ethosomes.

3.2. Effect of ethanol and soybean lecithin concentration on particle size and zeta potential of ethosomes

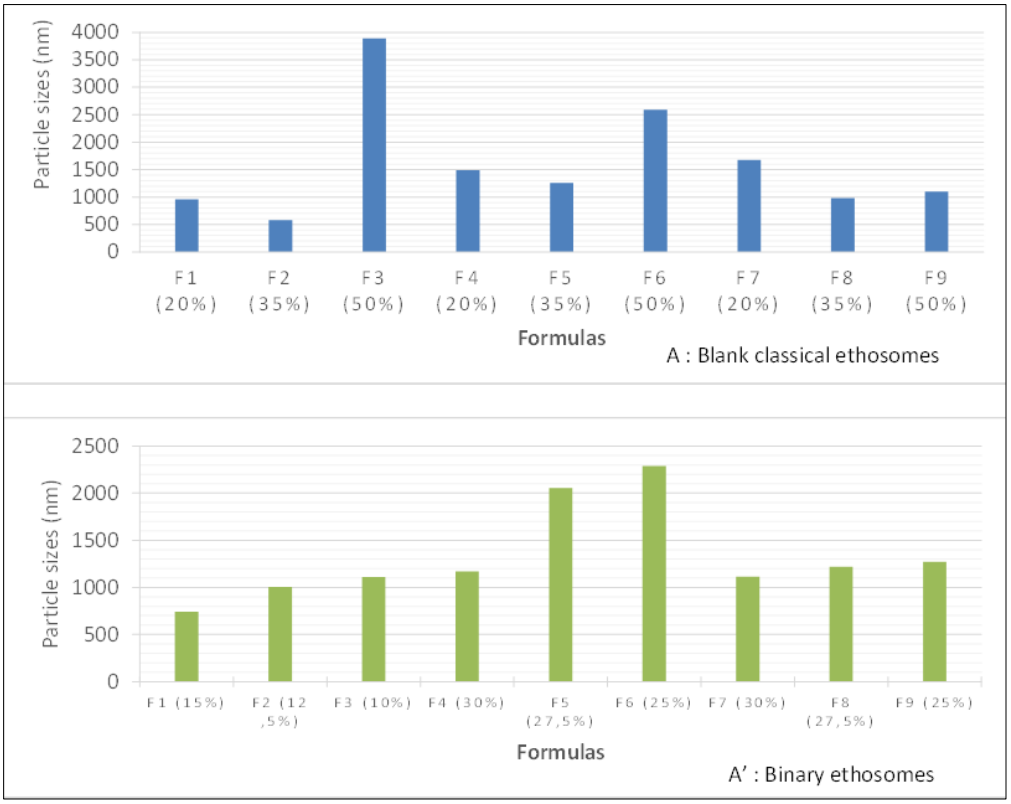

Figure 2 Effect of ethanol concentration on the particle size of blank classical ethosomes (A) and binary ethosomes (A')

On one hand, the effect of ethanol concentration on the particle size of blank conventional ethosomes was studied by fixing the concentration of soybean lecithin, as shown in Figure 2 (A). It was noted that the particle size of some ethosomal formulas (F1, F2, F4, F5, and F7) tended to decrease when the concentration of soybean lecithin varied from 2% to 5% in the presence of ethanol concentrations ranging from 20% to 35%. In contrast, higher ethanol concentrations, reaching 50% in formulations F3, F6, and F9, led to an increase in particle size.

On the other hand, decreasing the ethanol concentration in the ethanol-IPA mixture across all binary ethosome formulas from F1 to F9, as shown in Figure 2 (A'), resulted in an increase in particle size.

The results obtained are consistent with those of Ehab R. Bendas and Mina I. Tadros, who showed that the relationship between ethanol concentration and particle size was inversely proportional [36]. On the other hand, Aljohani, A.A and al. revealed that the decrease in particle size with increasing ethanol concentration is likely due to the modification of the net surface charge of the vesicles by this solvent, leading to steric stabilization and a reduction in particle size [7]. For their part, Abdulbaqi and al. in their study stated that increasing the ethanol concentration beyond a certain value would lead to an increase in vesicle size [37]. This suggests that there is an optimal ethanol concentration beyond which the disintegration of the structural integrity of the ethosomes would occur, leading to an increase in particle size [7].

Additionally, the effect of soybean lecithin concentration on particle size was also studied by varying its concentration from 2% to 5%, while fixing the ethanol concentration at 20% for the blank ethosomes (F1, F4, and F7), 35% (F2, F5, and F8) and 50% (F3, F6, and F9), and at 35% for the binary ethosomes from formula F4 to F9.

The blank conventional ethosomal formulations developed showed an increase in particle size, as shown in Figure 3 (A). In contrast, a decrease in particle size was observed for the binary ethosomes, as shown in Figure 3 (A').

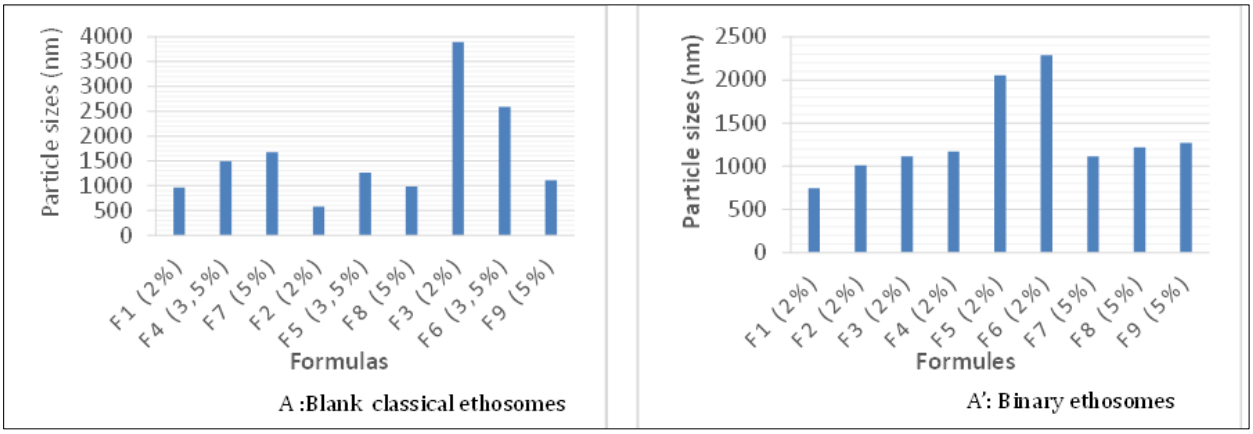

Figure 3 Effect of soybean lecithin concentration on the particle size of blank classical ethosomes (A) and binary ethosomes (A')

Regarding the blank classical ethosomes, the results obtained are in perfect agreement with previous studies, which reveal that an increasing concentration of phospholipids slightly or moderately increases the vesicle size [38-40]. As mentioned in the literature, the recommended amount of phospholipids that can be used in an ethosomal formulation is between 0.5% and 5% [41], this was applied in the present experimental study. Comparable results have also been reported by Nasr and al [42]. These results were also consistent with those reported by Morsi and al, who stated that increasing the lecithin concentration can result in an increase in particle size [43].

For the binary ethosomes, comparing formulations with similar ethanol concentrations but increasing soybean lecithin concentrations shows a decrease in the particle size of the ethosomes. The same results were observed in the study of Alhanouf A. Aljohani and Al [7].

Furthermore, the zeta potential values obtained ranged from -8.15 ± 0.1 mV to -41.1 ± 0.2 mV for the blank classical ethosomes and from -9.91 ± 0.1 mV to -48.4 ± 0.35 mV for the binary ethosomes, as indicated in Tables 3 and 4, respectively. Figures 4 (A) and (A') and Figure 5 (A) and (A') show that the zeta potential values do not depend on the ethanol and soybean lecithin concentrations.

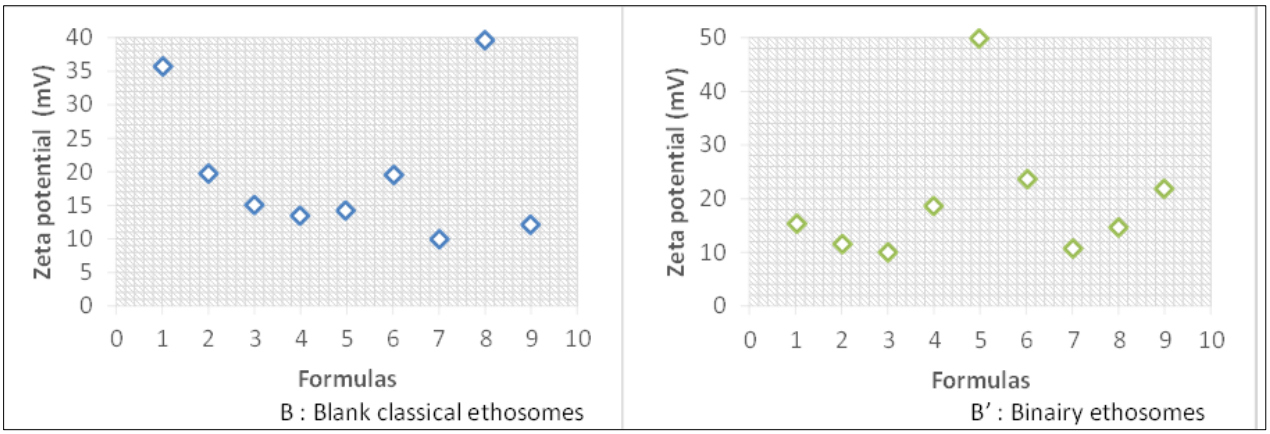

Figure 4 Effect of ethanol concentration on the zeta potential of blank classical ethosomes (A) and binary ethosomes (A')

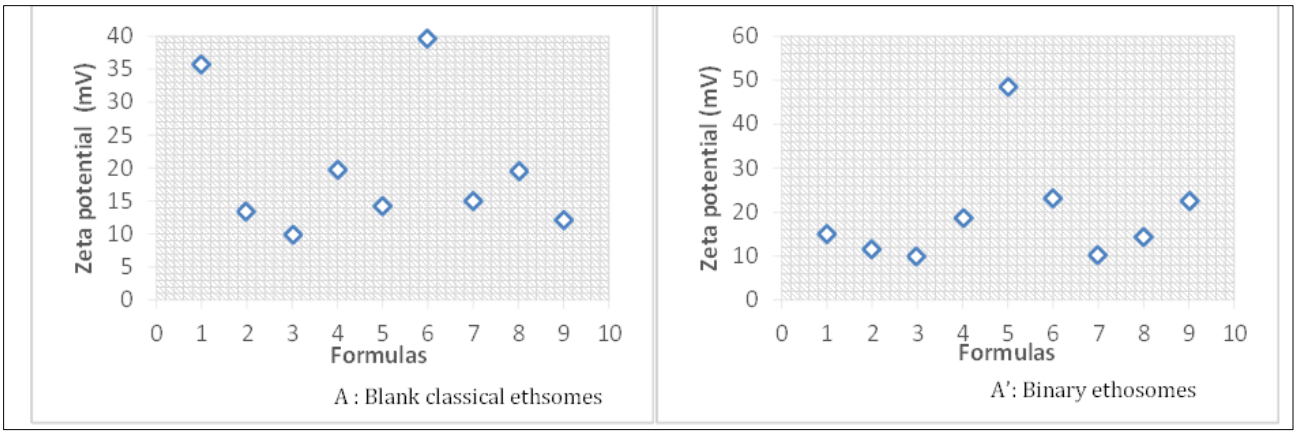

Figure 5 Effect of soybean lecithin concentration on the zeta potential of blank classical ethosomes (A) and binary ethosomes (A')

The zeta potential is a measure of the magnitude of electrostatic repulsion or attraction and it is known to affect stability [30-32, 44]. Its measurement helps to better understand the causes of dispersion, aggregation, or flocculation and can be used to improve the formulation of ethosomes [15, 30-32]. It was also mentioned by Ogiso and al. that the negative charge of the zeta potential of the ethosomal system is mainly attributed to the high ethanol content in these nanovesicles; indeed, ethanol imparts negative charges to the polar groups of the phospholipid heads, which would create electrostatic repulsion [6, 30, 31, 45]. According to the literature, a zeta potential lower than 20 mV in absolute value indicates good stability, while a value lower than 5 mV shows rapid aggregation [6, 44]. Therefore, the obtained zeta potential values suggest a satisfactory stability for all ethosomal formulations, regardless of the ethanol and sybean lecithin concentration, which is consistent with the results of the study by Y Zhai and al [46].

3.3. Effect of ethanol and soybean lecithin concentration on the entrapment efficiency (EE%) of binary ethosomes

Regarding the effect of ethanol concentration on the entrapment efficiency of Fluconazole in the binary ethosomes (Figure 6), the results showed values ranging from 48.89 ± 3.5% to 89.89 ± 0.1% . It was noted that increasing the ethanol concentration from 20% (F1, F2, and F3) to 35% (F4, F5, and F6) while maintaining the soybean lecithin concentration at 2% resulted in a decrease in the EE% of Fluconazole from 89.89 ± 0.1% to 74.24 ± 3.1%. Moreover, increasing the soybean lecithin concentration from 2% (F4, F5, and F6) to 5% (F7, F8, and F9), while maintaining the ethanol concentration at 35%, also had a negative impact on EE%; it decreased from 88.62 ± 0.05% for formula F4 to 48.89 ± 3.5% for formula F9.

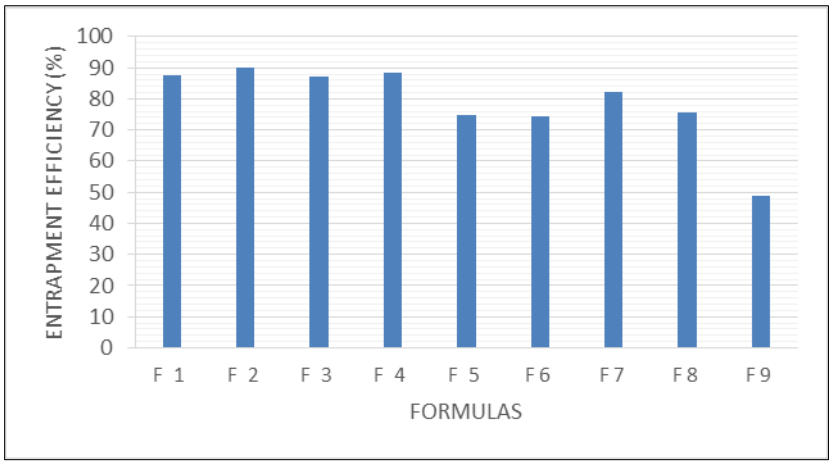

Figure 6 Effect of ethanol and soy lecithin concentration on the entrapment efficiency of binary ethosomes

The results obtained revealed that the entrapment efficiency of Fluconazole was dependent on the concentrations of soy lecithin and ethanol used. However, these results do not align with those in the literature. Indeed, it has been reported that increasing the ethanol concentration from 20% to 40% would increase the EE% in a linear relationship [37, 47]. The result obtained in this experiment suggests that, since the size of the ethosomal particles was reduced due to the ethanol concentration, the central cavity of the ethosomes was small, which negatively impacted the entrapment efficiency of Fluconazole. Furthermore, the membrane permeability would have been increased by the high ethanol concentration, which likely caused the solubilization of the phospholipids and subsequent leakage of the active ingredient through the lipid bilayer [16, 48, 49].

On the other hand, Abdulbaqi and al. in their study revealed that the range of phospholipid concentrations in an ethosomal formulation should be between 0.5% and 5%, and that increasing the phospholipid concentration significantly enhances the EE %. However, they added that this relationship holds true only up to a certain concentration, beyond which further increases in phospholipid concentration have no effect on the EE% [37].

4. Conclusion

After a preliminary study, this work successfully developed and synthesized binary ethosomes loaded with Fluconazole, and identified the formulation factors that may influence their characteristics. Indeed, the developed binary ethosomes exhibited relatively small vesicle sizes, acceptable zeta potential values, and satisfactory entrapment efficiency of Fluconazole. Other parameters could be evaluated as future perspectives of this work, including the study of Fluconazole release kinetics and the stability of the ethosomal formulations over the medium and long term.

Compliance with ethical standards

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Disclosure of conflict of interest

We declare that there are no conflicts of interest related to this document.

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