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Growth of Stable Nano-Sized Transfersomes as a Rectal Colloid for Improved Delivery of Cannabidiol

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Unpredictable release and absorption are problems with current cannabidiol (CBD) formulations. A workable substitute may be offered by the sensible design of a rectal colloid delivery system. In order to create a nano-sized transfersome that would produce increased bioavailability, stability, and absorption of CBD as a rectal colloid, the intrinsic physiochemical characteristics of transferosomes were utilized in this study. Using thin-film evaporation, create transfersomes made of soy lecithin, cholesterol, and polysorbate 80. These were then evaluated for size, entrapment effectiveness (%), shape, release of CBD, ex vivo permeability, and physicochemical stability. With a hydrodynamic particle size of 130 nm, a PDI value of 0.285, and a zeta potential of 15.97 mV, the formulation that was optimized for rectal distribution was able to entrap up to 80.0 ± 0.077% of CBD. The transfersomes were unilamellar, spherical vesicles, which matched the increased ex vivo penetration across the excised rat colonic membrane, according to the morphological analysis using SEM and TEM. Additionally, transfersomes demonstrated great promise in promoting rectal tissue permeation with superior stability and providing regulated release kinetics of CBD, a plant-based medication having intrinsically low bioavailability. They also enhanced the CBD's encapsulating stability for up to six months at room temperature.
Keywords: cannabidiol; transfersomes; rectal colloid; nanoencapsulation; permeation; stability.

1. Introduction

Because of their inherent physiochemical characteristics, colloidal-based drug carriers offer numerous benefits for the transport of bioactives. Colloidal vectors can be readily adapted to deliver a large range of tiny compounds to specified sites. The second most potent component of cannabis is cannabidiol (CBD), a potential tiny molecule that has no known psychotropic adverse effects, including addiction or hallucinations [1]. Only a small number of medicinal-grade products are now in use, despite mounting evidence that the CBD molecule has a variety

of pharmacological effects, including anti-inflammatory, anti-cancer, analgesic, and anti-anxiety actions [2]. Despite its high potency, CBD's effectiveness is restricted by its poor oral bioavailability, which ranges from 13 to 19% because of its quick metabolism, stomach instability, poor chemical stability (photo and thermosensitive), and low water solubility, which results in reduced and unpredictable absorption [3-6]. In an effort to optimize its possible therapeutic effects, a number of alternative delivery methods for CBD have been studied, including but not limited to transdermal [7], pulmonary [8], oromucosal [9], and intranasal [10]. While intranasal delivery of CBD provides a non-invasive method for achieving a quick beginning of action, this route of administration is limited by the possibility of irritation of respiratory and mucosal tissues as well as dosage variability. Research has also demonstrated that oromucosal administration for systemic absorption can improve the pharmacokinetics of CBD; however, absorption may not always take place through the transmucosal route [11]. To guarantee delivery effectiveness through this route, adequate mucosal residual duration and a strategy to avoid saliva washout are essential [12]. As far as we are aware, only few research has looked into rectal delivery of CBD as a potential helpful strategy for poorly accessible substances like CBD [13, 14]. Transmucosal administration, including that through the rectal route, is becoming more and more popular among patients receiving palliative care [15]. For instance, in cases of dysphagia or aphagia, or when the absorption of sensitive bioactives is necessary regardless of food effects, and/or when contact with GIT fluids must be avoided, rectal administration can offer both local action and systemic absorption of medications that can help particular patient groups [16]. A two-patient clinical study revealed that a rectal cannanbinoid formulation enhanced the systemic bioavailability in comparison to an oral formulation, despite the fact that there are few pharmacokinetics studies that explicitly compare the bioavailability between the rectal and oral administration [17]. Therefore, methods to attain more consistent and efficient drug absorption from the rectal region, such utilizing nanotechnology, are required to guarantee that this alternative mode of administration continues to be feasible for a greater range of bioactives. Furthermore, colonic disorders like colorectal cancer (CRC) can be managed using nano formulations because of their potential to function as diagnostic nanoplatforms and their capacity to decrease systemic toxicity through targeting [18]. In particular, lipid-based nanocarriers are among the best options since they may be functionalized with active targeting agents, provide a range of regulated particle sizes, and enable controlled release [19]. For the treatment of colorectal cancer, rectal administration is not the most recommended method. Wang and associates conducted a proof-of-concept study in which they demonstrated that a lipid-based nanoparticle rectal delivery system reduced in vitro and orthotopic murine growth of colorectal cancer [20]. Lipid nanocarriers are therefore very beneficial for the creation of rectal medication delivery systems. In order to obtain preferred and more stable bioactive absorption via the rectal route for the model botanical medicinal CBD, this study investigated the utilization of nano-sized transfersomes formed as a rectal colloid [21]. Like (nano) liposomes, transfersomes are ultra-thin, elasticized, biocompatible nanocarriers [22, 23]. Because transfersomes are more lipophilic and elastic than (nano) liposomes, they can help move bioactives between cells more profoundly and through biological barriers like the rectum for better absorption and penetration [24]. Phospholipids with an exterior complex lipid bilayer and an aqueous core structure make up transfersomes [25]. The unique flexibility required for self-optimizing the transfersome structure's deformability during permeation across a range of pore sizes is

provided by the incorporation of an edge activator [26]. Bilayered softeners known as edge activators, which are composed of single-chain surfactants such sodium cholate, sorbitan esters, and polysorbates, lower interfacial tension and destabilize lipid bilayers in vesicles to allow for easier deformation and intercellular transit. [27]. Here, we outline how the physicochemical characteristics of transfersomes were utilized to create a rectal colloid that would increase CBD's stability, absorption, and bioavailability. The findings that show the rectal route may be a feasible route for the steady distribution of CBD with optimal release and absorption were supported by thorough in vitro characterization and ex vivo investigations.

2. Literature review

Pulmonary delivery, a non-invasive method of administration that can circumvent first-pass metabolism and prevent uneven gastrointestinal adsorption, is a potential approach [28]. Because milk's composition varies seasonally and geographically, it is difficult to use milk as a platform for pharmaceutically regulated medication delivery. As a result, infant formula is being considered as a substitute milk-based formulation [29] because of its highly regulated nature. It has been demonstrated that co-administration of Epidiolex®/Epidyolex® with food increases the oral bioavailability of CBD in healthy volunteers [30], and that there is an increased exposure after consuming bovine milk [31]. With a 9.1-fold higher bioavailability, a 71-fold higher peak concentration, a 118-minute less time to attain optimal focus, and a 25-fold lower metabolite-to-CBD ratio, inhaled CBD outperformed oral delivery in terms of PK profile. These lend credence to the viability and effectiveness of breathing CBD as opposed to taking it orally [32]. However, CBD's low water solubility (0.1 µg/mL) restricts its ability to enter the body and reach the therapeutic concentration [33]. Only two inhalable powders have been created thus far, and none of them address the solubility problem. Devinsky et al.'s spray-dried CBD powder contained 65% fumaryl diketopiperazine (FDKP) by mass, 10% 1, 2-distearoyl-sn-glycero-3-phosphocholine (DSPC), and 25% CBD [34].

3. Materials and Methods

Triton X-100, phosphate-buffered saline (PBS) tablets, methanol, chloroform, soybean lecithin (SL), polysorbate 80, cholesterol, and hydrocortisone, mannitol, and CBD1.0 mg/mL in methanol standard. Sigma-Aldrich, located in St. Louis, Missouri, USA, was the source of Millipore®. Waters Corp. (Billerica, MA, USA) provided the membrane filters (0.22 m) and the high-pressure liquid chromatography (HPLC) column (4.6 mm 150 mm: 3.7 m). The isolation of cannabidiol (CBD) was a gift. The University of Witwatersrand Central Animal Service (CAS) provided the colorectal tissue. Other reagents utilized in the investigation complied with analytical standards, and for instrumental analysis, high purity solvents were employed.

3.1 Development of the CBD-Loaded Transfersome Based-Rectal Colloid Using Thin Film Hydration

To synthesize transfersomes for the best CBD encapsulation, a modified thin layer hydration technique with different lipid ratios was employed (Figure 1). A mixture of soy lecithin,

polysorbate 80, cholesterol, and 50 mg of CBD was used to create the lipid phase. After that, the we used an organic solvent to dissolve the lipid phase. that contained methanol and chloroform in a 2:1 ratio (1). At room temperature, the ingredients were stirred magnetically at 100 rpm until a homogeneous mixture was obtained. A rotary vapor was used to remove the organic solvent for five minutes, until a thin lipid coating was created, in a water bath set at 40^0 C and 100 rpm (2).

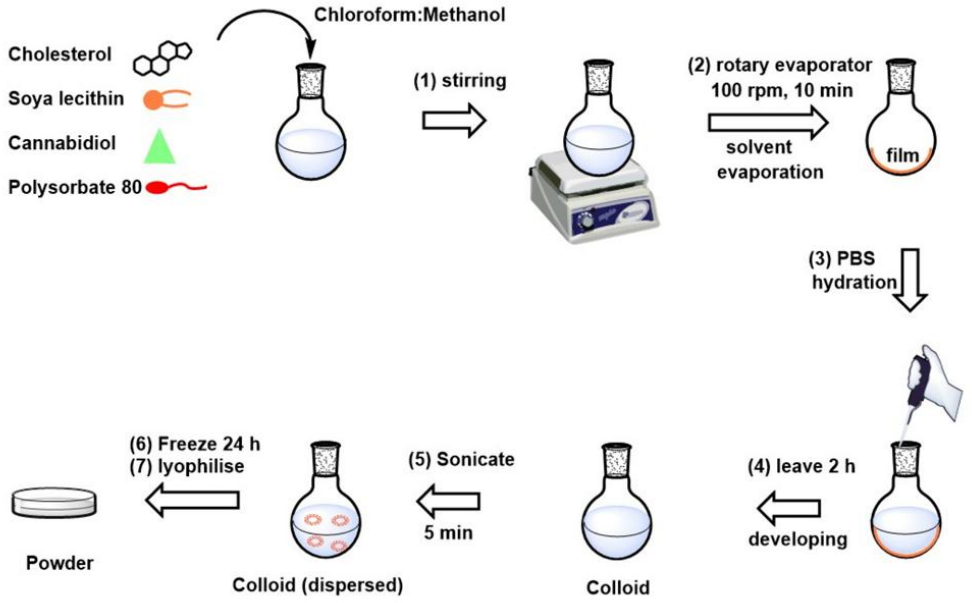

Fig 1: Diagrammatic illustration of the process used to prepare transfersomes loaded with CBD

Table 1: Lipid and solvent composition utilized in the creation of transfersomes loaded with CBD

Formulation	CBD (mg)	Polysorbate 80
T1	-	25
T2	-	50
T3	-	100
T4	60	25
T5	60	50
T6	60	100

3.2 Purpose of Morphology, Particle Size as well as Zeta Potential of CBD-Loaded Transfersomes

In order to prepare a sample for transmission electron microscopy using the FEI Tecnai T12 (FEI Company, Hillsboro, OR, USA), a diluted transfersomal dispersion loaded with CBD was made with distilled water (1:10) and then sonicated for 5 minutes using a bath sonicator

(Sonics Vibra Cell, CT, USA). A drop of the dispersion was then let to adhere to a copper grid covered with carbon. Before adding the uranyl acetate 2% aqueous solution stain, the grid was rinsed twice with deionized water for three to five seconds after any excess dispersion was carefully removed using filter paper. Overnight, the sample was left to dry naturally. A 6 kV scanning electron microscope (SEM) (Jeol JSM-120, Tokyo, Japan) running at high vacuum was used to assess the size and morphology of the transfersomes. An appropriate amount of sample was put on an aluminum stub, allowed to air dry, and then coated with gold-palladium for SEM analysis.

3.3 Determination of CBD Entrapment Efficiency (%EE)

Utilizing high-performance liquid chromatography, the amount of quantity of CDB (HPLC) is proposed. Using a mobile phase consisting of methanol: distilled water 85:15% at a flow rate of 1 mL/min, isocratic elution was performed. A Symmetry® C18 reversed phase column with a pore size of 3.5 m and diameters of 4.6 150 mm was used for the process. At 220 nm, a 20 L sample aliquot was injected and analyzed. 5.6 minutes was the retention period of cannabidiol, 2.6 minutes was the retention duration of hydrocortisone used for normalization, and 10 minutes was the total run time. The amount of CBD entrapped was measured by dissolving 5 mg of lyophilized transfersomes in 2 mL of 1% v/v Triton X-100 mixed with PBS and then agitating the mixture for 2 hours using a magnetic stirrer. A 0.2 m polycarbonate membrane filter was used to filter the resultant combination. HPLC was used to evaluate a 0.5 mL sample that had been further diluted with 0.5 mL of methanol. The following equations (Equation (1) were used to determine the percentage EE in accordance with reported procedures after the drug amount was determined.

$$EE = \frac{mass\ of\ \ CBD\ \ encapsulated}{Mass\ of\ \ CBD\ added\ to\ \ formulation} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

3.4 Using Thermal and Spectroscopic Analysis to Determine Excipient Compatibility

Mannitol (cryoprotectant), pure CBD, T3, and T6 were thermally characterized using differential scanning calorimetry (Mettler Toledo, DSC1, STARe System, Swchwerzenback, Switzerland). In perforated aluminum pans, samples weighing approximately 10 mg were crimped and heated in an inert nitrogen environment. With an insertion temperature of 10^0 C and a scanning temperature range of 10^0 C upto 300^0 C, a scanning rate of 10 C/min was used. For every sample, tests were performed in triplicate. Pure CBD, T3, and T6 were subjected to thermogravimetric analysis in order to assess their thermal degradation characteristics. Samples were obtained under inert conditions between 30 and 900 C (10 C/min) after an appropriate sample quantity was placed in the crucible. The data was analyzed using the PyrisTM tool. A PerkinElmer® Spectrum Series was used to record the FT-IR spectra of mannitol (cryoprotectant pure CBD, T3, and T6). The procedure, which was modified from the literature, was as follows: FT-IR measurements were conducted between 650 and 4000 cm^{-1} after suitable sample volumes were set up on a diamond stage.

3.5 Kinetics Evaluation of the Release Study of CBD from the Transferosomes

The Higuchi model, Koresmeyer–Peppas, zero order, and first order mathematical models were used to investigate the kinetics of cumulative drug release from transfersomes [35]. The

following equations were employed in this study:

$Q = K_0 t$ for zero order

$In(Q_t) = In(Q_0) + K_1 t$ for first order

$Q = K_H t^{1/2}$ for higuchi method

$Q = K\ .t^n$ for koresmeyer-peppas model

When t is the time in minutes, K_0 denotes zero order rate constant, and Q is the total amount of medication released at time t. The first order constant is indicated by K_1. K_H is Higuchi's dissolution constant. K denotes constant that combines the geometrical and structural characteristics of the dosage form. The release exponent is denoted by n.

3.6 Purpose of Ex Vivo Permeation of CBD-Loaded Transferosome

Wits Central Animal Service (CAS) granted an ethics waiver for the collection of colon tissue from Sprague Dawley rats weighing 200 – 300 g. Following its collection, the tissue was preserved in formalin. In order to forecast permeation, it was then immersed in PBS that was kept at 37^0 C. To evaluate the permeability of CBD and T6, a Franz diffusion cell with a diffusional area of 1.77 cm^2 was employed. The 12 mL receptor chamber was filled with PBS (pH 7.4; 37^0 C) containing 2% w/v polysorbate 80. A heat exchange jacket, which simulates rectal temperature, kept the temperature at 37^0 C. The donor compartment and receptor chamber were separated by the 0.056 cm thick colorectal tissue. Weighed and dispersed in 3 mL of PBS (pH 7.4; 37^0 C), the CBD isolate (3 mg) and T6 (300 mg) were evenly distributed on the membrane separating the donor and receptor compartments. Over the course of seven hours, samples (1 mL) were taken out of the receiving compartment medium at predetermined intervals. The volume of samples that were drawn was used to replenish the buffer. The HPLC technique was employed to measure the amounts of CBD in the samples. The rate of permeation was determined using the flow.

3.7 Colorectal Membrane Integrity Assessment

FT-IR and ionic conductivity measurements were used to evaluate the integrity of the colorectal membrane both prior to and following exposure to T6 and cannabidiol. Ionic conductivity was assessed using a Seven Multi S40 pH/electrical conductivity meter (Mettler–Toledo, Greifensee, Switzerland) both prior to and following exposure to CBD and T6. FTIR spectroscopy was used to examine the structural integrity of colorectal tissue both before and after exposure to CBD and T6 (PerkinElmer, UK).

4. Results and Discussion

Only the impact on the quantity of surfactant used to prepare the CBD-loaded transferosomes was examined in this investigation. Particle size, potential, and in vitro CBD release were among the various physicochemical characterizations of CBD-loaded transfersomes that were examined in order to infer alterations. Based on EE%, appropriate vesicular size and high drug

content quantity released (low retention of CBD), the best formulation for a CBD-loaded transfersome was chosen. Further investigations into the formulation's stability, morphological characterization, and penetration through the excised colonic membrane were conducted for the optimized formulation.

4.1. Conception of Vesicular Shape as well as Surface Morphology

An electron microscope (80 kV) was used to assess the size and shape of the ideal transferosomal dispersion (T6). The TEM images showed spherical vesicles by a unilamellar shape (Figure 2A), which matched TEM images of transfersomes stained with uranyl acetate that had been previously published. The average diameter, as determined by Image J software, was 87.31 12.62 nm. The mean diameter was predicted to be somewhat lower aimed at TEM analysis because the DLS measurements are carried out on solvated transfersomes, and this pattern was consistent with earlier research.

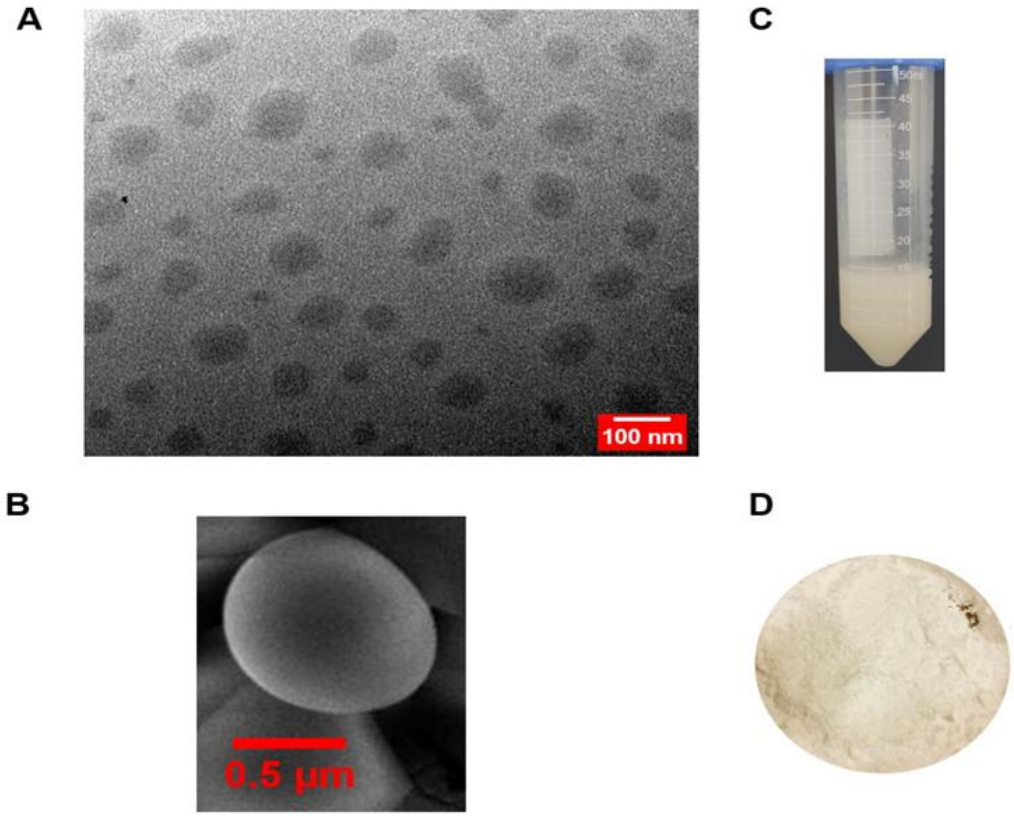

Fig 2: Images of enhanced CBD-loaded transfersome preparation (T6), both macro as well as microscopic. (A) TEM image (scale bar 100 nm); (B) SEM image; (C) the transfersomes' physical appearance (T6); and (D) the transferomes' lyophilized free-flowing powder.

SEM imaging was used to evaluate the surface morphology, and the findings revealed spherically cally-shaped transfersomes (Figure 2B). The preparations were kept as a dry lyophilized powder (Figure 2D) as well as rectal colloids were evenly distributed with no

discernible phase separation (Figure 2C).

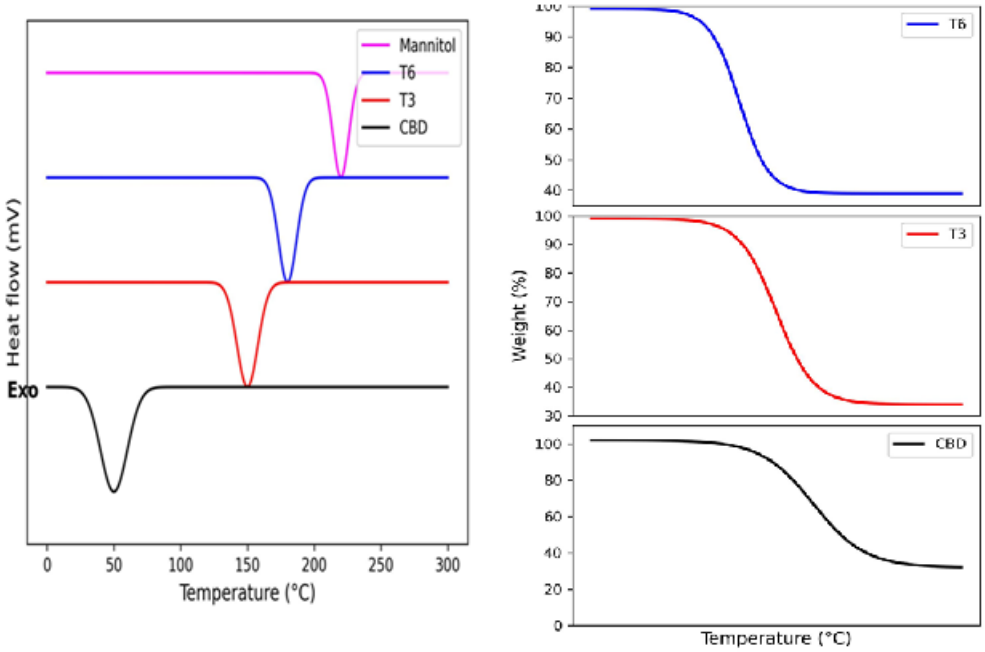

Fig 3: (A) Thermograms of pure CBD, transfersomes (T3), and CBD-loaded transfersomes (T6) were obtained using DSC and TGA, respectively.

In Figure 3A, the DSC thermogram is shown. According to published research, a distinct endothermic peak for CBD was seen between 60 and 75^0 C, which is also its melting point. The existence of mannitol, which was included as a cryoprotectant and may have existed in various crystalline forms during freeze drying, was confirmed by the observation of two distinct endothermic peaks in the T3 and T6 formulations at 130 and 150 C and 150 and 169 C, respectively. The lack of CBD endothermic peaks in formulation T3 confirms that CBD was successfully loaded into the transfersomes, and thermal phenomena in formulation T3 consist of comparable to those of formulation T6. When compared to free CBD, the temperature at which transfersome-loaded CBD began to degrade was noticeably higher. It is evident that CBD has been incorporated into the hydrophobic tails of the lipid bilayer because there is no temperature difference between the CBD and the empty -laden transfersomes colloid. This suggests that when CBD was encapsulated into transfersomes, its thermal stability was enhanced (Figure 3B).

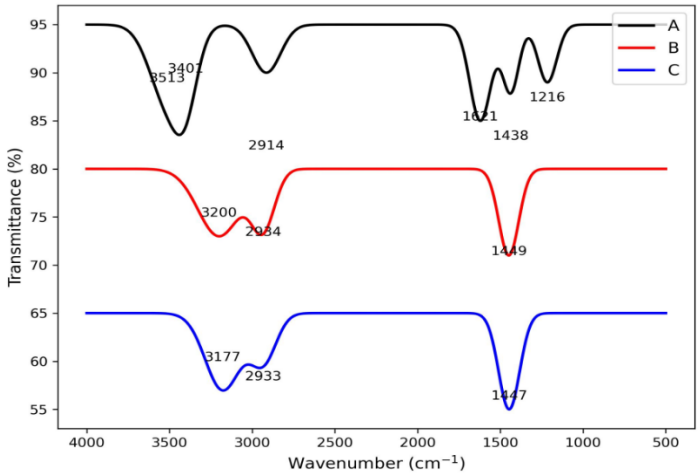

Fig 4: FTIR spectra of optimized CBD-loaded transfersomes (T6), CBD-free transferosomes, and virgin CBD (A).

Figure 4 shows FT-IR spectra of pure CBD, T3, and T6. FT-IR sheds light on how medications and excipients interact molecularly. Significant molecular vibrations in the 3401–3513 cm^{-1} core region of the CBD FT-IR spectrum (Figure 4 (A)) responded to the O-H (aromatic) stretching vibrations, while the bands at ~3000 cm^{-1} were attributed to C-H stretching (phenyl), ~2914 cm^{-1} to methyl as well as methylene groups, ~1575 cm^{-1} to C=C stretching (phenyl ring), and ~1216 cm^{-1} to C-O stretching vibrations.

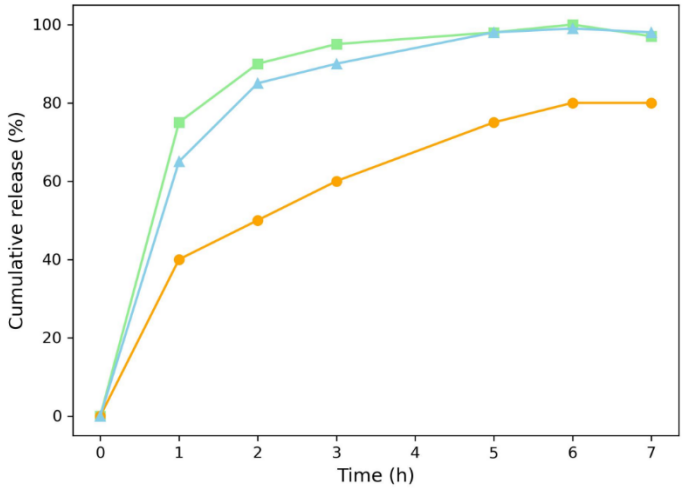

Fig 5: The cumulative release profile of CBD from the transferosomes over a 7-hour period at 37°C (n = 3) for T4: 25 mg polysorbate 80-loaded transfersomes, T5: 50 mg polysorbate 80-loaded transfersomes, and T6: 100 mg polysorbate 80-loaded transfersomes.

Figure 5 shows the graphic representation of the drug release from CBD-loaded transfersomes (T4 to T6) throughout an even hour at 37^0 C. About 95% of the medication was released aimed

at formulations T4 and T6, while about 80% of the medication was made available for formulation T5. The release was finished at 7 hours. For T4, T6, and T5, an initial fast release was seen. For an encapsulated hydrophobic drug, similar release patterns have been documented, with a quick initial release preceding a slow-release phase through suggested diffusion of drugs out of the lipid bilayer. T4 and T6 in particular displayed rapid CBD release. Since the surfactant promotes drug solubility in aqueous conditions, Greater ratios of surfactants have been shown to provide rapid drug release. On the other hand, T5's release profile indicated a relative barrier to drug release. This might result from the surfactant's increased ordering of the lipid bilayers at the ratio employed. However, T6's release pattern resembles T4's at very high surfactant concentrations.

Table 3: Kinetic modelling of CBD release from transfersomes in vitro

Formulation	Zero Order Model (R^2)	First Order Model	Higuchi model	Korsmeyer-peppas model
T4	0.5644	0.43251	0.7654	0.1457
T5	0.9444	0.7358	0.8365	0.2658
T6	0.7365	0.7452	0.7984	0.2547

The drug release investigation demonstrated that the transfersome formulation's reservoir effects can sustain drug release based regarding the quantity of edge activator. To get the best fit, the drug release results were fitted to drug release kinetics. mathematical model, as Table 3 displays. T5 showed zero order drug release, however the formulations T4 and T6 showed Higuchi mode, according to the correlation coefficient value (R2). Zero order indicates that a consistent amount of drug is released and that the drug concentration added has no effect on the drug release per unit of time. According to the Higuchi model, drug penetration occurs via diffusion, suggesting regulated release. These results were further corroborated by the Korsmeyer–Peppas model, which classified Fickian diffusion with n values obtained for T4–T6 smaller than 0.5. When the release is fast at first and progressively slower over time, this is known as Fickian diffusion. Diffusion triggered by a chemical gradient is the release mechanism.

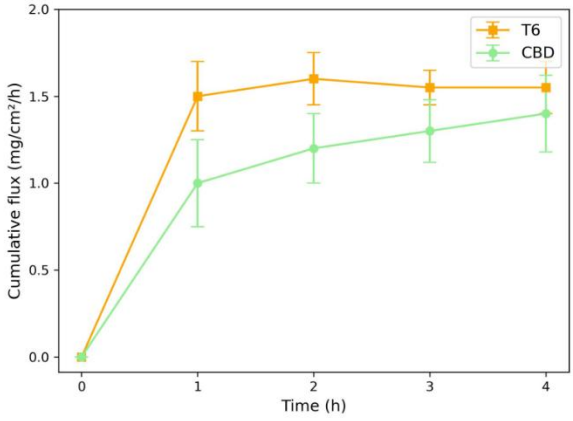

Fig 6: Over the course of seven hours at 37^0 C, three individuals' worth of pristine CBD and optimized CBD-loaded transfersomes cumulatively penetrated the excised colonic membrane (T6).

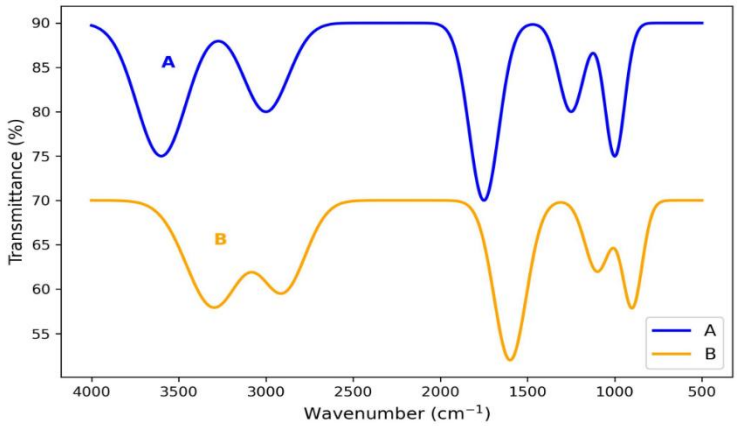

Fig 7: Alterations in the colorectal membrane's FTIR spectra that were noted both before and after the permeation study.

Important details regarding the drug's passage through cell membranes and into the bloodstream are provided by ex vivo permeation experiments. Transcellular passage across the cell membrane is the process by which the rectum allows for penetration or absorption. Permeation tests were conducted using T6, which was determined to be the most appropriate formulation for rectal CBD delivery based on drug release studies, physiochemical characteristics, and entrapment efficiency. For five hours, the permeation characteristics of formulation T6 were evaluated. The penetration of transfersomes and CBD was compared using cumulative flux. As seen in Figure 6, the transfersomes completely penetrated the colonic membrane in an hour, with a flux of roughly 1.7 mg/cm2/h as opposed to the 1 mg/cm^2/h of CBD alone. Additionally, following exposure to CBD and T6, the colorectal membrane remained intact in comparison to Figure 7 (A) (Figure 7 (B)). This is clear from the fact that the peaks at 2923 and 2853 cm^{-1} showed no discernible alterations, indicating that the membrane stayed intact during the investigation.

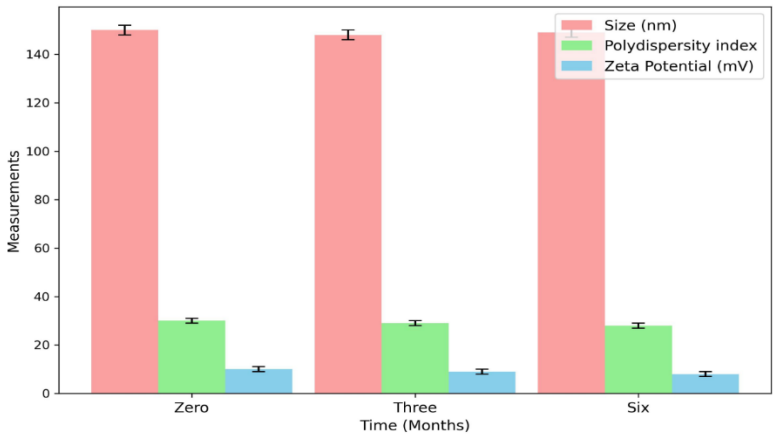

Fig 8: Based on measurements of the particle size, polydispersity index, and zeta potential at

room temperature (25^0 C) and ambient humidity, the stability of the transfersomes colloid over a six-month period is shown graphically.

The stability of transfersomes assessed over a period of six months based on size, PDI, and zeta potential is shown in Figure 8. The size increased insignificantly from 146.63 0.23 to 148.00 2.00, which is consistent with other studies' findings. The PDI decreased noticeably from 0.25 0.0056 to 0.23 0.0081. After six months, the zeta potential dropped somewhat from 29.13 1.3 to 27.91 0.4. Nevertheless, while the zeta potential drop was minimal, this finding still demonstrates that lyophilized transfersomes increased CBD stability. Additionally, the entrapment efficiency percentage for months 0 and 6 was determined to be 80.10% 0.25 and 78.51% 0.22, respectively, to determine stability in terms of CBD content. This outcome demonstrates that the transfersomes can improve CBD stability over a six-month timeframe.

5. Conclusion

The purpose of the current study was to evaluate the possible application of transfersomes as a cannabidiol encapsulating technique for rectal medication delivery. The solvent, lipids, edge activator, and hydration media were chosen for their appropriateness for rectal delivery according on this methodology. For the pre-made formulations, the effect on the surfactant concentration was examined in terms of particle size, zeta potential, and %EE. To improve the absorption of CBD in the rectal cavity, nano-sized transferosomes loaded with CBD were effectively created and included into a rectal colloid. The findings showed that stable transfersomes with an average particle size of 102.2–130.1 nm could effectively entrap 55.7–80.0% of CBD with different edge activator compositions that promoted lipid bilayer relaxation for better CBD encapsulation. According to the diffusion investigation, in vitro release experiments also showed a pattern of consistent CBD release kinetics with congruent absorptivity. Given these results, it is possible to prolong the use of CBD-loaded transfersomes in patients undergoing palliative care by embedding them in a colloidal enema or suppository that has steady rectal absorption. It would be simple to include the developed CBD-loaded transfersomes into a suppository basis, and additional optimization could be done to regulate the release and start of activity. To properly illustrate the advantages of the suggested lipid nanocarriers for CBD, in vivo testing with an appropriate CBD control should then be conducted.

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