

Molecular Dynamics Simulations of Polyoxyl-35 Castor Oil Microemulsions using Mesodyn

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Received: September 8, 2024 Last Revision: October 15, 2025 Accepted: January 13, 2026 Available online: October 07, 2026

Abstract

Microemulsions (MEs) are transparent, thermodynamically stable mixtures of water and oil, stabilized by surfactants and cosurfactants, which facilitate their formation by reducing interfacial tension and enhancing solubility. Mesoscopic dynamics (Mesodyn) simulation has emerged as a powerful analytical approach for investigating the phase behavior and dynamic evolution of surfactants in solvents under real-world conditions. The objective of the current study was to utilize Mesodyn simulation tools to examine the phase behavior of MEs. In this study, coarse-grained models of Kolliphor EL (Polyoxyl-35 Castor Oil), Capmul MCM NF, and Transcutol P were developed. The phase morphologies, aggregation behavior, and miscibility of the components in the ME systems were studied. An attempt was made to correlate the Mesodyn predictions for pharmaceutical MEs with experimental measurements. Transcutol P (cosurfactant), Kolliphor EL (surfactant), and Capmul MCM (oil phase) were selected as the ME components based on solubility studies conducted in wet-laboratory experiments. ME formulations were chosen based on the pseudo-ternary phase diagram. Oil and Smix (1:1) proportions varying from 1:9 to 9:1 were used to develop various MEs, which were then studied for their phase behavior. Various mesoscopic metrics, including density maps, order parameters, and thermodynamic parameters, were carefully analyzed. The simulation studies demonstrated the representation of the various ME components in Mesodyn using density maps. Homogeneity was observed in both the wet-laboratory and simulation studies for the 1:9 and 2:8 ME systems. By analyzing the P values of all systems, it was found that as the concentration of Smix and water increased, the rate of ME formation also increased. In this study, it was observed that as the oil-phase concentration increased, the hydrophobic effect became dominant, leading to a decrease in the Smix concentration. This led to immiscibility and phase separation, possibly due to higher interfacial tension. The 1:9 and 2:8 ME systems showed very low Gibbs free energy (ΔG) values, indicating the stability and spontaneity of ME formation. The laboratory results for ME formulations with 1:9 and 2:8 ratios were consistent with the simulation results, confirming that both formulations were homogeneous. Based on these findings, the ME system with a 1:9 (oil:Smix) composition was selected for further molecular dynamics (MD) simulations incorporating a drug. Despite the higher ΔG value, homogeneity was maintained upon drug incorporation. The order parameters remained almost unchanged compared to the blank system, indicating the suitability of the ME system for drug incorporation.

The outcomes of this work provide valuable insights for the future design and development of Kolliphor EL-based MEs loaded with BCS Class II drugs using the coarse-grained model.

Keywords: Materials studio; Microemulsions; Mesodyn simulations; Coarse-grained model; Phase morphology; Molecular dynamic simulations; Solubility Studies.

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Cite this article as: Mahajan D., Kaur H., Kumar M., Kaur J., Silakari O., Sapra B. Molecular Dynamics Simulations of Polyoxyl-35 Castor Oil Microemulsions using Mesodyn. Iran. J. Pharm. Sci., 2026, 22 (1): 328-341.

DOI: <https://doi.org/10.22037/ijps.v22i1.46624>

1. Introduction

Microemulsions (MEs) are effective transdermal drug delivery vehicles for both hydrophilic and hydrophobic medications due to their dual lipophilic and hydrophilic properties. In 1943, Schulman was the first to identify these systems, observing that the addition of a cosurfactant (pentanol) transformed a milky emulsion into a clear, more stable emulsion [1,2]. MEs can vary significantly in terms of their component organization, behavior, content, and arrangement, forming structures such as water-in-oil, oil-in-water, or bicontinuous phases. MEs have numerous applications in fields such as food, medicine, cosmetics, and oil extraction [3]. As versatile drug delivery systems, MEs are increasingly used because they offer enhanced stability, solubility, and bioavailability. The composition and shape of water, oil, and surfactants influence the self-assembly of these systems. Significant progress has been made in understanding the chemistry of MEs, particularly in developing interfacial characteristics that enable hydrophilic and hydrophobic domains to interact closely at the mesoscale. Understanding phase transitions and the stability of MEs at the mesoscopic level is essential for realizing their full potential as drug delivery systems [4]. These systems contain organized microstructures that vary depending on the conditions and chemistry of their components, making them scientifically and practically significant. From a thermodynamic perspective, the entropy and enthalpy effects influence the Gibbs free energy (ΔG) of ME formation, providing valuable insights into their stability.

Recently, computer-assisted simulations have proven to be valuable tools for providing additional information at the molecular or mesoscopic scale, addressing the limitations of practical experimental investigations [5]. Wheeler and Widom conducted the first computational study on MEs using a lattice model to investigate phase transitions [6]. Today, Mesodyn simulations have become an effective tool for analyzing various phase behaviors and the dynamic evolution of surfactants or polymers in solvents under real-world conditions [7].

Akzo Nobel developed Mesodyn in the 1990s. It links the speed of molecular kinetics to the thermodynamic relaxation of macroscopic properties, ensuring accurate representation of a system's thermodynamic behavior,

phase behavior, order parameters, and more. The Mesodyn model incorporates various types of bead interactions: spring-like potentials for intramolecular connections to represent flexible chain models, and an average-field approach for all other interactions [8], as shown in **Figure 1**.

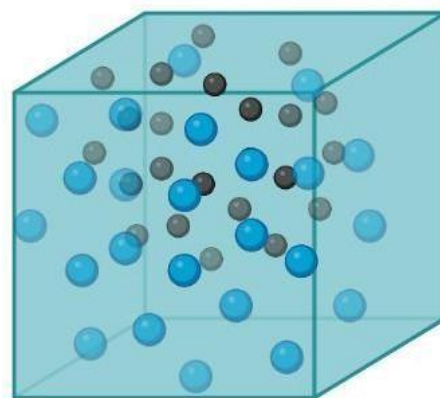


Figure 1. Box depicting beads

The purpose of the simulation is to predict the time evolution of density distributions for various types of beads. Mesodyn uses density functional theory (DFT) and assumes that all thermodynamic functions are derived from the free energy and depend on its local density. Each bead represents covalently bonded atom groups, such as one or a few chain structural units of the components and different compositions present in the system. It employs a coarse-grained approach, defining molecules as "Gaussian chains of beads." Mesodyn accepts input in the form of material factors, including bead diffusion coefficients, noise parameters, number of steps, grid dimensions, and bond lengths of the bead components [9].

Functional Langevin equations characterize the system's dynamics. They represent the noise-accounting diffusion equation in component densities:

$$\frac{\partial \rho_i}{\partial t} = M v_j \nabla \rho_i \rho_j \nabla [\mu_j - \mu_i] + n$$

$M v_j$ = The mobility of the bead

$\nabla \rho_i \rho_j$ = Kinetic coefficient

n = Blank Gaussian noise

μ = Diffusion coefficient

Integrating the equation generates Boltzmann density fields. Mesodyn describes the free energy within a non-uniform system, which drives component segregation, as a function of spatial density variations over time [10]. Order parameters are calculated to quantify the degree of

phase separation. The formula employed to determine the order parameter is:

$$P_i = 1/V \int_V [n_i^2(r) - n_i^2] dr$$

P_i = Time-dependent order parameter of a component

V = Cell volume

$n_i^2(r)$ = Local density distributions of a component within the sampling distance r

n_i^2 = Average system density distribution of the component beads

P describes the local bead distribution deviation from the assumed homogeneous mean distribution. A rise in P (or local bead order) indicates that component separation has occurred. Consequently, it is possible to observe distinct P values for every individual constituent as time progresses.

Many factors influence the emulsification region or phase behavior of the ME system. Most researchers focus primarily on the experimental aspect, despite certain inherent limitations. Numerous wet lab methods, including neutron scattering, small-angle laser diffraction, fluorescence labeling, X-ray diffraction, and dynamic light scattering, have been employed to investigate the phase behavior of MEs. However, it is practically challenging to characterize or observe the interactions of oil, surfactant, or cosurfactant at the molecular level.

Recently, the science of MEs has increasingly focused on molecular simulations. Theoretically, molecular dynamics (MD) simulations can be used to examine surfactant behavior at the atomic level. MD simulations are particularly valuable in constructing liquid structure systems at the atomic scale [11].

In the present study, coarse-grained models of Kolliphor EL, Capmul MCM NF, and Transcutol P are presented for the first time. The phase morphologies, phase behavior, aggregation behavior, and miscibility among the components of the ME systems have been investigated. Additionally, an attempt has been made to correlate the Mesodyn predictions of pharmaceutical MEs with experimental measurements. The simulations were conducted using the Mesodyn module within the commercially available BIOVIA Materials Studio 2022 software.

2. Materials and methods

2.1. Materials

The subsequent reagents were utilized as acquired: Terbinafine hydrochloride (Smilax Healthcare Pvt. Ltd.,

Baddi, India); Labrafac WL, Lauroglycol 90, Capryol 90, Peceol, Labrasol, Transcutol P (Gattefosse India Pvt. Ltd., Mumbai, India); Capmul MCM NF, Captex 200 (Abitec Corporation, Mumbai, India); Tea tree oil, Eucalyptus oil, Neem oil, Lemongrass oil, Lavender oil (Shanti aromatics, Ghaziabad, India); Peppermint oil, Methanol (Loba Chemie Pvt. Ltd., Mumbai, India); Tween 20, Tween 80, Polyethylene glycol, Propylene glycol (S.d. Fine Chemicals Ltd., Mumbai, India); Tween 40 (Sigma Aldrich Co. St. Louis, USA); Kolliphor EL BASF India Ltd., Mumbai, India.

2.2. Identification of Drug

2.2.1. Melting Point Determination

The melting point of Terbinafine hydrochloride (TH) was determined by capillary method using a digital automated melting point apparatus (Labtronix, India).

2.2.2. FTIR- ATR Spectroscopy

The TH spectrum was recorded on an FTIR-ATR spectrophotometer (Alpha, Bruker, Berlin, Germany). The spectrum was recorded in the region 400 to 4000 cm^{-1} and the sample was compared with those given in the literature.

2.2.3. Differential Scanning Calorimetry (DSC)

A sample of TH (6-8 mg) was sealed in aluminum crucibles of 30 μL capacity and heated from 40° to 300° C in a nitrogen atmosphere at a steady heating rate of 10°C/min using a differential scanning calorimeter (Setaram, LabSys Evo, France). The melting temperature, peak transition, and enthalpy of fusion were determined from the DSC thermogram and compared with literature values.

2.3. Screening of Components for MEs

2.3.1. Solubility Studies

To identify the appropriate oil phase, surfactant, and co-surfactant for the ME System, the dissolution of TH was assessed in different oils, surfactants, and co-surfactants. Among oils, natural herbal oils (Peppermint oil, Lavender oil, Eucalyptus oil, Neem oil, Tea tree oil, Lemongrass oil) and synthetic oils (Labrafac WL, Captex 200, Lauroglycol 90, Capryol 90, Peceol, and

Capmul MCM NF) were used. Due to the thick consistency, the drug dissolves poorly in various surfactants. (Tween 20, Tween 40, Tween 80, Kolliphor EL, and Labrasol) was assessed. To choose the co-surfactant, dissolution was evaluated in Transcutol P, Polyethylene glycol 400, and Polypropylene Glycol. Drug dissolution was assessed in the selected oils, surfactant, and co-surfactant by adding an excess amount of the drug to 2 ml Eppendorf tubes containing 1 ml of oil, surfactant, or co-surfactant. The mixture was agitated for 48 hours at room temperature using a rotary mixer. Following agitation, the balanced samples were spun at 5000 rpm for 15 minutes. The supernatant layer was collected, diluted in methanol, and analyzed by UV spectrophotometry at 283 nm.

2.3.2. Construction of Pseudo Ternary Phase Diagram

2.3.2.1. Formulation of MEs

The water titration approach was used to determine the ME zone and to evaluate the possibility of producing MEs with different combinations of oil, surfactant/co-surfactant, and aqueous phase, resulting in pseudo-ternary phase charts [12].

Drop by drop, the right amount of distilled water was added to the oil and Smix mixture until an opaque, colorless liquid was achieved. After mild magnetic agitation for 15 to 20 minutes, the blend was permitted

to settle and achieve equilibrium. All MEs with and without drugs were then housed at room temperature.

2.3.2.2. Particle Size Measurements

Particle size was determined using Malvern Z Zetasizer (Malvern Instrument Ltd., Malvern, UK). Portable polystyrene cuvettes were employed for evaluation, which were measured at 90°C and 25°C.

2.4. In-Silico Studies

2.4.1. Structure Preparation

Based on the drug solubility studies Capmul MCM NF, Kolliphor EL, and Transcutol P were chosen as oil, surfactant, and cosurfactant, respectively. ChemDraw was a tool for sketching their structures.

2.4.2. Computational Approach

In this study, all simulations were done in the Mesodyn module in the commercially available software Biovia Material Studio 2022. The condensed-phase optimized molecular potentials for atomistic simulation studies (COMPASS) force field was used to perform geometry optimizations and dynamics simulations [7]. Smart Minimizer was utilized to optimize the geometry and minimize the energy. 3D structures of all molecules are depicted in **Figure 2**.

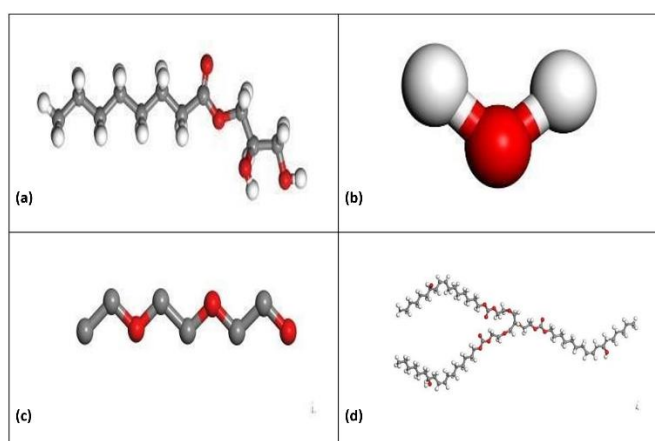


Figure 2. 3D structures of (a) Capmul MCM NF, (b) Water, (c) Transcutol P, and (d) Kolliphor EL

2.4.3 Building of Coarse-Grained Model

The system's components were represented as coarse-grained models before the Mesodyn simulation. A novel coarse-grained model for the surfactant Kolliphor EL, represented by the notation (K1(K2)₃), was added to the present Mesodyn simulation. The hydrophilic segment in this model is symbolized by bead K1, the hydrophobic segment by bead K2, water (H₂O) by bead W, Capmul MCM NF by bead CA, and Transcutol P by bead T in **Figure 3**. The initial variables for the Mesodyn simulation were selected and are summarized in **Table 1**. Furthermore, the simulation box with its components is depicted in **Figure 4**.

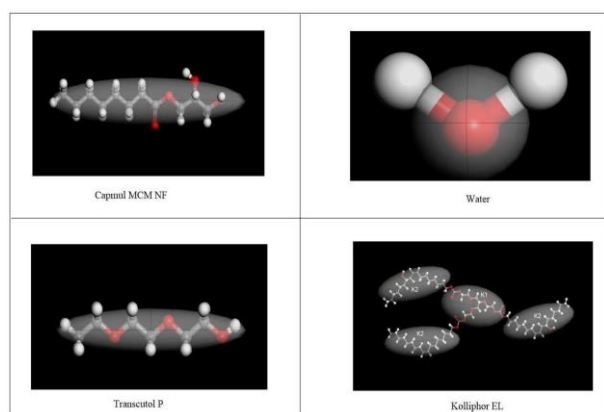


Figure 3. The coarse-grained models of Capmul MCM NF, H₂O, Transcutol P, and Kolliphor EL in the Mesodyn simulation.

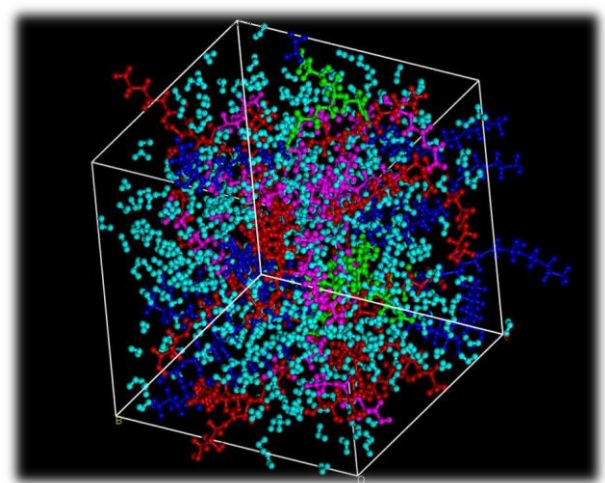


Figure 4. Depiction of simulation box with Capmul MCM NF (Red), Kolliphor EL (Green & Royal blue), Transcutol P (Pink), and Water (Turquoise blue)

Table 1. Mesodyn simulation parameters.

Cubic grid size	32 × 32 × 32 nm
Bond length	1.1543 nm
Bead diffusion coefficient (μ)	10 ⁻⁷ cm ² s ⁻¹
Noise parameter	100
Compressibility parameter	10 kt
Temperature	298K
Total time steps	100000 ps
Total simulation time	2000 μ s
Grid spacing	1nm
No. of steps	20000

3. Result and Discussion

3.1. Identification of Drug

3.1.1. Melting Point Determination

The melting point of TH obtained by the capillary method was 209° C, which was in agreement with the literature value (204 – 208 °C) [13,14].

3.1.2. ATR- FTIR Spectroscopy

The spectra of the pure drug were analyzed using an ATR-FTIR spectrophotometer. TH showed distinctive bands at 2964.56, 2428.61, 1639.56, 1465.58, 1361.24, and 957.16 cm⁻¹ (**Figure 5**), which matched those reported in the literature [15].

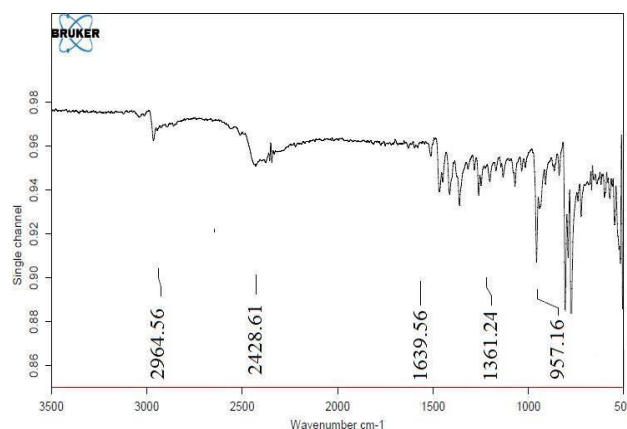


Figure 5. ATR-FTIR Spectra of TH

3.1.3. Differential Scanning Calorimetry (DSC)

DSC analysis exhibited a distinct endothermic melting peak at 212.2°C, confirming the melting point of TH. Various researchers have reported similar peaks at the same temperature [16,17]. At 92.4°C, an additional

endothermic peak was observed, which may have been due to the drug's water content (**Figure 6**).

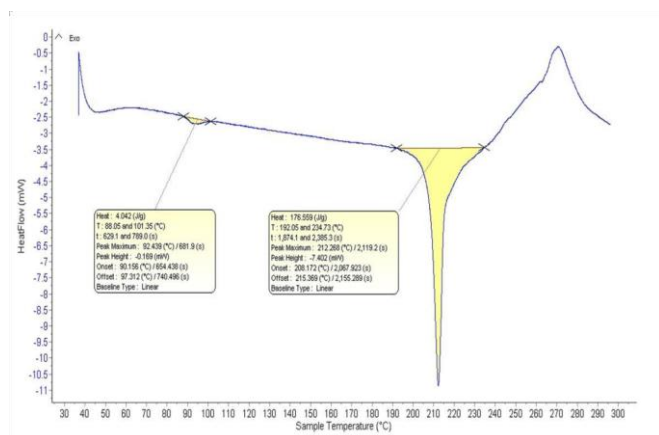


Figure 6. DSC Thermogram of TH

3.2. Screening of Components for MEs

3.2.1. Solubility Studies

To identify suitable ingredients for ME formulation, the solubility of the drug (TH) in various oils, surfactants, and co-surfactants was evaluated. Among the oils tested, herbal oils showed lower drug solubility than synthetic oils. This reduced solubility in herbal oils can be attributed to their larger molecular volume, which limits their solubilizing capacity [18]. The highest drug solubility was observed in Capmul MCM (41.46 ± 0.424 mg/ml), while among herbal oils, peppermint oil demonstrated the highest solubility (11.4 ± 0.195 mg/ml). Based on these findings, Capmul MCM was selected as the oil phase for MEs preparation.

Among the surfactants, Kolliphor EL showed the highest drug solubility, at 36.68 ± 0.727 mg/mL. For co-surfactant selection, the drug's solubility was assessed in Transcutol P, Polyethylene Glycol 400, and Propylene Glycol. Transcutol P exhibited the highest solubility (26.45 ± 0.210 mg/ml) among the tested co-surfactants.

3.2.2. Construction of Pseudo-Ternary Phase Diagram

3.2.2.1. Formulation of MEs

The literature extensively documents that a single surfactant may not always achieve the necessary reduction in the water-oil interfacial tension. Combining short- to medium-chain alcohols, such as Transcutol P, with single-chain surfactants can alleviate interfacial stress by

enhancing fluidity at the interface. Medium-chain alcohols also facilitate better mixing of the aqueous and lipid phases by partitioning between the two [19]. Based on solubility studies, Transcutol P (co-surfactant), Kolliphor EL (surfactant), and Capmul MCM (oil) were selected for ME formulation using the water titration method (**Figures 7a & 7b**).

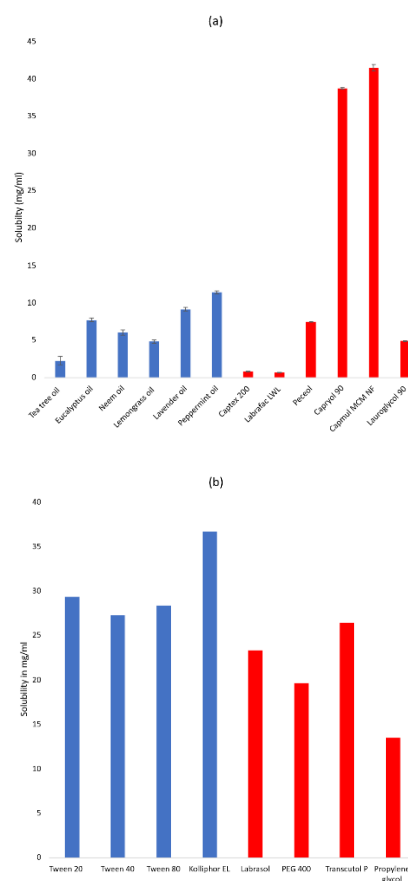
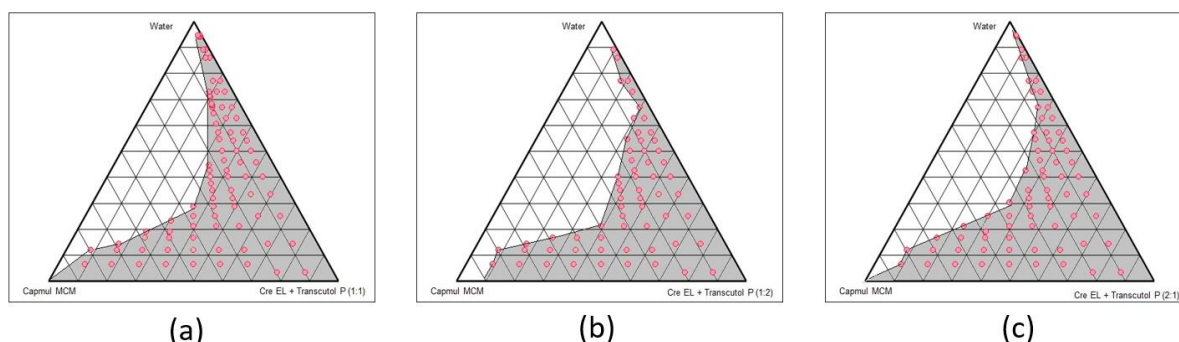


Figure 7. Solubility of drug in (a) surfactants (blue) and co-surfactants (red); (b) herbal oils (blue) and synthetic oils (red)

In the current study, MEs formed spontaneously when the ingredients were combined at a controlled temperature of 25° ± 0.5°C. As previously mentioned, phase diagrams of the MEs were constructed to identify the ME region (**Figure 8**). Kolliphor EL and Transcutol P were blended in varying weight ratios (1:1, 1:2, and 2:1) to create a surfactant/co-surfactant mixture for phase diagram construction. The phase study revealed that the largest ME region (18.23 ± 0.51 cm²) was achieved with Kolliphor EL and Transcutol P at a 1:1 weight ratio (**Table 2**). Consequently, formulations were developed using a 1:1 surfactant/co-surfactant ratio.

Table 2. Composition of ME formed by using Surfactant: Cosurfactant (Smix: 1:1).

S.NO	Oil: Smix	% Oil (w/w)	% Smix (w/w)	% Water (w/w)
1	1:9	0.5660	5.0943	94.3396
2	2:8	1.1320	4.5283	94.3396
3	3:7	8.1818	19.0909	72.7272
4	4:6	22.2222	33.3333	44.4444
5	5:5	35.7142	35.7142	28.5714
6	6:4	46.1538	30.7692	23.0769
7	7:3	56.7567	24.3243	18.9189
8	8:2	68.5714	17.1428	14.2857
9	9:1	79.4117	8.8235	11.7647

**Figure 8.** Pseudo ternary phase diagrams containing Capmul MCM NF, Smix (Kolliphor EL; Transcutol P) (a) 1:1 (ME area: $18.23 \pm 0.51 \text{ cm}^2$) (b) 1:2 (ME area: $14.24 \pm 0.97 \text{ cm}^2$) (c) 2:1 (ME area: $14.81 \pm 0.55 \text{ cm}^2$)

3.2.2.2. Particle Size Measurements

The 1:1 Smix ratio for the ME system was also evaluated for particle size and polydispersity index (PDI), with the results presented in **Table 3**. The findings revealed that the 1:9 and 2:8 ratios produced particle sizes within the desired range for MEs, compared to other tested ratios.

Table 3. Particle size and PDI for (1:1) Smix.

Ratio	Particle size (nm)	Polydispersity index (PDI)%	Zeta potential
1:9	145.35	28%	-10.3mV
2:8	168.12	24.5%	-15.9mV
3:7	205.6	10.9%	-4.5mV
4:6	216.4	0.351	-7.1mV
5:5	222.3	25.3%	-6.7mV
6:4	231.7	25.7%	-7.6mV
7:3	257.5	20.4%	-6.7mV
8:2	288.7	25.1%	-9.1mV
9:1	398.4	5.4%	-0.968 mV

3.3. In-Silico Studies

3.3.1. Phase Morphology Profiles of the System 1:1 (Smix) Set

To study the phase behavior of MEs containing Capmul MCM, Kolliphor EL, and Transcutol P, a 1:1 Smix ratio was selected. This system demonstrated the largest ME area ($18.23 \pm 0.51 \text{ cm}^2$) during the experiments. Kolliphor EL is known for its dual hydrophilic and hydrophobic alkyl chains, represented by K1 and K2 beads. In this representation, the K1 bead corresponds to the hydrophilic portion, while the K2 bead represents the lipophilic component of the surfactant. During simulations, the K1 bead was considered the backbone, and three K2 side chains formed the hydrophobic portion. Thus, the morphology of the Kolliphor EL assembly was modeled as K1 and (K2)₃ beads (**Figure 3**).

The content of each component in the system was determined based on the weight proportions derived from wet-lab experiments. The basic phase morphology of the ME systems, with oil and Smix (1:1) ratios ranging from 1:9 to 9:1, showed no noticeable spherical formations at 20,000 simulation time steps. To improve

observation, the water phase was excluded from visualization.

The chemical properties of the components suggest that the polar head of bead CA (oil phase) likely forms hydrogen bonds with the hydrophilic part of the surfactant (bead K1). At the same time, its nonpolar tail interacts hydrophobically with the hydrophobic part (bead K2). Similarly, the polar head of bead T (co-surfactant) is expected to form hydrogen bonds with bead K1. Both bead CA and bead T contain hydroxyl groups capable of intermolecular hydrogen bonding with the water phase, thereby influencing the miscibility of the components in the ME system. These interactions can be further analyzed using order parameters.

A related study by Lee and colleagues examined the phase morphology of Pickering dispersions containing nanocellulose (NC) in a poly-1-butene (PB)-water mixture. Their findings showed that the -CH groups on the glucose-pyranose rings of NC exhibited lipophilic properties. In contrast, the hydroxyl and methyl hydroxyl groups in other regions were hydrophilic and interacted with the water phase. Initially, the hydrophobic PB self-aggregated into transient PB beads before interacting with NC atoms, forming a more stable Pickering emulsion. This study provided valuable insights into the aggregation behavior and interactions within such formulations, which could be extrapolated to the ME systems under investigation [20].

3.3.2. Thermodynamics of the Smix (Surfactant: Cosurfactant 1:1)

MEs form when droplets or micelles spontaneously emerge during blending, while maintaining thermodynamic equilibrium. This phenomenon is driven by the need to balance the entropic effects associated with stabilizing the oil-water interface [20]. According to ME literature, the interfacial tension between water and oil is reduced to extremely low levels due to the adsorption of surfactant and co-surfactant molecules at the globule interface. Additionally, the system's free energy decreases as the overall concentration of surfactants and co-surfactants decreases, reducing their chemical potential at the interface and within the core [21].

The thermodynamic stability of MEs is primarily determined by the net change in free energy resulting from these processes, which may turn negative. For an ME to be thermodynamically stable, the free energy of formation must be negative [22]. In this study, the Gibbs free energy of the system was calculated to understand the energetic interactions during component blending, revealing favorable associations that provide insights into the ME system's stability.

In molecular dynamics (MD) simulations, assessing system equilibrium is essential for obtaining reliable, accurate results. While Gibbs free energy is rarely directly measured for actual systems and may not always align with experimental data, it remains a valuable indicator of the stability of ME systems. The progression of Gibbs free energy serves as a robust measure of system integrity [23].

* ΔG was calculated by using the following equation:

$$\Delta G = H - T\Delta S$$

ΔG = Gibbs free energy

H = Enthalpy

$T\Delta S$ = Temperature * Entropy

The thermodynamic parameters of ME systems with Smix (1:1) ratios ranging from 1:9 to 9:1 are summarized in **Table 4**. The ΔG values were found to be indirectly dependent on the Smix percentage. The comparison of various systems at 20,000-time steps highlights the ΔG values for different 1:1 Smix systems at this time point.

A study conducted by Zhao and colleagues investigated the ΔG values for a Brij97/isopropanol/isoamyl acetate/water system. They compared two systems, 1:9 and 2:8, and observed that the 2:8 system exhibited a higher ΔG than the 1:9 system, indicating greater stability of the ME in the 1:9 ratio. The ΔG values effectively reflect the robustness of the ME system, both before and after grouping [7].

The thermodynamic spontaneity of the process is further demonstrated by the minimal free energy required to form an emulsion. According to Craig and coresearchers, an operation is considered thermodynamically favorable when the free energy needed to create an emulsion is very low [24].

Table 4. Summarization of thermodynamic parameters of the systems at 20000-time steps.

ME systems	%Smix	Enthalpy (ΔH)	Entropy*Temperature ($T*\Delta S$)	Gibbs free energy (ΔG)
1:9	5.0943	0.1082	0.7680	-0.6598
2:8	4.5283	0.1099	0.7570	-0.6470
3:7	19.0909	0.4542	0.1668	0.2873
4:6	33.3333	0.7160	-0.2616	0.9776
5:5	35.7142	0.7459	-0.3126	1.0585
6:4	30.7692	0.7030	-0.2355	0.9386
7:3	24.3243	0.6250	-0.1035	0.7285
8:2	17.1428	0.5019	0.0952	0.4066
9:1	8.8235	0.3548	0.3382	0.0166

3.3.3. Prediction of Phase Separation by Mesodyn (Order Parameters)

Mesodyn simulations are used to predict the accumulation behavior and phase differentiation within ME systems. To better understand the cluster growth process, various variables are analyzed, with phase separation effects captured through the mean-square deviation from uniformity, which determines the order parameter (P). Phase separation occurs when the order parameter differentiates between various beads [25].

The order parameter (P) is a crucial metric for assessing the compatibility or incompatibility of system components. High values of P indicate significant phase segregation, while low values suggest a uniform system with good miscibility. Previous studies have shown that an increase in P (or local bead order) indicates the presence of distinct phases, enabling tracking of P value variations over time [10]. When P values approach zero, it indicates that the components are highly dispersed [7].

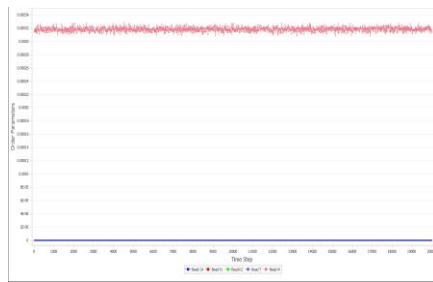
In this study, order parameters were calculated for the Smix (1:1) ratio across ME systems with oil-to-Smix ratios ranging from 1:9 to 9:1. In the 1:9 system (**Figure 9A**), homogeneity was observed throughout the simulation (20,000time steps) at 298K. The P value for bead W was 0.00032, and the P values for bead CA, bead K1, bead K2, and bead T were nearly zero, indicating no aggregate formation. A similar observation was made for the 2:8 system (**Figure 9B**), though the pattern showed slight differences.

In the 3:7 system (**Figure 9C**), a slight difference in P values between the beads was noted, suggesting the onset of phase separation. The P value of bead W ranged from 0.0007 to 0.0005, while bead CA, bead K1, bead

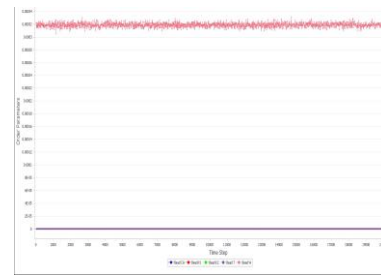
K2, and bead T remained close to zero. Despite this, practical separation was not visible. In the 4:6 (**Figure 9D**), 5:5 (**Figure 9E**), and 6:4 (**Figure 9F**) systems, distinct heterogeneity patterns were observed, with the P values of bead W decreasing from 0.0009 to 0.0004. The P values of bead CA increased from 0.00045 to 0.0006, and bead T fluctuated between 0.0003, 0.00018, and 0.0002 across the systems. For bead K1 (hydrophilic) and bead K2 (hydrophobic), the P values were near zero, around 0.00005 for K1 and 0.00025 to 0.00015 for K2.

In the 7:3 (**Figure 9G**), 8:2 (**Figure 9H**), and 9:1 (**Figure 9I**) systems, bead CA's P -values were 0.00065, 0.00048, and 0.00054, respectively. The 9:1 system (**Figure 9I**) showed greater fluctuations in bead CA, indicating greater oil bead aggregation than in other systems. In these systems, the other bead P values were close to zero, suggesting minimal aggregation among the beads. The patterns indicate that the oil phase becomes more distinct from the other components in the latter three systems, with consistency between simulated and experimental observations.

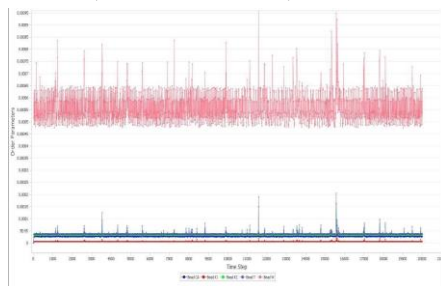
Overall, as the oil-to-Smix ratio increased from 1:9 to 9:1, the separation in order parameter values increased, indicating a lower rate of ME formation. In other words, as the quantity of Smix and water increased, the rate of ME formation accelerated. Zhao and co-investigators also observed that the rate of ME formation for Brij97/isopropanol/isoamyl acetate/water was faster when comparing the P values of oil and water beads in 1:9 and 2:8 systems [7].



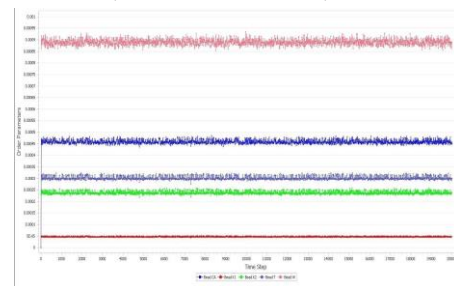
9 A). 1:9 system (Capmul MCM-0.56%, Kolliphor EL-2.5%, Transcutol P-2.5%, Water- 94.33%)



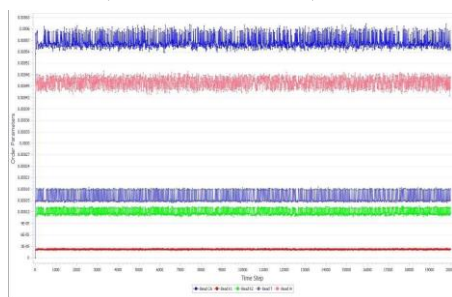
9 B). 2:8 system (Capmul MCM -1.13%, Kolliphor EL-2.26%, Transcutol P- 2.26%, Water 94.3%)



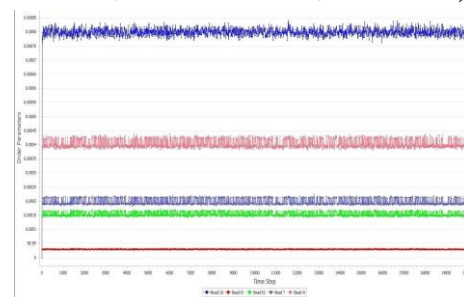
9 C). 3:7 system (Capmul MCM-8.18%, Kolliphor EL-9.54%, Transcutol P-9.54%, Water-72.7%)



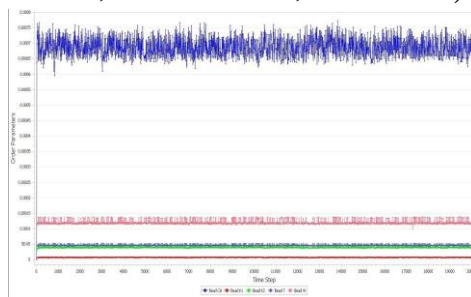
9 D). 4:6 system (Capmul MCM -22.2%, Kolliphor EL-16.6%, Transcutol P-16.6%, Water-44.4%)



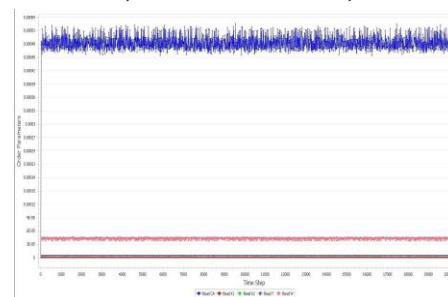
9 E). 5:5 system (Capmul MCM-35.71%, Kolliphor EL-17.85, Transcutol P-17.85, Water- 28.57%)



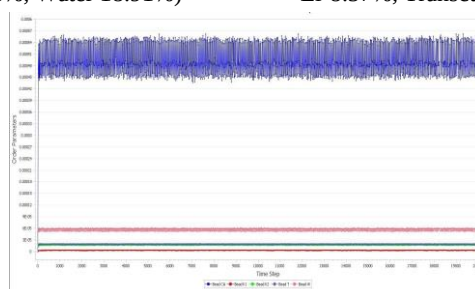
9 F). 6:4 system (Capmul MCM-46.15%, Kolliphor EL-15.38%, Transcutol P- 15.38%, Water- 23.07%)



9 G). 7:3 system (Capmul MCM-56.75%, Kolliphor EL-12.16%, Transcutol P-12.16%, Water 18.91%)



9 H). 8:2 system (Capmul MCM-68.57%, Kolliphor EL-8.57%, Transcutol P-8.57%, Water 14.28%)



9 I). 9:1 system (Capmul MCM -79.41%, Kolliphor EL 4.41%, Transcutol P -4.41%, Water 11.7%)

Figure 9. Mesodynamic order parameters (P) for 1:1 (Smix) ME systems from ratio 1:9 to 9:1 (A-I) for 20000 simulation time steps.

The kinetics of phase separation can be further evaluated using density maps from the Mesodyn method, which assess the miscibility and immiscibility characteristics of the ME systems [23]. The adsorption of surfactant/cosurfactant at the oil-water interface reduces the surface stress of the ME system [26]. As the oil phase increased, the hydrophobic effect became dominant, leading to reduced Smix concentrations and, in turn, immiscibility and phase separation due to higher interfacial tension.

3.3.4. Density Maps of the Beads in the Systems 1:9, 5:5, 9:1

Density maps of the various beads present in the ME system were generated at 20,000 time steps for three different systems (1:9, 5:5, and 9:1). These maps were based on bead distribution rather than field volume, providing insight into the accumulation behavior of the Kolliphor EL-based ME system. The distribution of the beads, represented by different colors, helps to explore the aggregation behavior within the system.

In this study, the bead system was structured as (K1(K2)3), where bead K1 (green) represents the hydrophilic portion, bead K2 (royal blue) represents the hydrophobic portion, bead W (turquoise blue) represents water (H₂O), bead CA (red) represents Capmul MCM NF, and bead T (pink) represents Transcutol P. The comparison of density maps across the three systems (1:9, 5:5, and 9:1) reveals that in the 1:9 and 2:8 systems, the particles are more uniformly dispersed, with greater homogeneity. This minimal aggregation is further supported by particle size and PDI experimental results

(Table 5). Zhao *et al.* (2017) also observed a similar increase in mean particle diameter in the 2:8 system of Brij97/Isopropanol/Isoamyl acetate/water compared to the 1:9 system.

Table 5. Particle size and PDI for (1:1) Smix.

Ratio	Particle size (nm)	Polydispersity index (PDI) %	Zeta potential
1:9	145.35	28%	-10.3mV
2:8	168.12	24.5%	-15.9mV
3:7	205.6	10.9%	-4.5mV
4:6	216.4	0.351	-7.1mV
5:5	222.3	25.3%	-6.7mV
6:4	231.7	25.7%	-7.6mV
7:3	257.5	20.4%	-6.7mV
8:2	288.7	25.1%	-9.1mV
9:1	398.4	5.4%	-0.968 mV

When comparing the density of bead CA across the three systems, it becomes evident that the density of bead CA is more widely dispersed in the 9:1 system compared to the 1:9 and 5:5 systems at the 20,000-time step. In the 1:9 system (Figure 9A), the P values for bead CA, bead K1, bead K2, bead T, and bead W are nearly zero, indicating no aggregation and uniformity. In the 5:5 system (Figure 9E), however, there is visible separation of P values among the beads, signaling immiscibility or phase separation. In the 9:1 system, a higher fluctuation in the P value of bead CA is observed, indicating greater aggregation. This observation is corroborated by the density maps of the 9:1 system (Figure 10), which show greater aggregation than those of the other systems.

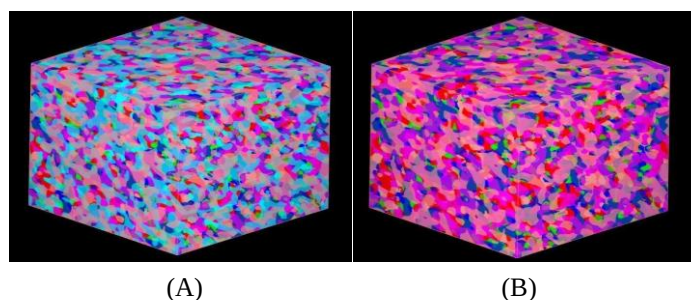


Figure 10. Phase morphology of 1:9 system with drug (TH): (A) 1:9 ME system with water; (B) 1:9 ME system without water.

3.3.5. Observations of the Drug in the 1:9 System

The Mesodyn simulation was also conducted for the 1:9 ME system, which, as observed experimentally and from MD studies, exhibits high homogeneity. The components of the ME system are listed in **Table 2**, including 1% of the drug (TH), represented as bead D (peach color). The system's phase diagram, both with and without water, is shown in **Figure 10**. The phase diagram indicates that the drug is evenly distributed throughout the system when water is present.

A comparison of the thermodynamic parameters for the 1:9 system, both with and without the drug, is summarized in **Table 4**. Although ΔG increased upon drug addition, homogeneity was maintained. The order parameters remained almost identical to those of the blank system, as shown in **Figure 11**.

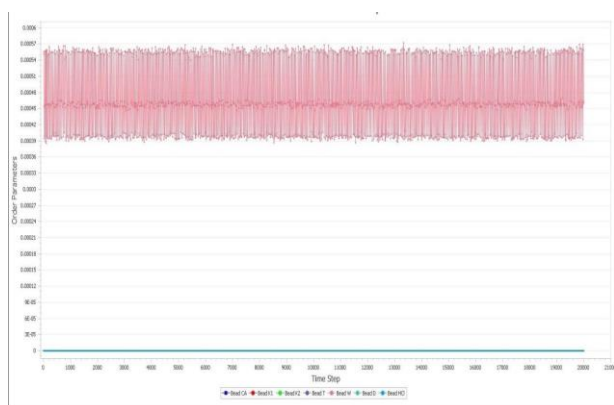


Figure 11. Order parameters of 1:9 system with the drug (TH) at 20000-time steps.

4. Conclusions

The research successfully determined the optimal solubility of a drug in various oils, surfactants, and cosurfactants for ME formulation. Transcutol P, Kolliphor EL, and Capmul MCM NF were selected for the development of the ME systems. The maximum solubility of the drug (TH) was observed in Capmul MCM (oil phase), Kolliphor EL (surfactant), and Transcutol P (cosurfactant). Phase diagrams of different surfactant/cosurfactant mixtures, with varying weight ratios, were constructed using the water titration method. Based on the pseudo-ternary phase diagram, a 1:1 Smix ratio with the largest microemulsion area (18.23 ± 0.51

cm²) was selected for formulating the MEs. MEs were then formulated with oil-to-Smix ratios ranging from 1:9 to 9:1.

Using order parameters and visualizations, dynamic simulations were performed with COMPASS force fields to investigate phase behavior and aggregation tendencies within the MEs. The coarse-grained model of Kolliphor EL (Bead K1(K2)3), Capmul MCM (Bead CA), Transcutol P (Bead T), and water (Bead W) was introduced in bead format. Predictions were made regarding the phase morphologies, phase behavior, aggregation behavior, and miscibility of ME components. Experimental observations were compared with Mesodyn predictions, and the two were found to be consistent. A range of mesoscopic metrics, including density maps, order parameters, and thermodynamic parameters, was carefully analyzed.

It was anticipated that the polar head of bead CA (oil phase) would form hydrogen bonds with the hydrophilic part of the surfactant (Bead K1), while its nonpolar tail would interact hydrophobically with Bead K2. Additionally, the polar head of Bead T was expected to form hydrogen bonds with Bead K1. Both Bead CA and Bead T contain hydroxyl groups that can engage in intermolecular hydrogen bonding with the water phase. These interactions could influence the miscibility of different ME components.

Both wet lab and simulation investigations of the 1:9 and 2:8 ME systems demonstrated homogeneity. The P values of each system showed that the rate of ME production increased with higher concentrations of Smix and water. As the oil phase increased, the hydrophobic effect became more dominant, and the Smix concentration decreased, leading to immiscibility and phase separation, likely due to higher interfacial tension.

Both the 1:9 and 2:8 ME systems exhibited very low Gibbs free energy (ΔG) values, indicating stability and spontaneous formation. Wet lab results showed that the 1:9 (145.35 nm) and 2:8 (168.12 nm) ME systems were within the desired range, consistent with the simulation findings of homogeneity.

The study found that the Gibbs free energy (ΔG) values of various ME systems reflected good thermodynamic stability, with more negative values indicating stronger stability. Phase separation, as shown

by order parameters, was more pronounced at higher oil concentrations than at Smix concentrations. Bead density maps, which aligned with PDI and particle size measurements, confirmed uniform dispersion in systems with higher Smix concentrations.

Ultimately, the use of Capmul MCM, Kolliphor EL, and Transcutol P to create stable MEs was validated through both experimental and *in silico analyses*, providing in-depth insights into stability, phase behavior, and the role of each component in ME formation.

Acknowledgments

Authors would like to thank the Department of Pharmaceutical Sciences and Drug Research, Punjabi University, Patiala for providing the experimental facilities and Department of Biotechnology for providing a computational facility under the Bioinformatics Centre, BIC (BT/PR39876/BTIS/137/7/2021), New Delhi, India.

Conflict of interest

No conflict of interest was disclosed by the authors.

Data availability

Data is available upon request from the corresponding author.

Authors Contributions

Dhriti Mahajan: methodology, experiments, *in-silico* work, and writing; Harpreet Kaur: writing (original draft); Jasveer Kaur: editing; Manoj Kumar, Om Silakari: *in silico* work; Bharti Sapra: writing, review, editing, and supervision.

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Funding

The authors are thankful to the anonymous reviewers and editors for the necessary suggestions. The authors would like to thank the Department of Biotechnology for providing a computational facility under the

Bioinformatics Centre, BIC (BT/PR39876/BTIS/137/7/2021), New Delhi, India.

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There was no use of artificial intelligence in the making of this article.

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