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SYSTEMATIC PREFORMULATION AND LIPID SCREENING OF THYMOL FOR THE DESIGN OF NANOSTRUCTURED LIPID CARRIERS (NLCS)

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ABSTRACT

Objective: This study will focus on conducting a systematic preformulation and lipid screening study on Thymol (THML) to enable the development of stable and efficient Nanostructured Lipid Carriers (NLCs) for use against leishmaniasis. **Methods:** The physicochemical properties of THML were studied using UV-Vis Spectroscopy, where the absorbance value (276.5 nm) and calibration curve were determined. The structural functional groups of THML were identified using FTIR Spectroscopy. Purity was established using capillary melting point technique. Lipophilicity was evaluated based on shake flask methodology to establish the log P value. Lipid screening was accomplished by performing solubility studies in different solvents at 37 °C. **Results:** Analysis of Characterization confirmed the high purity of THML. Infrared spectrum showed typical peaks at 2956.70 cm^{-1} and 1417.80 cm^{-1} that verified the presence of isopropyl groups and aromatic group, respectively. The log P value calculated was 3.3, suggesting strong hydrophobicity, whereas results from solubility test offered a scientific basis for selection of lipid for better drug loading. **Conclusion:** The results indicate that THML has all the essential physicochemical characteristics for efficient entrapment in NLC. This preformulation baseline study bridges the current knowledge gap by providing the critical parameters necessary for the formulation of more sophisticated lipid carriers for the treatment of Leishmaniasis.

KEYWORDS: Thymol, FTIR, UV-spectroscopy, Nanostructured Lipid Carriers (NLCs), Preformulation, Partition Coefficient, Solubility.

INTRODUCTION

The compound thymol (THML) is a monoterpenoid phenol obtained from thyme oil and has been gaining much attention in medicine because of its strong pharmacological properties. The compound thymol is known for its ability to combat various pathogens effectively through its strong antimicrobial and antifungal activity in treating fungi infections.[1,2,3,4] Furthermore, THML also exhibits considerable anti-inflammatory action, thus emphasizing its multifunctionality and possibilities for future use in healthcare. In recent times, there has been considerable interest shown in the possibility of using THML for therapeutic purposes in treatment of various infections, specifically Leishmaniasis, which is a disease caused by parasites from the Leishmania genus of protozoa.[5,6,7,8]

The available treatment options often suffer from issues such as toxicity, resistance, and inadequate efficacy, underscoring the urgent need for novel therapeutic strategies.

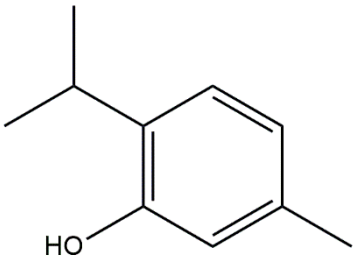

Fig. 01: Structure of THML.

NLCs, also referred to as nanostructured lipid carriers, are relatively new approaches to drug delivery technologies that were specifically developed to counter a number of shortcomings associated with conventional treatment techniques. The distinctive feature of NLCs is the combination of two types of substances: solid and liquid lipids that create a special environment for drugs. The presence of both components allows for increased solubility of hydrophobic medicines.[5,9,10]

The most unique characteristic about NLCs is that they have a property where they allow the slow and regulated delivery of encapsulated drugs, thereby extending the drug's effect and minimizing the drug dosage frequency. This regulated delivery system not only ensures that the drug level in blood is maintained at an optimum value but also avoids any adverse effects of rapid drug delivery.[11,12]

For instance, encapsulating THML, which is an active substance found in nature and characterized with antimicrobial and anti-inflammatory actions, within NLCs will improve its biological performance substantially. Through NLCs, it becomes easier to ensure increased stability and bioavailability of THML, making it possible to target particular cells effectively. Such encapsulation makes it possible to prevent toxicity, since it ensures a gradual release of THML into the body.[1,2,3,13,14,15]

However, selection of appropriate lipids for the development of nanoliposomes (NLCs) is important for effective use of this technique for delivery of drugs. However, current literature reveals that there is an existing lack of study in evaluating lipid matrices for entrapment of THML with regard to treatment of Leishmaniasis.[14,15,16,17]

The present study will attempt to evaluate several lipids to determine their compatibility and usefulness in the formation of NLCs loaded with THML. In this context, the present study attempts to bridge some of the gaps in the

available literature and provide important information regarding the preparation of NLCs for effective treatments for Leishmaniasis.

MATERIAL

THML (99%, Molychem India LLP, Mumbai, India), methanol (HPLC grade) was obtained from Merck (Darmstadt, Germany), ethanol (99%, Sigma-Aldrich, Mumbai, India), Acetone (≥99.5%, Sigma-Aldrich, Mumbai, India), and ethyl acetate (≥99.5%, Sigma-Aldrich, Mumbai, India) were obtained. All the remaining analytical-grade chemicals were utilized for the intended study.

METHODS

Preparation of stock solution: A 1000 µg/ml stock solution was prepared by placing 10 mg of THML, which was accurately measured, into a calibrated volumetric flask and dissolving it in 10 ml of methanol. To achieve the target concentration of 10 µg/ml (Stock II solution), Stock I solution is combined with a methanol solvent.[18,19,20]

Determination of wavelength of maximum absorbance (λmax): The Stock-II solution was examined in full scan mode throughout the entire UV and visible spectra, ranging from 400 to 200 nm, using methanol as a blank. After the spectrum was obtained, the software was utilized to determine λmax.[18,19,21]

Preparation of a Calibration Curve. To make six calibration standards with concentrations of 10, 20, 40, 80, 100, and 150 µg/ml, the stock II solution was diluted. We measured the absorbance of each calibration standard at a set λmax of 276nm using a fixed wavelength measuring mode. The calibration curve illustrated how absorbance changes with concentration. The method described above was done five times to get the same results each time.[18,20]

FTIR studies: We used the sample method to look at THML. An FTIR spectrophotometer scanned the spectra of samples that were put in a KBr crystal.

Determination of Melting Point: The capillary tube method was used to determine the melting points. Samples were placed into a capillary and sealed with a flame. A capillary tube was inserted into the equipment to determine the melting point. This method was implemented three times (n=3) to achieve better results.[20,22]

Determination of the Partition coefficient: The partition coefficient is a key preformulation technique that measures a drug's lipophilicity, affecting its affinity for the lipid matrix in Nanostructured Lipid Carriers (NLCs). Using the shake-flask method (n=3), the drug is distributed between an organic phase (like n-octanol) and an aqueous phase (like phosphate-buffered saline). After equilibrium, the drug concentrations are measured, and the ratio, expressed as (log P), predicts the drug's encapsulation ability in the lipid core and its stability.[21,23]

Solubility studies: An abundance of the drug in various solvents, such as water, ethanol, methanol, phosphate buffer, and n-hexane by solubility studies. Solubility studies were conducted by adding excess THML to 5 mL of solvent, maintaining the mixture at 37°C ± 0.5°C in a shaker bath for 24 hours to reach equilibrium. Samples were filtered (0.45 µm), diluted, and analyzed (n=3), Samples underwent filtration through a 0.45 µm membrane filter and were then analyzed using a UV spectrophotometer.[18,19,21]

Screening of lipids based on solubility study: The screening method started by evaluating the solubility of the model drug, THML, in many stable lipids and oils. A solubility study was necessary in order to determine lipids that would be able to successfully encapsulate and hold the drug. The quantity of lipids was quantified and subsequently placed into individual glass tubes.[21,23] THML was slowly incorporated into the melted lipids while mixing until saturation was reached. Solubility of lipids was reached once no additional solubilization was possible, leaving an excess of undissolved drug.[20,21,23,24]

When the samples reached their saturation point, equilibrium was achieved, and the un-dissolved drugs were separated from the solutions. The quantity of drugs that had successfully dissolved in the lipids was estimated through the re-weighing process of the un-dissolved fractions. This method helped to establish the solubility of the drug in the lipids and provided vital information in identifying the appropriate lipids that maximize solubilization and hence increase drug loading in the new drug delivery systems.

Data analysis: R^2 indicates the degree to which the regression line aligns with the data points from the standard reactions for each specific CT. If the value of R^2 is 1.00, this means that there is a perfect fit between the regression line and the data points. The desired R^2 is 0.997.

RESULTS

Determination of wavelength of maximum absorbance: The measurement of the wavelength by quantitative UV spectroscopy that results in the greatest absorption value. In general, solutions with an absorbance less than 1 are suitable for measuring the wavelength of maximum absorption. The full scan function of the UV-visible spectrophotometer was employed to measure the maximum wavelength of the THML solution, considering the relevant requirements. The UV analysis program was used to analyse the entire scan range to determine λmax. From Figure 2, it can be seen that the wavelength of 276.5 nm was measured for THML.[18,19,20]

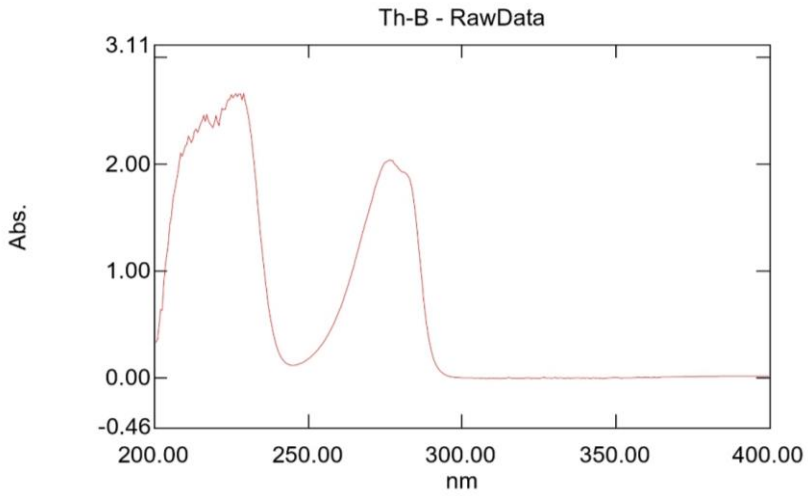

Fig. 2: Maximum absorbance (λ_{max}) of THML at 276.5 nm.

Preparation of standard curve: Every instrumental analytical technique, including UV-visible spectrophotometry, requires a consistent calibration curve and a formula that describes the relationship between concentration and response to measure unknown substances. The previously mentioned method is more commonly recognized and reproducible

than the graphical method.[21,25,26] Five important calibration standards are used to generate the THML calibration curve, emphasizing the efficiency of the quantitative analysis of THML. In Figure 3, the absorbance of several calibration standards at 276.5 nm was measured using a UV-visible spectrophotometer operating in fixed-wavelength mode.[18,19,20]

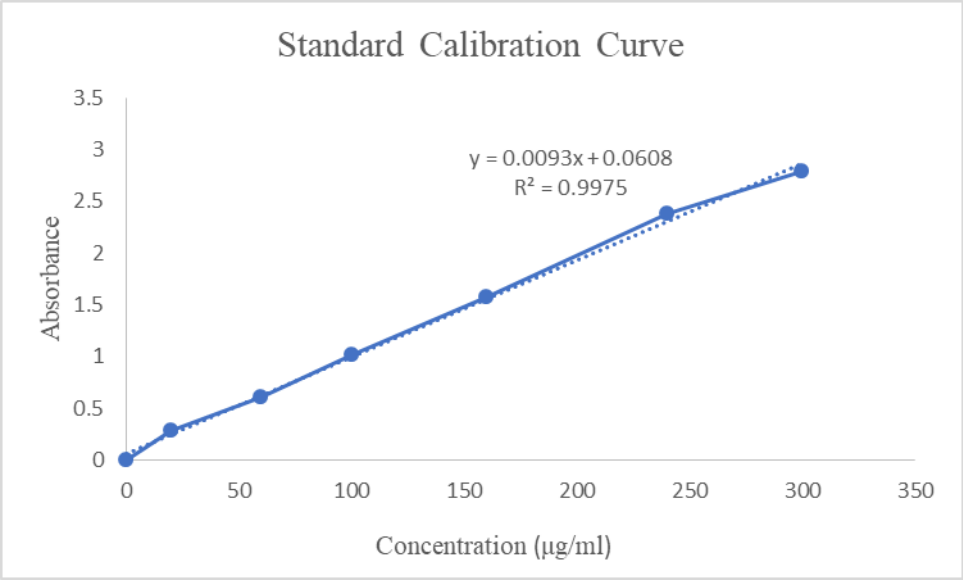

Fig 3: Standard curve of THML in Methanol.

FTIR: FTIR spectroscopy serves to identify substances by elucidating their structural characteristics and purity.

Organic substances vibrate when subjected to infrared radiation. Absorption takes place when these vibrations align with the radiant energy. Employing the infrared spectrum aids in recognizing a drug's functional characteristics. The prominent absorption bands found in the range of 400-4000 cm^{-1} were measured through Fourier Transform Infrared spectroscopy of drugs and drug formulations via an FTIR spectrophotometer.[27,28,29,30,31] The results are shown through Figures 4 and Table 1, which show the occurrence and nonoccurrence of absorption bands, as well as the creation of any new absorption bands.

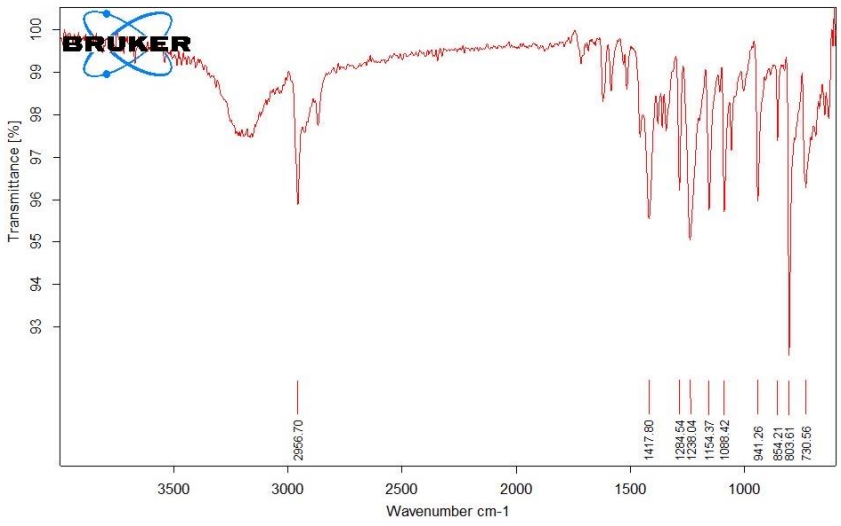

Fig. 4: FTIR spectra of THML.

Table no. 01: Infrared absorption spectrum range of THML.

S. No.	Optimized Range (cm^{-1})	Functional Group	Outcome	References
01.	2956.70	C-H (Aliphatic) stretch	CH_3 and CH of the isopropyl group	[1], [3], [4], [28]
02.	1417.80	C=C (Aromatic) Skeletal vibration	Aromatic ring breathing	[1], [3], [4], [28]
03.	1284.54	C-O Stretching	Phenolic C-O bond	[1], [3], [4], [28]
04.	1238.04	C-O Stretching	Phenolic C-O bond	[1], [3], [4], [28]
05.	854.21- 803.61	C-H (Aromatic) Out-of-plane bending	Aromatic ring substitution (para-substituted)	[1], [3], [4], [28]

Melting Point: The determination of the melting point of THML was done through capillary tube method in order to ascertain the quality and stability of the compound (Fig 5). It exhibited an exact and consistent melting point range of 50 °C, reflecting the pure nature and crystal-like structure of the compound (n=3).[26,32] The slight difference in values obtained indicates consistency, thus, indicating absence of contaminants or decomposition.[26,32,33]

Fig 5: The determination of the melting point of THML (n=3).

Solubility study

The solubility of Thymol (THML) was evaluated in various polar and non-polar solvents—including ethyl acetate, acetone, methanol, ethanol, NaOH, and water—to establish the optimal vehicle for drug dissolution. The results of this study are illustrated in the graph below (Fig 06).

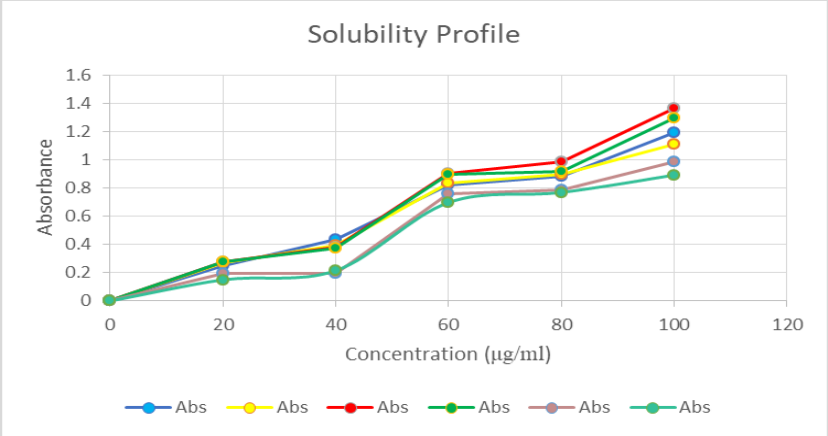

Fig 6: Solubility profile in various solvents.

The solubility of thymol (THML) in the various solvents is different from one another, which is seen from the variations in their absorbances at the same concentration. From the absorbances recorded, it can be observed that organic solvents such as methanol, ethanol, ethyl acetate, and acetone have greater solubility power for THML than NaOH and H_2O.

Partition coefficient: The partition coefficient (log P) of THML is estimated to be 3.3, indicating a tendency towards hydrophobicity. The term "log P" is an indication of a better affinity of THML towards lipids or non-polar substances compared to polar substances. This implies that THML has a greater probability of dissolving in fats, oils, or organic compounds rather than water. This high log P value is indicative of the capacity of THML to diffuse across cell membranes which are lipophilic.[21,29,30,34,35]

Table No. 02: Summary report of the results.

S. No.	Parameter	Result/Value	Outcome
01.	Wavelength of Maximum absorbance (λmax)	276.5nm	Validates purity and enables quantitative UV analysis.
02.	Melting Point (°C)	50	Confirms the crystalline nature and high purity.
03.	Partition Coefficient (log P)	3.3	Indicates the high lipophilicity for NLCs encapsulation.
04.	FTIR analysis (FTIR Spectra peaks in cm^{-1})	2956.70, 1417.80, 1284.54, 1238.04, 854.21- 803.61	Confirms the structural identity and functional groups.
05.	Solubility Studies	Higher solubility than aqueous	Provides rationale for lipid matrix selections.

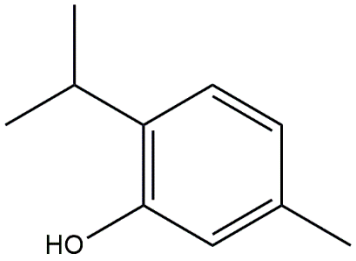

Fig. 01: Structure of THML.

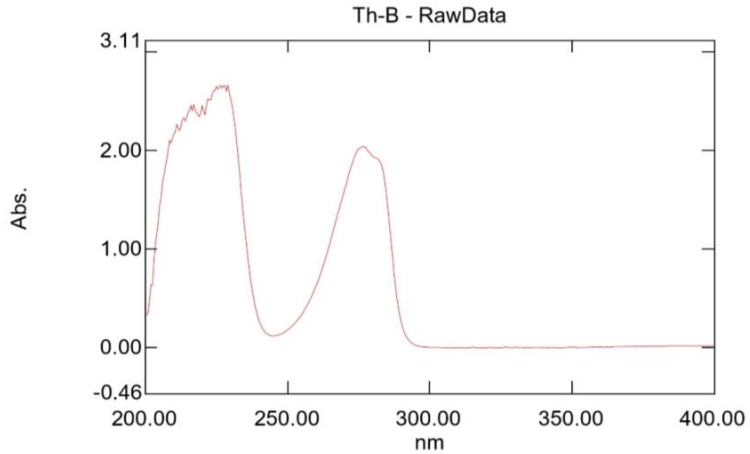

Fig 2: Maximum absorbance (λ_{max}) of THML at 276.5 nm.

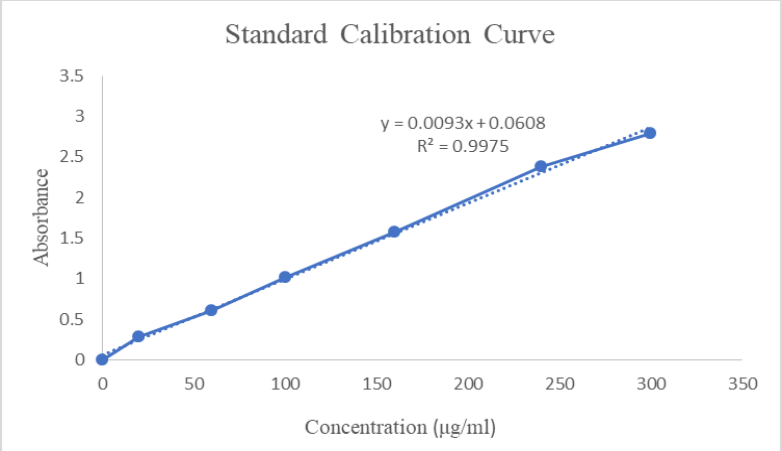

Fig 3: Standard curve of THML in Methanol.

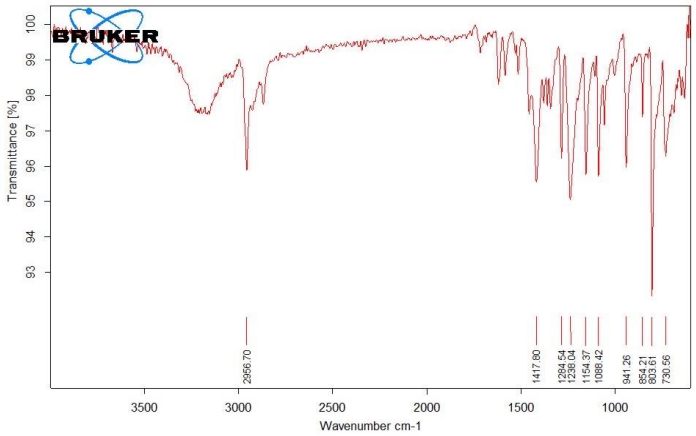

Fig 4: FTIR spectra of THML.

Fig 5: The determination of the melting point of THML (n=3).

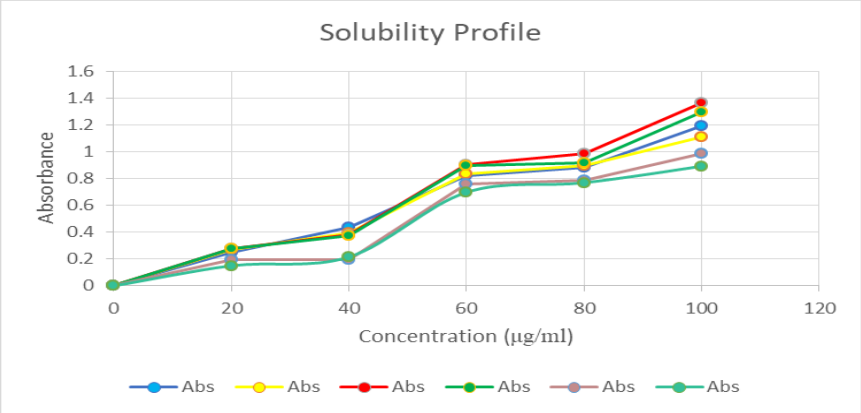

Fig 6: Solubility profile in various solvents.

DISCUSSION

The preformulation study of Thymol (THML) on a systematic level provides an excellent basis for developing the chemical into a potent drug to target Leishmaniasis. From the results obtained using the analytical methods, Thymol (THML) was found to have a λmax of 276.5nm in UV-visible spectrophotometry analysis, whereas the capillary melting point analysis revealed its high purity with a melting range of 50 °C.

The presence of the isopropyl moiety was also confirmed by FTIR analysis through its vibration band at 2956.70 cm^{-1} along with para-substituted benzene rings with vibration bands between 803.61 and 854.21 cm^{-1}, which are important parameters for tracking stability.[3,4,28]

The log P value of 3.3 indicates that the compound exhibits considerable lipophilic properties, which implies a higher affinity for lipid matrices than water-based environments; this is an essential requirement for preparing efficient nanocarriers (NLC).[3,14]

Additionally, studies on saturation solubility in different solvents, where organic solvents such as methanol, ethanol, ethyl acetate, and acetone exhibited higher solubilization capacity than polar solvents, enabled the choice of appropriate lipid matrices based on scientific reasoning.

Altogether, the results are able to bridge important gaps in the literature on the preformulation of THML, and provide the necessary guidelines to develop an optimized strategy to treat Leishmaniasis using NLC systems.

CONCLUSION

The preformulation study of Thymol (THML) has been systematically conducted, allowing for the creation of an important physicochemical and analytical background, proving that the drug is suitable for the incorporation into NLCs. The purity and molecular integrity of the starting material have been confirmed by means of accurate determination of its melting point (50 °C) and FTIR spectroscopy that has revealed characteristic phenolic and aromatic functional groups. The calculation of log P equal to 3.3 proves that the drug exhibits considerable lipophilicity, thus being strongly attracted to lipid matrices but not water. Besides, lipid screening and solubility studies carried out within the framework of this research project are valuable sources of information for the rational choice of lipid matrices, which is an integral aspect of effective drug loading and obtaining stable formulations. Therefore, this research makes

up a critical part in addressing gaps in preformulation data on THML, providing an important basis for the development of efficient NLC-based treatments of Leishmaniasis.

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ABBREVIATION: THML (THML), LDBS (Lipid-based delivery systems), FTIR (Fourier Transform Infrared), NLCs (Nanostructured lipid carriers), λmax (Lambda max), log P (Partition coefficient).

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