

Original Research Article

Impact of lipophilic carriers on metronidazole lipophilicity

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Abstract

Purpose: To optimize the lipophilicity of metronidazole using two lipophilic carriers (stearic acid and cholesterol).

Methods: The partition coefficient of compounds was determined using the shake flask method. The classical method included shaking a sample in a flask to equilibrate it between an aqueous phase and an organic phase. The concentration of the analyte of interest was determined in both phases using spectrophotometry. Two lipophilic substances, stearic acid (SA) and cholesterol (CH), were included at varying concentrations. Specifically, stearic acid was incorporated at 0.25 % and 0.5 %, while cholesterol was incorporated at 0.1 and 0.25 %. A full factorial design with 4 base formulas was utilized to provide a comprehensive analysis of both the main and interaction effects of the two additives on metronidazole lipophilicity.

Results: Statistical model portrayed that both SA and CH significantly affected Log P values (SA: $p = 0.003$, CH: $p = 0.006$). Notably, interaction between SA and CH was highly significant and of a negative interaction coefficient ($p = 0.000$, Coef = -0.04209), indicating that higher cholesterol levels reduce the lipophilicity-enhancing effect of stearic acid.

Conclusions: Lipophilicity of metronidazole may be optimized using optimum amounts of stearic acid and cholesterol. This could offer new formulation strategies for the purpose of enhanced drug delivery.

Keywords: Lipophilicity, Full Factorial Design, Metronidazole

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INTRODUCTION

In pharmaceuticals, optimization of drug properties, which augments therapeutic efficacy and alleviates adverse effects, constitutes one of the primary objectives of drug development. Among diverse physicochemical characteristics of drugs, lipophilicity stands out as a significant determinant of pharmacokinetic and pharmacodynamic profiles, contributing to the solubility and permeability of drugs through membranes, drug's potency and toxicity [1,2]. Lipophilicity is quantitatively assessed through partition coefficient, Log P, which quantifies the

equilibrium partitioning of a solute between water and an immiscible organic solvent [3]. Several experimental methods are utilized to determine the partition coefficient of compounds featuring shake flask method, liquid chromatography and capillary electrophoresis. The shake flask method, acclaimed for its straightforwardness, is favoured at small-scale settings owing to its reliability and minimal requirements for sophisticated equipment with relatively simple setup and procedure. The shake flask which is a classical method includes shaking a sample in a flask to equilibrate it between an aqueous phase and an organic phase. The concentration of the

analyte of interest is determined in both phases afterwards, using spectroscopic or chromatographic methods [4].

Metronidazole is a widely used antibiotic and antiprotozoal medication known for its hydrophilic properties, portrayed by a low log P value of about -0.1 [5]. It has a molecular weight of 171.16 g/mol, with water solubility of 1 in 100 parts and ethanol solubility of 1 in 200 parts [5]. According to the Biopharmaceutics Classification System (BCS), metronidazole is typically classified as a Class I drug, indicating its high solubility and permeability, and making it well-suited for oral administration. However, Kasim *et al* [6] reported that metronidazole falls under Class III instead, characterized by high solubility but low permeability. Regardless of that, metronidazole is not limited to oral dosage forms, as topical formulations have also been developed [7], where optimized lipophilicity is essential for efficacy.

The main objective of this study is to optimize the lipophilicity of metronidazole using two lipophilic materials (SA and CH) and determine which excipient significantly alters the lipophilicity. In addition, the study also aims to analyze the synergistic or antagonistic effects of SA and CH on the partitioning of metronidazole, which can later offer new formulation strategies.

EXPERIMENTAL

Materials

Metronidazole was kindly supplied by Sama Al-Fayhaa Co., Basra, Iraq. Cholesterol and stearic acid were purchased from Albasheer Office, Baghdad, Iraq.

Construction of metronidazole calibration curves in octanol and water

Ultraviolet spectral analysis was used to quantify the concentration of metronidazole following the preparation of dilution series. Wavelength of maximum absorbance of metronidazole was identified within the specific parameters of this investigation in both distilled water and octanol through spectral analysis of a 0.2 mg/mL solution. Identification was performed in triplicate. Calibration curves were constructed by preparing a 0.5 mg/mL stock solution for each system and generating a dilution series: 10 concentrations for distilled water and 7 concentrations for octanol. Each dilution was done in triplicate. Due to technical errors, two dilution points in the octanol system were excluded from the analysis. Curves

and mathematical models were generated using Minitab statistical software [8].

Design

The effect of SA and CH on metronidazole lipophilicity was evaluated using a 22 full factorial design with 8 total runs and each run was done as per Table 1. The independent variables were chosen at specific concentrations according to the FDA inactive ingredient database [9].

Table 1: Full factorial design

Run Order	Blocks	SA (%)	CH (%)
1	1	0.25	0.10
2	1	0.25	0.25
3	1	0.50	0.10
4	1	0.50	0.25
5	2	0.25	0.10
6	2	0.25	0.25
7	2	0.50	0.10
8	2	0.50	0.25

Log P was established as the dependent variable, representative of the drug optimized lipophilicity and was calculated according to Eq 1. The runs were conducted in blocks to control and mitigate any effects of nuisance variables, improve precision of the experiment, and manage practical constraints [10]. Design space generation and data analysis were done using Minitab statistical software [8].

$$\text{Log P} = \log_{10} (\text{CA/CB}) \dots\dots\dots (1)$$

Where CA = Concentration in octanol and CB = Concentration in water

Separation process

In this investigation, eight runs were performed. For each run, a 0.5 mg/mL solution of metronidazole was prepared, and different concentrations of SA and CH were added according to the full factorial design. Ethanol served as the solvent for the mixtures. A volume (10 mL) of each mixture was transferred to a separatory funnel containing 30 mL each of distilled water and n-octanol. The separatory funnel was shaken and left to stand for 72 h at room temperature to achieve complete phase separation [11]. Since metronidazole is light sensitive, separatory funnels were wrapped in aluminum foil to prevent photodegradation [12]. Water and n-octanol phases were collected separately and the concentration of metronidazole in each phase was quantified using UV spectroscopy.

UV analysis

Each phase was diluted with its corresponding solvent in a 1:4 dilution ratio. Triplicate dilutions were prepared for each phase, and these were analyzed for quantification of metronidazole. The average absorbance values for each phase were calculated, and metronidazole concentrations were subsequently determined using the established calibration curves.

Fourier transform infrared spectroscopic analysis (FTIR)

An FTIR scanning of KBr pellets containing powder samples of pure drug, dried formula and its physical mixture was performed in the wave number range 400 - 4000 cm^{-1} at a resolution of 4 cm^{-1} with speed of 2 mm/sec [13].

X-ray diffraction analysis

A Diano X-ray diffractometer (USA) fitted with a Co-K α radiation (45 kV, 9 mA, scanned from 3 ° to 50 ° at 2 angles) was used to analyze samples in powdered form including pure drug, dried formula and its physical mixture [13].

RESULTS

Octanol phase calibration curve

The wavelength of maximum absorbance was observed at 311 nm. The absorbance of serial dilution of metronidazole was used to plot the

calibration curve (Figure 1) with an r^2 of 0.998. The analysis of variance, as shown in Table 2, revealed a high F-value, which suggests that the model fit is statistically significant. The Regression Sum of Squares (SS) was high relative to the Total SS, meaning that a great portion of the variability in UV absorbance of metronidazole in n-octanol is explained by variation in metronidazole concentration [8]. Furthermore, the Error Sum of Squares (SS) is very small, indicating that the residuals are minimal and the predictions of the model are very close to actual observations.

Water phase calibration curve

In the waste phase, the wavelength of maximum absorbance was observed at 321 nm. The calibration curve shown in Figure 2 disclosed an r^2 value of 0.999. Analysis of variance exhibited an extremely high F-value as seen in Table 3, making the model fit statistically significant. Similar to the n-octanol system, the Regression Sum of Squares was very high relative to the Total SS and the Error Sum of Squares was also very small, indicating minimal residuals.

Log P results and full factorial analysis

A full factorial design was employed to assess the effect of carriers on the lipophilicity of metronidazole, with SA and CH each at two concentration levels. Full factorial design data are exhibited in Table 4.

Table 2: Analysis of variance in n-octanol system

Source	DF	SS	MS	F	P
Regression	1	0.233	0.233	1313.69	0.000
Error	3	0.0005	0.0001	-	-
Total	4	0.234	-	-	-

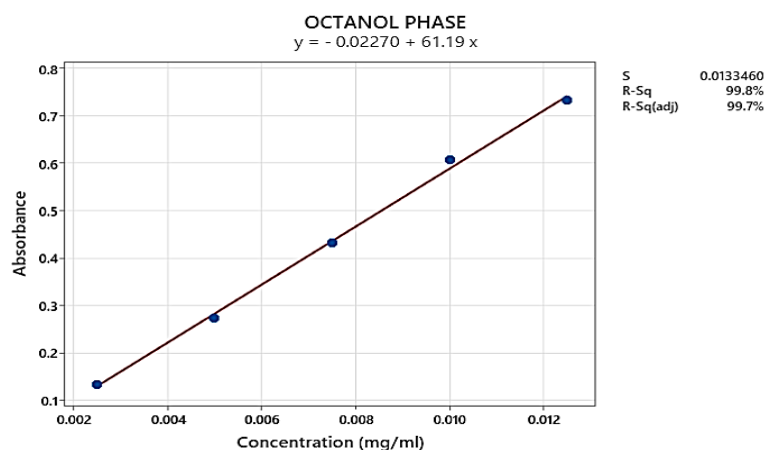


Figure 1: Calibration curve and regression equation of metronidazole in octanol system

Table 3: Analysis of variance in aqueous system

Source	DF	SS	MS	F	P
Regression	1	1.128	1.128	5766.2	0.000
Error	8	0.001	0.0002	-	-
Total	9	1.13	-	-	-

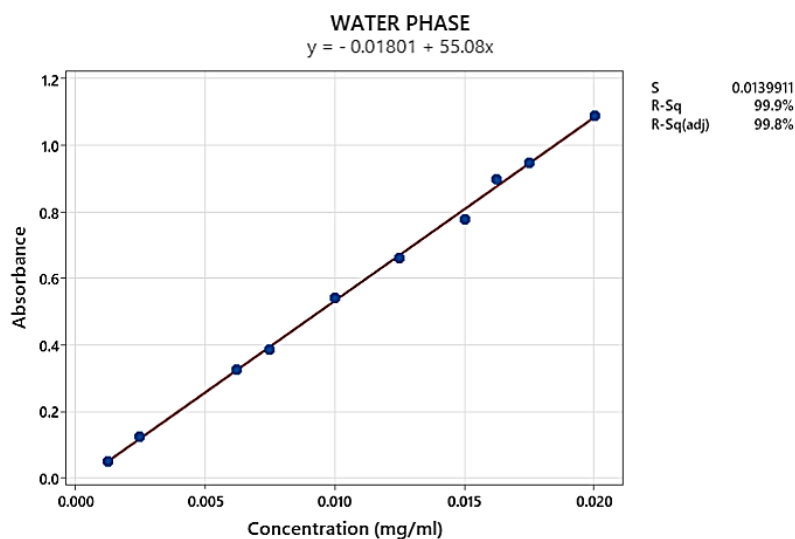

Figure 2: Calibration curve and regression equation of metronidazole in aqueous system

Table 4: Full factorial design data

SA	CH	Log P
0.25	0.10	0.036±0.026
0.25	0.25	0.142±0.031
0.50	0.10	0.056±0.012
0.50	0.25	0.010±0.021
0.25	0.10	0.011±0.040
0.25	0.25	0.151±0.043
0.50	0.10	0.064±0.019
0.50	0.25	0.020±0.028

The analysis of variance, presented in Table 5, indicates that the overall model is statistically of high significance ($p = 0.001$), suggesting that the chosen concentrations and their interactions significantly impact Log P values. Both SA ($p = 0.003$) and CH ($p = 0.006$) have significant main effect, as increasing the concentrations of both

could lead to a potential increase in log P. A particularly noteworthy finding is the interaction between SA and CH, which is reflected by a highly significant p -value ($p = 0.000$). The significant interaction term ($F = 128.04$) implies that the effect of one excipient is dependent on the level of the other excipient. Table 8 specifically addresses the interplay between the two excipients. The interaction coefficient of -0.04209 and the CH main coefficient of -0.01939 suggest that the presence of CH reduces the lipophilicity-enhancing effect of SA. To display the standardized effects of the factors and their interactions on the value of log P, a Pareto chart was generated (Figure 3). The bars for the interaction (SA*CH), Stearic Acid (SA), and Cholesterol (CH) all extended beyond the threshold line, which further signifies their effects statistically [8].

Table 5: ANOVA of full factorial design

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	3	0.021711	0.007237	65.39	0.001
Linear	2	0.007540	0.003770	34.06	0.003
SA	1	0.004533	0.004533	40.95	0.003
CH	1	0.003007	0.003007	27.17	0.006
2-Way Interactions	1	0.014171	0.014171	128.04	0.000
SA*CH	1	0.014171	0.014171	128.04	0.000
Error	4	0.000443	0.000111		
Total	7	0.022154			

Table 6: Full factorial design coefficients

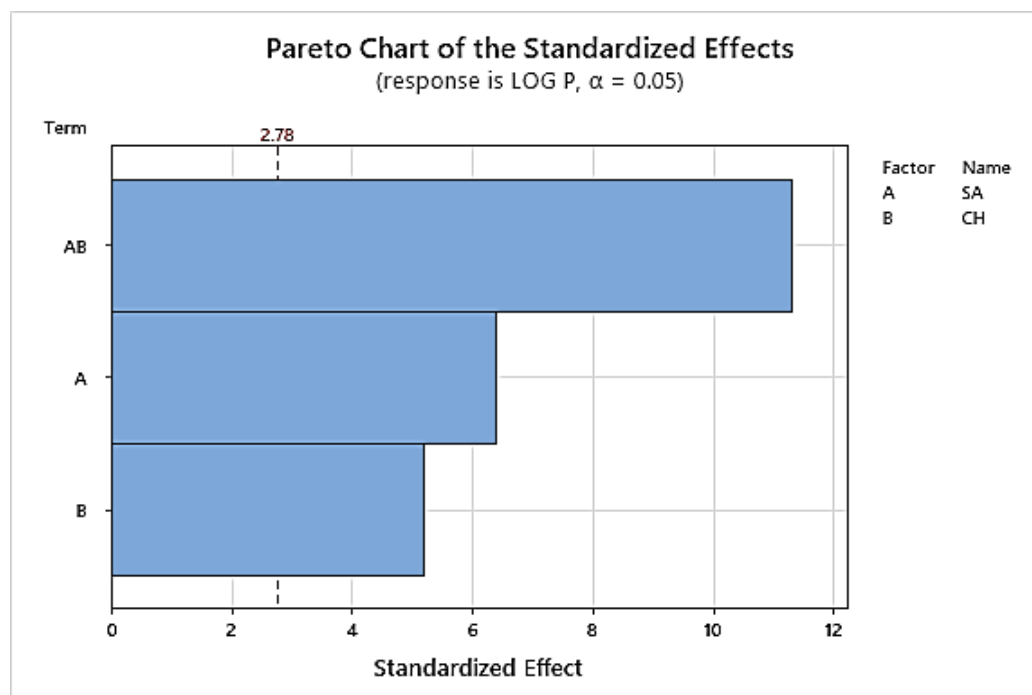
Term	Coef	SE Coef	T-Value	P-Value
Constant	0.06184	0.00372	16.63	0.000
SA				
0.25	0.02380	0.00372	6.40	0.003
CH				
0.10	-0.01939	0.00372	-5.21	0.006
SA*CH				
0.25*0.10	-0.04209	0.00372	-11.32	0.000

However, the largest standardized effect on log P is depicted by the longest bar of SA*CH, highlighting the importance of considering the interaction between excipients upon their addition to the formulation.

Most of the important peaks were found in the selected formula, indicating absence of chemical interaction between metronidazole and involved excipients or applied conditions in method of preparation and this outcome is clearly seen in Figures 5 and 6.

Fourier transform infrared analysis

Regarding the functional groups of metronidazole, the most important characteristic peaks are illustrated in Table 7 and Figure 4.

**Figure 3:** Pareto chart of the standardized effects**Table 7:** The FTIR characteristic peaks of pure metronidazole (standard and observed)

Region range	Observed IR peak (cm ⁻¹)	Assignment	Comment
600-1000	640.4, 740.67, 825.5	C-H, C-C, C-N stretching	
1000-1300	1188	C-O	
1300-1600	1427, 1481, 1535, 1373	-CH ₂ -CH ₃ bending, NO asymmetric and symmetric stretching	Finger print
1600-2000	1805.37	C=C alkene	Double bond
2000-2700		No Triple bond	
2700-4000	2854.65, 3101.54, 3217.27	O-H, N-H and vinyl C-H	Bond to H atom

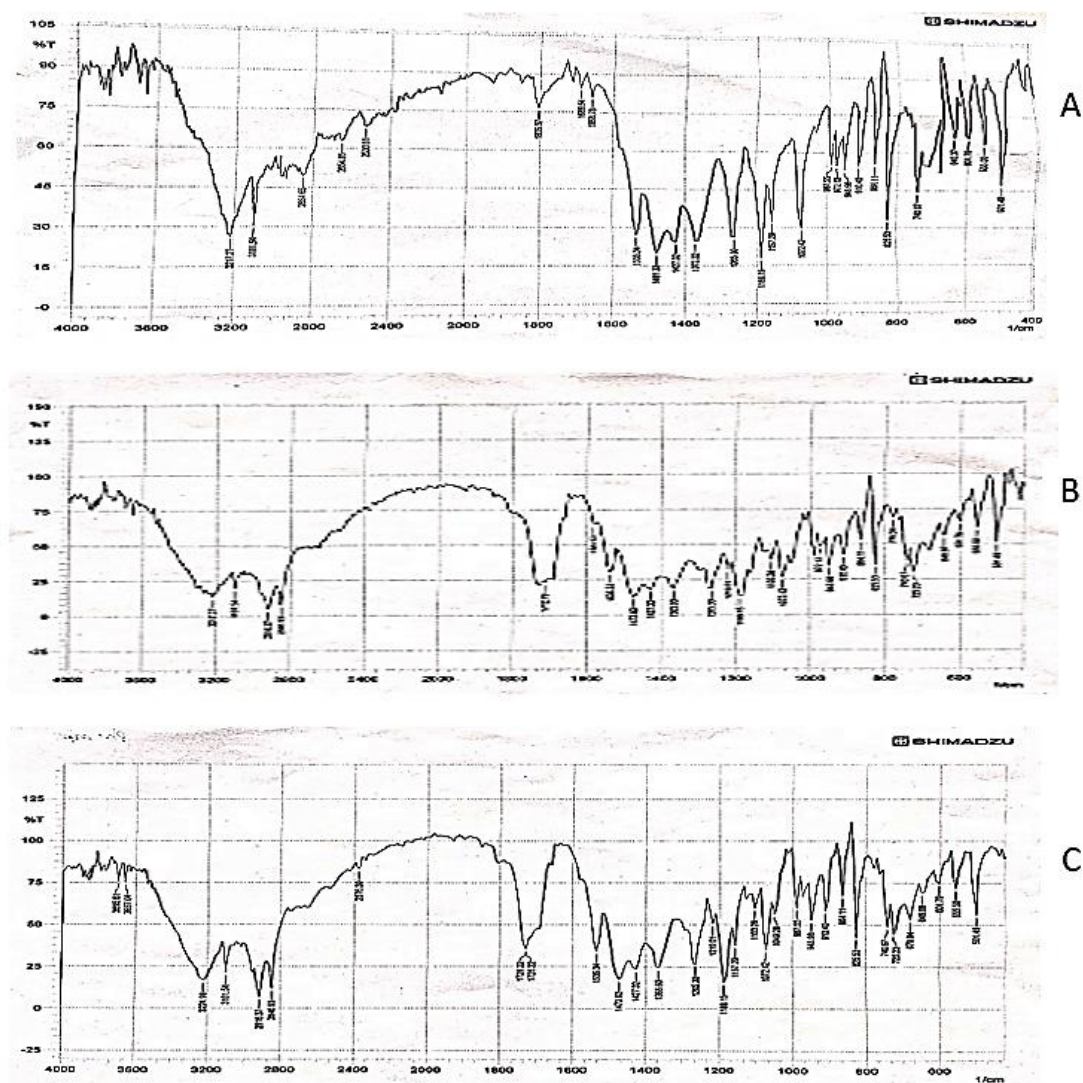


Figure 4: FTIR spectrum of (A) metronidazole (B) selected formula (C) Physical mixture of selected formula

X-ray diffraction

The results shown in Figure 5 A - C indicate absence of physical incompatibility between metronidazole, excipients and methods, reflecting no change in crystallinity state except some reduction in peak intensity, which may be attributed to sampling conditions or particle size change.

DISCUSSION

In this investigation, the effects of two carriers, stearic acid (SA) and cholesterol (CH), on the lipophilicity of metronidazole were explored. While SA alone increases the lipophilicity, its effect diminishes when CH is added at higher concentrations, signifying an inverse relationship between both carriers. The qualitative trend observed (decreased partitioning with higher cholesterol) aligns with the findings of Wennberg *et al* [14] despite the differences in methodology.

The findings demonstrated that cholesterol significantly reduces the partitioning of solutes into the lipid tail region of membranes [14], which can be attributed to the energy barrier for solute partitioning, a phenomenon generated by enhanced van der Waals interactions and tighter packing within the lipid layer [15]. Since the consideration of octanol as an epoxy to physiological phospholipid bilayers, this insight can be extrapolated to the octanol/water system, where pre-mixing cholesterol with metronidazole likely results in similar molecular interactions that hinder the partitioning of drug into the octanol phase. The addition of cholesterol creates a more ordered environment, thus raising the energetic cost for metronidazole to transition from the aqueous phase to the octanol phase, analogous to the reduced partitioning observed in lipid membranes. However, validation of this theory requires more understanding of how exactly octanol reacts with cholesterol.

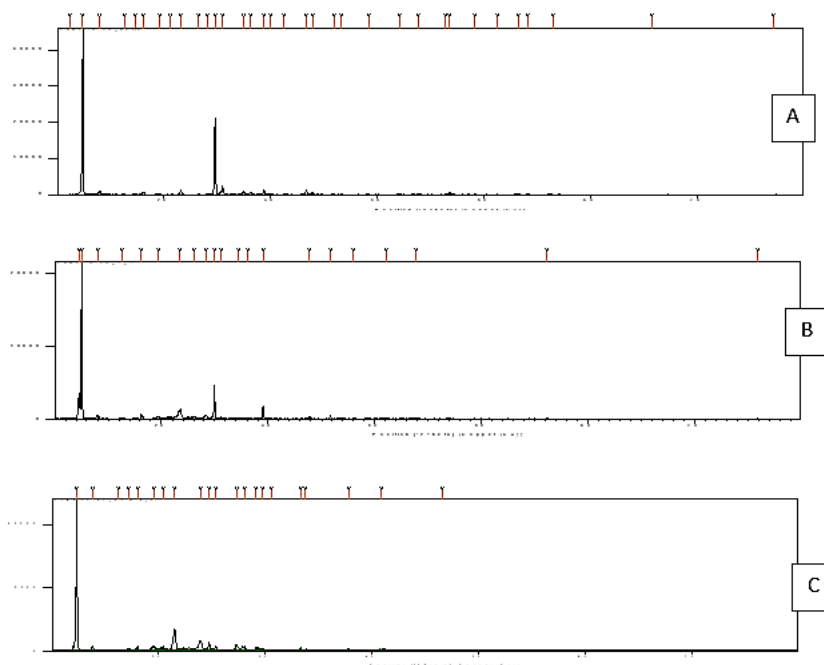


Figure 5: XRD crystallography of metronidazole (A), selected formula (B) and physical mixture (C)

A particular consideration of using ethanol as the sole solvent should be accounted for while interpreting the results. Ethanol, which is miscible with water, influences the measurement of Log P by changing solubility [16] and distribution behaviors [17], leading to different Log P values compared to systems that do not utilize ethanol. Moreover, the presence of ethanol might also cause excipients to interact differently with metronidazole [18], which in turn affects the Log P measurement. However, ethanol was used consistently, maintaining an exact concentration across all runs, which ensured that changes in Log P values are due to the concentrations of stearic acid and cholesterol rather than variations in solvent. This consistency is beneficial for comparing relative effects but might not accurately reflect absolute Log P values compared to measurements in a purely aqueous environment.

An alternative procedure that does not involve the use of ethanol was considered, which involves melting stearic acid and cholesterol to create a homogeneous mixture with metronidazole. This method requires heating the lipid components slightly above their melting points, then mixing metronidazole into the molten mixture before cooling and solidifying the mixture. However, this approach is unfortunately not applicable because of the possible thermal degradation of metronidazole at elevated temperatures [19].

Another alternative method would involve dissolving the lipids in minimal amounts of organic solvent and allowing the solvent to evaporate to form a lipid film, which can then be hydrated. However, this method requires very careful conditions to prevent metronidazole from degradation in response to light. Time constraints also limited the choice of an optimal experimental procedure.

CONCLUSIONS

Optimized lipophilicity of metronidazole using stearic acid and cholesterol reveals a significant synergistic contribution of both carriers to the increase in log P measurements. However, an initial reduction in the lipophilicity-enhancing effect of stearic acid is observed in the presence of cholesterol at higher concentrations. This interplay between the two carriers could offer new formulation strategies, especially in drug delivery using vesicular carriers.

DECLARATIONS

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None.

Ethical Approval

None provided.

Use of Artificial Intelligence/ Large language models

The authors confirm that AI-based tools were used solely for language editing and manuscript polishing purposes (e.g., grammar, phrasing, and clarity). No AI tools were used to generate content, interpret data, or influence study design, results, or conclusions. All intellectual contributions, study analyses, and interpretations reported in the manuscript are the original work of the authors.

Availability of data and materials

The datasets utilized and/or analyzed in this study are available from the corresponding author upon request.

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

The authors affirm that the study was conducted by the named contributors, who bear the responsibility for the content.

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